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The Laureates of the Nobel Prize in Physiology or Medicine 2013: left – James E. Rothman (born 3 Nov 1950, Haverhill, MA, USA; Yale University, New Haven, CT, USA); center – Randy W. Schekman (born 30 Dec 1948, St. Paul, MN, USA; University of California, Berkeley, CA, USA); right – Thomas C. Südhof (born 22 Dec 1955, Goettingen, Germany; Stanford University, Stanford, CA, USA). They were awarded for discoveries of machinery regulating vesicle traffic, a major transport system in our cells (see Editorial, p. 991–2).

Dobitnici Nobelove nagrade za fiziologiju ili medicinu u 2013. godini: levo – James E. Rothman (rođen 3. novembra 1950, Haverhill, MA, USA; trenutno zaposlen na Yale University, New Haven, CT, USA); centar – Randy W. Schekman (rođen 30. decembra 1948, St. Paul, MN, USA; University of California, Berkeley, CA, USA); desno – Thomas C. Südhof (rođen 22. decembra 1955, Goettingen, Germany; trenutno zaposlen na Stanford University, Stanford, CA, USA). Oni su nagrađeni za otkrića u vezi sa mehanizmima uključenim u regulaciju prometa vezikula, glavnih transportnih sistema u našim ćelijama (vidi Uvodnik, str. 991–2).

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

The Nobel Prize in Physiology or Medicine 2013

Nobelova nagrada za fiziologiju ili medicinu 2013. godine

Gordana Šupić

Institute for Medical Research, Military Medical Academy, Belgrade, Serbia and Faculty of Medicine of the Military Medical Academy, University of Defense, Belgrade, Serbia

Ever since pioneering electron microscopic studies revealed the internal morphology of cells, cell biologists have been fascinated by the complex intracellular membrane structure and organization, particularly the endoplasmic reticulum (ER) and the Golgi complex. The ER is the site where the newly made membrane and secretory proteins has been sythetized and they then pass through the Golgi complex, where they undergo many posttranslational modifications, including carbohydrate modifications. Protein transport between ER, Golgi apparatus to cell surface is based on budding of small vesicles and subsequent fusing of these vesicles with target organelle. This complex intracellular transport process, termed vesicle trafficking, is highly organized and critical for diverse processes as cell growth, endocytosis, hormone release, and neurotransmission [1].

The 2013 Nobel Prize honors three scientists, James E. Rothman, Thomas C. Südhof and Randy W. Schekman, who have elucidated the precise molecular mechanisms of vesicle trafficking, a major transport system in our cells [2]. Randy Schekman identified three classes of genes that were required for vesicle traffic [3, 4]. James Rothman discovered that a protein complex enables vesicles to dock and fuse with their target membranes [5, 6]. Thomas Südhof identified molecular machinery that responds to an influx of calcium ions and directs calcium-sensitive proteins in nerve cells to rapidly bind vesicles and release their cargo to the outer membrane of the nerve cell, with temporal precision and on command [7, 8].

Vesicle trafficking is regulated by specific protein complexes. The first group is coat protein complexes, which induce forming a vesicle out of a donor membrane, clathrin or coat protein complex I and II (COPI and COPII) [9, 10]. The second group of proteins are vesicle and target-specific identifiers to dock the vesicle, membrane proteins SNARES (the abbreviation for SNAp REceptors). The third group of proteins are the proteins that help fuse the docked vesicle, NSF (for N-ethylmaleimide Sensitive Factor) and SNAP proteins (for Soluble NSF Attachment Protein) [1, 11].

Secretory proteins travel from the ER to the Golgi apparatus in transport vesicles coated with the protein complexes [1]. Clathrin-coated vesicles mediate transport from the Golgi apparatus and from the plasma membrane or lysosomes. COPII-coated vesicles bud from the ER, and COPI coated vesicles bud from Golgi cisternae. Initially, the cargo molecules bind to specific receptors, which triggers interaction with coat proteins. Coat proteins spontaneously polymerize into a cage-like structure that surrounds the vesicle. Formation of coat structure initiates the "budding" of the membrane. During the COPII budding process, cargo proteins and v-SNAREs are concentrated in designated regions of the ER [8–11]. Docking and fusion of vesicles to the target membrane is regulated by SNARE proteins [1].

SNAREs are receptor protein superfamilies that target and dock specific vesicles to the correct compartment. SNARE proteins have a central role in providing specificity and in catalyzing the fusion of vesicles with the target membrane. They are either vesicle (v-SNARE) or target (t-SNARE) membrane specific proteins. According to the SNARE hypothesis, the road map of vesicular transport is determined by the pattern of localization of SNAREs among compartments. Current evidence suggests that SNARE complex formation promotes membrane fusion by simple mechanical force. The paired v-SNARES, from transport vesicles and t-SNARES, from target membranes, wrap around each other, firmly interact and form stable SNARE complex, which lock the two membranes together. Water molecules are displaced from the interface of the vesicle and target membrane, thus phospholipid membranes could adhere and fuse [6, 7, 11]. Following specific vesicle docking, NSF and SNAP help fuse the docked vesicle by assembling to initiate fusion, with the hydrolysis of ATP, ensuring that the vesicle fuses at the right location and that cargo molecules are delivered to the correct destination [7, 8].

SNAREs have been best characterized in nerve cells, where they mediate the docking and fusion of synaptic vesicles at the nerve terminal plasma membrane. Neurotransmitters are released by synaptic vesicle exocytosis at the active zone of a presynaptic nerve terminal. [12, 13]. Upon arrival of an action potential the neurotransmitter is released from presyn-

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aptic terminals within a few hundred microseconds, and is Ca^{2+}-regulated [12, 13]. Action potential opens Ca^{2+}-channels, and transiently increases the local Ca^{2+}-concentration at the presynaptic active zone. Ca^{2+} then triggers neurotransmitter release by activating synaptotagmin Ca^{2+}-sensors, and induce mechanical activation of the membrane fusion machinery [13]. The SNARE complexes at neuron terminals are the as select targets for various neurotoxins causing botulism and tetanus. These toxins are highly specific proteases that cleave SNARE proteins in the nerve terminals, thus blocking the release of a neurotransmitter [1].

Through their discoveries, the three Nobel Laureates Rothman, Schekman and Südhof, have revealed one of the most fundamental processes in cell physiology. These exquisite discoveries identify how vesicles, carrying cellular molecular cargo, are precisely delivered, in a timely manner. This mechanism is critical for a variety of physiological processes in which vesicle fusion must be controlled, from neurotransmission to release of hormones and cytokines. Disorders in vesicle transport have damaging effects and could contribute to neurological diseases, diabetes, and immunological disorders.

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ORIGINAL ARTICLES

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The analysis of the connection between plaque morphology of asymptomatic carotid stenosis and ischemic brain lesions

Analiza povezanosti morfologije plaka asimptomatske karotidne stenoze i ishemijske moždane lezije

Djordje Milošević*, Janko Pasternak*, Vladan Popović*, Dragan Nikolić*, Pavle Milošević†, Vladimir Manojlović*

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Abstract

Background/Aim. A certain percentage of patients with asymptomatic carotid stenosis have an unstable carotid plaque. For these patients it is possible to register the existence of lesions of the brain parenchyma – the silent brain infarction. These patients have a greater risk of ischemic stroke by modern imaging methods. The aim of this study was to analyze the connection between the morphology of atherosclerotic carotid plaque in patients with asymptomatic carotid stenosis and the manifestation of silent brain infarction, and to analyze the influence of risk factors for cardiovascular diseases on the occurrence of silent brain infarction and the morphology of carotid plaque. **Methods.** This retrospective study included patients who had been operated for high grade (> 70%) extracranial atherosclerotic carotid stenosis at the Clinic for Vascular and Transplantation Surgery of the Clinical Center of Vojvodina over a period of 5 years. The patients analyzed had no clinical manifestation of cerebrovascular insufficiency of the carotid artery territory up to the time of operation. The classification of carotid plaque morphology was carried out according to the Gray-Weale classification, after which all the types were subcategorized into two groups: stable and unstable. Brain lesions were verified using preoperative imaging of the brain parenchyma by magnetic resonance. We analyzed ipsilateral lesions of the size > or = 3 mm. **Results.** Out of 201 patients 78% had stable plaque and 22% unstable one. Unstable plaque was prevalent in the male patients (male/female ratio = 24.8% : 17.8%), but without a statistically significant difference ($p > 0.05$). The risk factors (hypertension, nicotinism, hyperlipoproteinemia, and diabetes mellitus) showed no statistically significant impact on carotid plaque morphology and the occurrence of silent brain infarction. Silent brain infarction was detected in 30.8% of the patients. Unstable carotid plaque was found in a larger percentage of patients with silent brain infarction (36.4% : 29.3%) but without a significant statistical difference ($p > 0.05$). **Conclusions**. Even though silent brain infarction is more frequent in patients with unstable plaque of carotid bifurication, the difference is of no statistical significance. The effects of the number and type of risk factors bear no statistical significance on the incidence of morphological asymptomatic carotid plaque.

Key words:
carotid stenosis; brain ischemia; magnetic resonance imaging; risk factors.

Apstrakt

Uvod/Cilj. Neki bolesnici sa asimptomatskom karotidnom stenozom imaju nestabilni karotidni plak. Kod ovih bolesnika savremenim metodama snimanja može se registrovati postojanje lezija moždanog parenhima, tj. nemih moždanih infarkta. Ovi bolesnici imaju veći rizik od razvoja manifestnog ishemijskog moždanog udara. Cilj rada bio je da se utvrdi povezanost morfologije aterosklerotskog karotidnog plaka i pojave nemih moždanih infarkta kod bolesnika sa asimptomatskom karotidnom stenozom, kao i uticaj faktora rizika od nastanka kardiovaskularnih bolesti na nastanak nemih moždanih infarkta i morfologiju karotidnog plaka. **Metode.** Retrospektivnom studijom obuhvaćeni su bolesnici koji su u petogodišnjem periodu operativno lečeni na Klinici za vaskularnu i transplantacionu hirurgiju Kliničkog centra Vojvodine zbog visokostepene (> 70%) ekstrakranijalne aterosklerotske karotidne stenoze. Analizirani su bolesnici koji do momenta operacije nisu imali kliničke manifestacije cerebrovaskularne insuficijencije karotidnog sliva. Podaci su dobijeni analizom podataka sadržanih u istorijama bolesti tih bolesnika. Klasifikacija morfologije karotidnog plaka izvedena je prema Gray-Weale klasifikaciji, a potom su svi tipovi klasifikovani u dva podtipa: stabilni i nestabilni plak. Moždane lezije su verifikovane pomoću preoperativnog snimka moždanog parenhima magnetnom rezonancom. Analizirane su ipsilateralne lezije veće od ili jednake 3 mm. **Rezultati.** Istraživanjem je analiziran 201 bolesnik. Kod

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78% bolesnika nalazio se stabilan plak, a kod 22% nestabilan plak. Nestabilan plak je u višem procentu bio prisutan kod pacijenata muškog pola (odnos muškarci/žene = 24,8% : 17,8%) ali bez statistički značajne razlike ($p > 0,05$). Faktori rizika (hipertenzija, nikotinizam, hiperlipoproteinemija, dijabetes melitus) nisu pokazali statistički značajan uticaj na morfologiju karotidnog plaka i nastanak nemih moždanih infarkta. Nemi moždani infarkt nađen je kod 30,8% bolesnika, a nestabilan karotidni plak kod većeg broja bolesnika sa nemim moždanim infarktom (36,4% : 29,3%), ali bez statistički značajne razlike ($p > 0,05$). **Zaključak.** Iako bolesnici sa nestabilnim plakom karotidne bifurkacije imaju češće dokazan nemi moždani infarkt, ta razlika nije statistički značajna. Broj i vrsta faktora rizika ne utiče statistički značajno na pojavu morfoloških oblika asimptomatskih karotidinih plakova.

Ključne reči:
aa. carotis, stenoza; mozak, ishemija; magnetska rezonanca, snimanje; faktori rizika.

Introduction

Stroke is one of the leading health problems of modern man. It is the third most frequent cause of death in industrially developed countries, closely behind cardiac diseases and cancer, and is the leading cause of long-term disability [1].

The results of epidemiological research show that 88% of all strokes have ischemic etiology, whereas other causes include intracerebral hemorrhage in around 9% cases and subarachnoid hemorrhages in around 3% of cases [1, 2].

Atherosclerosis of the carotid and vertebral arteries is the most frequent cause of extracranial cerebrovascular disease (ECD) which leads naturally to artery stenosis. Atherosclerotic stenosis of extracranial segment of the internal carotid artery is the cause of around 20% of ischemic strokes [1, 2].

Stenosis of carotid arteries can be symptomatic and asymptomatic. The majority of extracranial carotid stenoses are asymptomatic [3, 4].

The treatment of atherosclerotic ECD can be medical or surgical and its basic aim is to prevent new or reccurent ischemic event, transient ischemic attack (TIA) or stroke. With symptomatic ECD the treatment is a form of secondary prevention, whereas with the asymptomatic ECD it is a primary prevention of stroke [4].

Both the North American Symptomatic Carotid Endarterectomy Trial (NASCET) and the European Carotid Surgery Trial (ECST) analyzed the advantages of medical and open surgical treatment of symptomatic ECD resulting with clearly defined views on medical and surgical treatment of symptomatic atherosclerotic ECD [4].

The Veterans Administration Centers Study (VACS), the Asymptomatic Carotid Atherosclerotic Study (ACAS) and the Asymptomatic Carotid Surgery Trial (ACST) analyzed the effects of medical treatment in comparison with surgical therapy in prevention of ischemic strokes in asymptomatic atherosclerotic ECD. Unlike symptomatic ECD where the benefit of surgical treatment has been proven in patients with extracranial carotid stenosis > 60% at an annual level, the studies of asymptomatic ECD showed that the benefits of surgical treatment in terms of prevention of ischemic stroke can only be observed in a five year period. According to the ACAS study, there was a decrease from 2% to 1% of the annual incidence of ischemic stroke in surgically treated asymptomatic patients; this means that there would be around 20 patients with asymptomatic ECD operated on annually in order to prevent one ischemic stroke per 5 years. According to that study there is a large number of patients operated for asymptomatic ECD with no direct benefit of the operation in terms of secondary prevention of brain lesions [5].

The research so far demonstrated the significance of atherosclerotic carotid plaque morphology as a factor contributing to ischemic cerebrovascular incidents in both manners: hemodinamically and as a source of thromboembolic ischemic events. Atherosclerotic plaque rupture is central for the ischemic cardiovascular incident. Plaques are usually composed of an extracellular lipid matrix, thin fibrous cap, smaller quantity of smooth muscle fibers and numerous macrophages and mastocites in most ruptures. Thin fibrous cap of the plaque can rupture as a consequence of diminished collagen synthesis, increased matrix degradation or as a result of external mechanical and chemodynamic stress. Plaques at the carotid artery branch point are exposed to strongest biomechanical and chemodynamic stress [6–9].

Histological researches on carotid plaque in symptomatic and asymptomatic patients show that the main features of unstable plaques found in symptomatic patients are: ulceration of the surface and rupture of the plaque, thinning of the fibrous cap and infiltration of the fibrous cap with microfags and T-lymphocytes [7].

Morphological assessment of carotid plaques can be determined preoperatively with the use of modern imaging methods – ultrasohography (US) and magnetic resonance imaging (MRI); or postoperatively with pathohistological analysis. Numerous researches prove the correlation of the morphological analysis of carotid plaque made by imaging methods with the pathophysiological data and thus pointing to the possibility of an adequate preoperative assessment of the morphology of carotid plaque [10, 11].

Silent brain infarction (SBI) is an ischemic change of the brain parenchyma without clinical symptomatology which can be registered by modern imaging methods – multislice computed tomography (MSCT) and MRI, and which is > or = 3 mm. The changes < 3 mm are not considered as SBI but as a consequence of perivascular space expansion (Wirchov-Robin) in the brain parenchyma [12]. Researches show that in most cases the origin of SBI has an thromboembolic etiology – either of cardiac or arterio-arterial origin, whereas the arterio-arterial SBI are usually connected with unstable carotid plaque. It has been proven that SBI is a significant risk factor for the development of clinically manifested ischemic stroke and a contributing factor in the devel-

opment of cognitive disfunction and psychiatric and neurological disturbances [12, 13].

There have been numerous studies on different risk factors for the development of ischemic brain lesions to detect and define a critical group of patients out of a large group with asymptomatic ECD. These would directly benefit from the operative treatment in terms of ischemic stroke prevention.

The aim of this study was to analyze the influence of atherosclerotic plaque morphology on the prevalence of SBI, as well as to analyze the impact of defined risk factors on atherosclerotic carotid plaque morphology and on the prevalence of SBI.

Methods

Medical documentation (medical history, computer data bases, operative protocols, archives and photo documentation) of the Clinic for Vascular and Transplant Surgery, Clinical Center of Vojvodina, Novi Sad, was analyzed for the period from January 1, 2005 to December 31, 2009 (5-year period). This retrospective study included the following methodological stages: stage I – selection of the material, that is medical documentation (medical history, anesthesia reports, surgery lists, intensive treatment lists, the accompanying clinical, radiological and laboratory documentation, hospital discharge, coroner's reports) of all the patients with asymptomatic ECD; the stage II – scope of data for each patient organized according to the following parameters: age, sex, risk factors for narrowing of carotid arteries (diabetes mellitus, hypertension, hyperlipoproteinemia, nicotinism, the presence of ischemic brain changes > or = 3 mm, morphology of atherosclerotic plaque; stage III – descriptive statistics for the entire population carried out as recommended by Glantz [14]. The parameters for the descriptive statistics were: middle value, standard deviation, median, minimum and maximum. The frequency of certain categories was examined for the nonparametric features included. In terms of comparative statistics, we used the Student's *t*-test in order to define the difference in the middle values of attributes between the groups tested; the stage IV – defining the features for comparative statistics regarding sex – subjects of male and female sex were compared in terms of descriptive statistical characteristics and the existence of ischemic brain lesion and participants comparison according of the characteristics to descriptive statistics.

The statistically significant difference was set at $p < 0.05$.

Asymptomatic ECD is narrowing of one or both internal carotid arteries without the existence of focal or global neurological symptoms accompanying artery stenosis: TIA, amaurosis fugax (AF) and ischemic stroke. Our study included all patients with high grade (> 70%) extracranial carotid artery stenosis during a 5-year period with no previous history of cerebral vascular insufficiency at all. Symptomatic were considered the patients with their last ischemic event within the last 6 months [15].

Morphological classification of atherosclerotic carotid plaque was carried out according to the Gray-Weale Duplex Ultra Sound (DUS) classification: type I – dominant echolucent plaque with thin echogenic cover, type II – echolucent lesions with smaller regions of common echogenicity; frequent exulceration, type III – lesions with dominant common echogenic reactivity, with small echolucent areas (less than 25%); stable plaque, provided that the luminal side kept its interior intact; type IV – uniform echogenic lesion, it corresponds to homogenous-fibrous plaque, with no signs of intraplaque hemorrhage or exulceration [11].

The carotid plaques were further classified according to the preoperative DUS results into two subcategories: unstable plaque – types 1 and 2 according to the Gray-Weale classification; and stable plaque – types 3 and 4 according to the Gray-Weale classification [7].

The classification of plaques into the stable and unstable ones was also carried out on the basis of intraoperative results.

The existence of SBI was determined on the basis of preoperative MRI image of the brain parenchyma and the SBI have been analyzed and localized in the ipsilateral hemisphere of the brain in relation to the localization of the carotid plaque.

The SBI include the changes of the brain parenchyma localized cortically and subcortically as hyperintensive lesions on T2 MRI images > or = 3 mm [11].

Stage V included comparative statistics in order to compare the differences in the intensity of the phenomenon between the observed groups, thus we used the Pearson χ^2-test for nonparametric features, and for the comparison of differences in the structure of the phenomenon the Spirman's correlation test. Calculations were performed within the StatSoft, Inc. package (2007). Stage VI covered analysis of the results. Thus, all the data were processed and presented in separate table for each group.

Results

In the period from January 1, 2005 to December 31, 2009 there was the total of 201 patients operated on for asymptomatic ECD at the Clinic for Vascular and Transplantation Surgery of the Clinical Center of Vojvodina in Novi Sad. Their average age was 66.8 years.

There were 11 patients with unstable plaque (22%) and 190 patients with stable carotid plaque (78%).

Table 1 shows the percentages distribution of the type of plaque in relation to risk factors and sex.

None of the analyzed risk factors showed statistically significant impact on the type of carotid plaque.

There was no statistically significant difference in relation to the morphology of the plaque between the sexes.

The distribution of the patients in relation to the presence/absence of ischemic changes in the brain bigger than 3 mm (positive MRI > or = 3 mm) is given in percentages (Table 2).

The distribution of the patients according to the presence/absence of ischemic changes > or = 3 mm in relation to the risk factors and sex is given in percentages (Table 3).

Table 1

Type of plaque in relation to the risk factors

Risk factor	Patients (%)		*p*
	stable plaque	unstable plaque	
Hypertensio arterialis	88.5	77.3	0.097
No hypertensio arterialis	11.5	22.7	0.097
Nicotinism	60.5	65.9	0.634
Without nicotinism	39.5	34.1	0.634
Hyperlipoproteinemia	45.2	38.6	0.544
No hyperlipoproteinemia	54.8	61.4	0.544
Non diabetes mellitus	75.2	70.1	0.209
Non insulin dependent diabetes mellitus	12.3	14.3	0.209
Insulin dependent diabetes mellitus	12.5	15.6	0.209
Sex (male/female)	75.2 / 82.2	24.8 / 17.8	0.318

Table 2

Presence of ischemic changes in the brain of patients with asymptomatic carotid plaque

Positive MRI changes > or = 3 mm	Patients (%)
Present	30.8
Not present	69.2

MRI – magnetic resonance imaging.

Table 3

Ischemic changes in relation to the risk factors

Risk factor	Patients with positive MRI > or = 3 mm (%)	Patients with negative MRI > or = 3 mm (%)	*p*
Hypertensio arterialis	82.3	87.8	0.411
No hypertensio arterialis	17.7	12.2	0.411
Nicotinism	62.9	61.2	0.937
Without nicotinism	37.1	38.8	0.937
Hyperlipoproteinemia	43.5	43.9	0.913
No hyperlipoproteinemia	56.5	56.1	0.913
Non diabetes mellitus	77.4	75.5	0.394
Non insulin dependent diabetes mellitus	11.3	17.3	0.394
Insulin dependent diabetes mellitus	11.3	7.2	0.394
Sex (male/female)	34.2 / 26.2	65.8 / 73.8	0.291

MRI – magnetic resonance imaging.

None of the analyzed risk factors showed a statistically significant impact in the presence or absence of ischemic changes in brain parenchyma.

There was no statistically significant difference in the presence of ischemic changes between the sexes.

Table 4 shows the percentage distribution of the type of plaque in relation to the presence/absence of ischemic changes > 3 mm.

Table 4

Type of plaque in relation to ischemic changes

Type of plaque	Patients with positive MRI changes > or = 3 mm (%)	
	presence	absence
Stable	29.3	70.7
Unstable	36.4	63.6

MRI – magnetic resonance imaging.

There was no statistically significant difference in terms of type of plaque in relation to the presence/absence of ischemic changes.

Discussion

Numerous epidemiological studies show that the greatest number of atherosclerotic ECD is asymptomatic. The basic aim of atherosclerotic healing ECD is the prevention of ischemic brain lesions. Studies on the treatment of asymptomatic ECD prove the preventive impact of operative treatment of carotid stenosis > 60%, but also that a treatment of a large number of asymptomatic patients would be take to prevent a relatively small number of ischemic strokes [5]. Taking into consideration the general operative morbidity and mortality of carotid surgery, the conclusion is that carotid surgery is not directly preventive for all the patients with asymptomatic ECD. It is estimated that around 70% of patients with asymptomatic ECD experience a massive ischemic stroke. Recent findings in relation to the treatment of asymptomatic ECD are directed towards finding the group of asymptomatic patients to benefit to a greater extent from the operative treatment [5]. Keeping in mind the fact that coronary atherosclerotic disease has a higher incidence of ischemic lesions in case of unstable atherosclerotic plaque, the supposition was that the incidence of ischemic changes also changed in relation to the morphology and type of atherosclerotic plaque in case of carotid atherosclerotic disease [8]. Within this study the total of 201 patients were analyzed who had underwent surgery for high grade (>70%) of asymptomatic stenosis. The analysis of the carotid plaque showed a higher percentage of stable atherosclerotic plaque (78%) with asymptomatic patients, whereas a smaller number of patients

had the unstable plaque (22%) which is in line with recent findings in the literature which state that around two thirds of atherosclerotic plaques in asymptomatic patients are stable plaques and only one third unstable [16]. It has also been noted that stable plaques are present in a larger percentage in female patients whereas unstable ones are more prevalent in male patients, although these results had no statistically significant difference. These findings are also in line with the data from the literature where it is stated that soft unstable plaques are more frequently found in men [3]. The literature shows that the presence of defined risk factors for cardiovascular diseases bears no statistically significant influence on the morphology and type of plaque. In this paper the risk factors were analyzed showing no significant effect on the morphology and type of plaque, and a greater percentage of unstable carotid plaques in smokers [3]. SBI existence analysis showed their presence in 30.8% and the absence in 69.2% of patients with asymptomatic ECD, with the greater percentage of SBI registered in male patients than in female. None of these factors showed a greater influence on the incidence of SBI in patients with asymptomatic ECD.

The largest percentage of registered SBI was present in patients with unstable exulceric carotid plaque but the results did not show statistically greater incidence of SBI in unstable carotid plaque. According to the data from the literature, the number of registered SBI in asymptomatic carotid stenosis > 60% is around 34%, and tending to increase with the increase of the artery stenosis level. Around 24% of SBI is registered in the area of ipsilateral hemisphere [5]. From the etiological point of view, SBI can be thromboembolic or nonthromboembolic. Thromboembolic SBI is a consequence of thromboembolism in silent zones of the brain parenchyma possibly of cardiogenic origin or it can be caused by artery emboli, which is the reason for the existence of unstable exulceric carotid plaque to be considered the most frequent cause of SBI in asymptomatic ECD [13, 16–18]. According to the Asymptomatic Carotid Stenosis and Risk of Stroke (ACSRS) study, most registered SBI in asymptomatic ECD, for stenosis from 60% to 79%, are registered in male patients and is around 41%. This number is close to the results of our study. According to this study, a significant number of SBI is registered in patients with carotid stenosis > 60% and it increases with the stenosis percentage, regardless plaque morphology. Unstable atherosclerotic carotid plaque is a contributing factor in the development of SBI, whereas most registered cases of SBI have ipsilateral distribution in the brain parenchyma [5].

Numerous researches point to a connection between the presence of SBI and the incidence of ischemic stroke regardless the etiology [5]. The results of the ACSRS study show that in patients with medium or high level of asymptomatic carotid stenosis and registered ipsilateral SBI, there is an increased risk of the development of ischemic stroke. According to this study, ischemic stroke will happen in 4.6% of patients with a significant asymptomatic carotid stenosis and ipsilateral SBI, whereas in patients without SBI ischemic stroke will happen in 2.4% cases annually, which is nearly 50% less [5]. In patients with low level carotid stenosis (< 60%) there is a small risk of ischemic stroke regardless the morphology of plaque and that it is approximately at 1.6% at an annual level [7]. The research conducted by Cao et al. [19] showed that ipsilateral SBI is found in 24% of patients with asymptomatic carotid stenosis > 60%. These researches point that at a 10-year level risk of ischemic stroke in patients with carotid stenosis > 60% and ipsilateral SBI is 21%, whereas in patients without SBI it is 11%, which is the level of a significant statistical importance.

Our results, regarding the incidence of SBI and asymptomatic high-grade carotid stenosis, the influence of carotid plaque morphology and defined risk factors for cardiovascular diseases, are in line with similar international researches. Analyzing these results, and those in the literature [20–22], the significance of preoperative assessment of the morphology of carotid plaque and of the brain parenchyma are stressed, thus separating a group of patients with moderated symptomatic carotid stenosis for whom the operative treatment has a considerable preventive effect on the development of ischemic brain lesions, out of a larger group of asymptomatic patients with high level carotid stenosis.

Conclusion

The role of (endo) surgical treatment in secondary prevention of ischemic stroke is clearly defined, whereas the primary prevention remains a moot point. Even though patients with unstable plaque of the carotid bifurcation have more frequently SBI, this difference bears no statistical importance. The effect of the number and type of risk factors of ECD is not statistically relevant for the incidence of morphological asymptomatic carotid plaques.

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ORIGINAL ARTICLE

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Finite element analysis in defining the optimal shape and safety factor of retentive clasp arms of a removable partial denture

Definisanje optimalnog oblika i faktora sigurnosti retencionih ručica kukica parcijalnih skeletiranih proteza metodom konačnih elemenata

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Abstract

Bacground/Aim. Retentive force of removable partial denture (RPD) directly depends on elastic force of stretched retentive clasp arms (RCAs). During deflection RCA must have even stress distribution. Safety factor is the concept which can be applied in estimating durability and functionality of RCAs. This study was based on analyzing properties of clasps designed by conventional clasp wax profiles and defining the optimal shapes of RCAs for stress distribution and safety factor aspects. **Methods.** Computer-aided-design (CAD) models of RCAs with simulated properties of materials used for fabrication of RPD cobalt-chromium-molybdenum (CoCrMo) alloy, commercially pure titanium (CPTi) and polyacetale were analyzed. **Results.** The research showed that geometrics of Rapid-flex profiles from the BIOS concept are defined for designing and modeling RCAs from CoCrMo alloys. I-Bar and Bonihard clasps made from CPTi might have the same design as CoCrMo clasp only by safety factor aspect, but it is obvious that CPTi are much more flexible, so their shape must be more massive. Polyacetale clasps should not be fabricated by BIOS concept for CoCrMo alloy. A proof for that is the low value of safety factor. **Conclusion.** The BIOS concept should be used only for RCAs made of CoCrMo alloy and different wax profiles should be used for fabricating clasps of other investigated materials. The contribution of this study may be the improvement of present systems for defining the clasps shapes made from CoCrMo alloys. The more significant application is the possibility of creating new concepts in defining shapes of RCA made from CPTi and polyacetale.

Key words:
denture, partial, removable; dental prosthesis design; dental clasps; dental alloys; titanium; polyacetylenes; safety.

Apstrakt

Uvod/Cilj. Retenciona sila parcijalne skeletirane proteze (PSP) direktno zavisi od elastične sile rastegnute kukice. Da bi uspešno obavile svoju ulogu, retencione ručice kukice (RRK) prilikom defleksije moraju imati što ravnomerniju raspodelu napona. Stepen sigurnosti je pojam koji se može primeniti u proceni trajnosti i funkcionalnosti RRK. Ciljevi ove studije bili su analiziranje svojstava kukica koje su urađene pomoću konvencionalnih voštanih profila za izradu RRK, kao i definisanje optimalnih oblika RRK sa aspekta raspodele napona i stepena sigurnosti. **Metode.** Analizirani su CAD (*computer aided design*) modeli RRK kojima su simulirana svojstva gradivnih materijala koji se koriste za izradu legura: CoCrMo, komercijalno čist titan (CPTi) i poliacetal. **Rezultati.** Rezultati su pokazali da je geometrija *Rapid-flex* profila, korišćenih u okviru BIOS, definisana za projektovanje i modeliranje RRK koje se izrađuju od legure (CoCrMo). I Bar i Bonihard kukice od CPTi mogu se uraditi po istom konceptu kao i legure CoCrMo sa aspekta stepena sigurnosti, međutim, titanijumske kukice bile su znatno elastičnije i stoga su morale biti masivnije. Kukice od poliacetala ne smeju se modelovati po BIOS konceptu za leguru CoCrMo. Dokaz za to je vrlo mali stepen sigurnosti. **Zaključak.** BIOS koncept može da se koristiti samo za RRK koje se izrađuju od legure CoCrMo. Za izradu kukica od ostalih ispitivanih materijala neophodni su drugačiji voštani profili. Doprinos studije predstavlja i poboljšavanje postojećih sistema za definisanje oblika RRK izrađenih od legura CoCrMo. Značajnija primena rezultata je i mogućnost stvaranja novih sistema za definisanje oblika RRK od CPTi i poliacetala.

Ključne reči:
zubna proteza, parcijalna, mobilna; zubni protetski modeli; zubne kukice; legure, stomatološke; titan; poliacetileni; bezbednost.

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Introduction

Removable partial dentures, present for several decades, even in the era of dental implants can be incorporated into modern dental trends. Prophylaxis, noninvasive nature, aesthetics, durability and functionality of dental restoration are the most important principles that are applied in modern methods of removable partial denture (RPD) planning and creation.

Prophylaxis is known as the first principle in planning RPD [1–5]. It refers to the reduction of the skeleton and avoiding the contact of solid parts of the prosthesis with free gingiva [6–12]. Methods of minimally invasive dentistry in the development of RPD, such as purposed fillings and selective grinding in the aim of defining proper guiding planes allows better therapeutic alternative to conventional dental procedures in order to week the same aesthetic and functional requirements with maximum preservation of healthy dental tissues [13, 14]. Aesthetic problems occur when the therapist is forced to plan retentive clasp arms (RCAs) in the visible sector. An attempt to improve the aesthetic effect by the reduction of RCA ends up with weakening of the retention force of RPD [15]. Retentive clasp arms of RPD are one of the factors that dictate the durability and function benefits of dentures [16]. It has been found that the durability of RCA depends on the material used as well as its design [17, 18]. Preference materials for RPD modeling include cobalt-chromium-molybdenum (CoCrMo) alloy, titanium and type IV gold alloys. Retentive clasp arms, made in the conventional way, out of CoCrMo alloy, from one-piece cast are not flexible enough to be able to respond the challenges in aesthetic areas. The aforementioned challenges imply that RCA can be placed in a position without being visually noticeable, or for the same reason, its length can be reduced. Elastic modulus for titanium alloy is approximately half the size of CoCrMo alloy, which enables the production of shorter retention elements and setting a clasp in deeper undercut area [19]. However, complicated titanium casting technology is one of the reasons for its poor commercial use.

Polyacetal copolymer can be an alternative to a cast metal alloy RCA [20, 21] and comparing with the metal alloy has a much better aesthetic due to the possibility of tooth and gingiva coloring and elastic properties. The results of previous studies suggest that more undercuts can be used with polyacetal clasps comparing to the already mentioned materials, but due to low elastic modulus the retention elements must be of much larger size [22].

RCAs can be applied in cases of all non-invasive methods for RDP modeling, but the question is whether this kind of denture, that meets the requirements for prophylaxis and non-invasive support, can meet the aestethic requirement, and still deliver durable and functional results.

RCAs are constantly exposed to elastic deformations resulting in the stress in the material. In order to perform its role successfully, the presence of uniform stress distribution in the cross section and along the clasp is essential [23–27].

Safety factor is the term that can be applied in assessing the durability and function of a RCA. Safety factor represents the relationship between working stress of clasp material originated by the action of a given load and stress within elastic limit, yield strength, respectively (data provided by the clasp material manufacturer). Safety factor is a term describing the structural capacity of a system beyond the expected loads or actual loads, and is represented by dimensionless number. The body or an object with safety factor, under force applied, of one or above, will not undergo plastic deformation under a given load [28–34]. In order to determine the safety factor successfully, it is necessary to clearly define which region of the arm is exposed to the highest stress during the application of retention force.

Whether plastic deformation will occur in RCA depends on the balance of strength and elasticity of the material it is made of. Stress distribution in RCA under forces applied, whether their application is balanced or there are regions particularly threatened and predisposed to plastic deformation, greatly influence the plastic deformation itself. This research should clarify the question regarding those particular regions within RCA made of CoCrMo alloys, commercially pure titanium or polyacetal.

Since the modeling process RCA implies wax models in many cases made of Rapid FlexTM wax profiles in accordance with the BIOS principles, the question is whether the BIOS can be used for RCA modelling out of various materials.

Based on the hypothesis that durability and functionality of retentive clasp arms in RPD made of various materials require clearly defined shapes, the aim of this research have was to determine and compare the safety factor of virtual RCAs on the models of premolars, modeled out of various materials by BIOS concept and to define optimal shape of RCAs on virtual models of premolars, made of various materials.

Methods

Designing a coronal part of a retentive tooth virtual model begins by scanning the enlarged plaster model of the upper first premolars. Scanning was conducted by the means of UHG 1500 device. Computera-aided design (CAD) program was used in defining the digital model of retention tooth. Scanned data were processed using the Auto Desc Inventor 7 software. Virtual premolars modeling in the aforementioned software was done in accordance with the average values of 0.25 mm for undercut depth and 30° for the angle of gingival convergence.

Defining virtual models of retentive clasp arms in the BIOS

In accordance with the BIOS system, virtual models of the I-Bar, Bonihard and circumferential clasps were defined within this experiment. Auto Desc Inventor 7 softwre was used in the modeling procedure. The hight and width ratio of the profiles was 10 to 8. Slope inclination of the profiles was 1.28, as measured on wax profiles (Rapidflex, Degusa) which are used in clasp modeling in the BIOS. The path of each clasps was defined by analyzing the undercut space of digital model on retentive tooth. Virtual models of clasps are planned in accordance with table values of BIOS profiles for CoCrMo alloy (Figure 1).

Fig. 1 – Virtual clasp models designed by the BIOS for CoCrMo alloy.

The dimensions of the cross section and inclination angle values of circumferential clasps are not found in the available literature, therefore those values were obtained by scanning a wax profile (Degussa-Rapid-Flex-System).

The finite elelment method in the process of stress and deflection analysis of refentive clasp arms

RCAs virtual models volume was divided into tetrahedral finite element shape. The finite element mesh consists of 2,994 nodes and 1,601 elements. The simulated force of 5 N was applied and directed towards the top of the clasp arm. The analysed clasp arm deflection, was 0.25 mm, due to the same undercut depth the clasp should overcome. Stress and displacement analysis of RCA models was done using the Autodesc Inventor 7 software. Stress values were expressed in MPa, while deflection in milimeters. Both values were represented by gradation – by differently coloured boxes. The analysis included factors that affect the intensity of the clasp retention force, such as the friction between the clasp and teeth where saliva is a lubricant. The average value of friction was 0.2 (Figure 2).

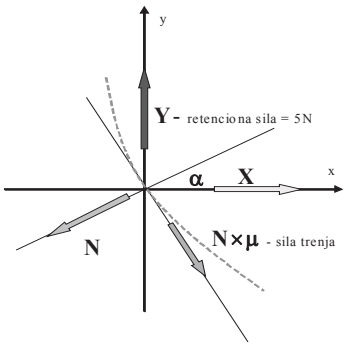

Fig. 2 – Illustration of the influence of forces on the retentive clasp arm.

The change of model parameters was related with the ability of changing elastic modulus and nominal yield strength, which enabled the analysis of virtual models that has the characteristics of different materials. Within the experiment, the analysis was conducted upon model properties of I-Bar, Bonihard and circumferential retentive clasps made of CoCrMo alloy, CP titanium and polyacetal (Table 1).

The constant values within this part of the experiment were: the undercut depth of 0.25mm, the angle of gingival convergence of 30° and friction between a retention tooth model and a RCA model 0.2. Variables were: the length, width and height of the clasp, elastic modulus of a material, nominal yield strength (0.2%) and clasp profile slope inclination.

Defining the optimal shape of a virtual RCA made of CoCrMo alloy

The clasp models were analyzed so that all the clasps were modelled by the BIOS. The length of the clasp path was measured on the digital tooth model. According to the BIOS table, rapid flex wax profile was reduced having the height and width ratio of 10 to 8.

As stated before, the wax profile was scanned by the means of optical scanner and its slope inclination was suggested to be 1.28°. All of these parameters were imported into the software obtaining RCA models that match real clasps planned by the BIOS. After the analysis the shape optimization of RCA was performed and the original models by the BIOS were called "initial BIOS models". Optimal models had the following characteristics: uniform stress distribution within the virtual clasp model, maxium utilization of material, deflection of 0.25 mm and the safety factor greater than 1. Optimization was carried out on the models with the aspect ratio of 10 to 8. Slope inclination angle of the profile was 1.28. "Optimal" RCA models were primely modeled by the parameters for Co,Cr,Mo alloy, having that "optimal" shape additionally examined for other tested materials. The premolar circumferential clasp model was used as the reference one.

On each successive model the dimensions of the profile cross section were changed at low stress intensity regions, towards the decrease in its dimensions. In cases of balanced stress distribution and stress intensity within the elastic limit, the model was accepted as "ideal" in relation to material utilization. The next stage in the optimization was deflection of 0.25 mm. An important term that optimal RCA had to meet was the safety factor value above 1.

The RCA in such way designed model was ideal in relation to stress distribution and material utilization, but missing the requirement of deflection at the clasp top of 0.25

Table 1

Elastic properties of the tested materials

Elastic properties	CoCrMo alloy	CP titan	Polyacetal
Ee	210 GPa	110 GPa	2.9 GPa
0.2% σ	610 MPa	450 MPa	87 MPa

Ee – elastic modulus; 0.2% σ – convential yield strength.

mm, under retention force of 5 N. To solve this problem, the requirement for maximal material utilization must be set aside as less important and to provide, by increasing the cross section dimensions, deflection of RCA tip model to be equal to the undercut value of 0.25 mm.

Comparative analysis of the safety factor of retentive clasp arms made of various materials by the BIOS

In order to illustrate the diversity of material properties used for fabricitaion of RPD retention elements, we conducted a comparative analysis of safety factors upon identical profiles of I-Bar, Bonihard and circumferential RCAs designed by the principles of the BIOS. The modulus of elasticity and conventional yield strength (0.2% σ) were variable parameters (Table 1).

Results

The results of the conducted comparative analysis of the safety factor in virtual RCAs models designed from different materials by the BIOS, showed that the same RCA modeling manner with various materials used was absolutely unacceptable (Table 2). Due to identical design of I-Bar and Bonihard clasp, except for the difference in the crescent-shaped end of the Bonihard clasp, which did not affect the level of security factor, only the results for the I-Bar clasp were presented (Figure 3).

Table 2

Safety factor comparative values

Clasp type	Simulated materials used for clasp model	
	CPTi	Polyacetal
I-Bar	1.34	0.24
Circumferential premolar	0.69	0.12

CPTi – commercially pure titanium.

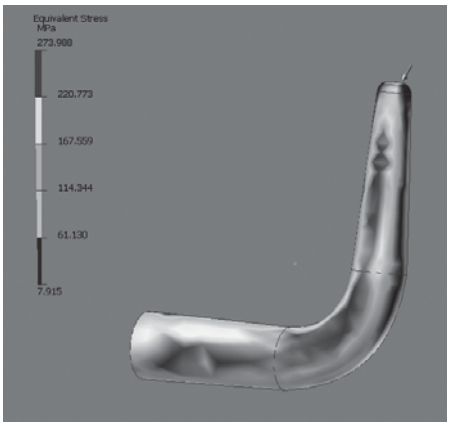

Fig. 3 – Stress distribution in the I-bar retentive clasp arms virtual model.

Optimization of the shape of RCAs made of CoCrMo alloy allowed the creation of clasp shape, by the change in cross section aspect ratio and the slope inclination of profiled, that provides displacement of 0.25 mm, a uniform stress distribution under the force of 5 N and safety factor greater than 1.

Optimization was achieved on the model with the profile aspect ratio of 5 to 10, horizontal gradient of 0.859°, curved area of 0.91° and 3° of vertical area. The required deflection of 0.25 mm defines geometry, thus I-Bar clasp safety factor reaches 1.00662, which indicates the possibility of providing an optimal clasp shape by changing the profile slope inclination and dimension.

The dimensions of optimal Bonihard clasp model made of CoCrMo alloy were compatible with I-Bar clasp. The retention force was deliberately moved to the crescent-shaped tip for the worst case of load to be simulated. Deflection of 0.3 mm was observed at the top of Bonihard clasp arm. Clasp arm-vertical area connection was deflected about 0.25 mm.

Optimization of the circumferential premolar clasp made of CoCrMo alloy was achieved by increasing dimension of the initial BIOS profile by 8% and changing the slope inclination by cubic parabolas. By such geometry modification a safety factor greater than 1 was obtained (Figure 4).

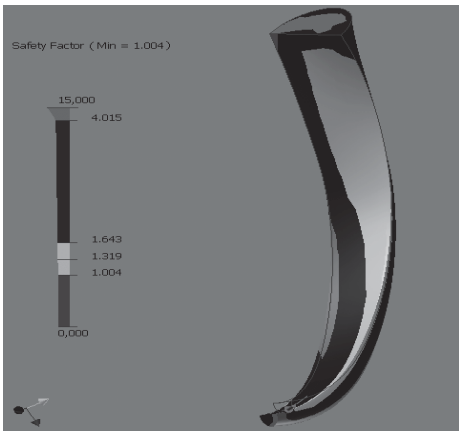

Fig. 4 – Illustration of the safety factor in the optimal premolar circumferential clasp made of CoCrMo alloy.

RCA optimal shapes made of CoCrMo alloy were modified because of the lower values for CPTi modulus of elasticity. By increasing the cross section dimensions of the premolar I-Bar clasp by 19%, the shape was obtained leading to the optimal I-Bar RCA model made of CPTi. There was a slope inclination of 0.98° on the horizontal region, of 0.91° on the curved, and of 3° on vertical. Stress distribution indicates its uniformity along the clasp. The highest values of measured stress were 169 MPa, and safety factor was 2.66.

Evenly distributed stress was observed in the illustration of safety factor distribution. In order to provide a sufficient clasp stiffness the optimal shape of premolar circumferential clasp model made of CPTi was increased by 19%, compared to the optimal clasp made of CoCrMo alloy. Safety factor was 1.67.

RCA optimal shapes made of polyacetals were significantly different from the other materials. Due to the high values of deflection, in addition to increase cross-section dimensions it was necessary to shorten the horizontal dimension of the clasp made of polyacetal. The cross section dimensions were 3–4 mm. The clasp had three times higher deflection than required, and low safety factor of 0.8.

Stresses on the optimal premolar circumferential clasps made of polyacetals were generally evenly distributed and the intensity was low. Deflection was 0.28 mm. Safety factors, although favorably distributed, exceed the values of 4 to 8. Optimization of premolar circumferential clasp models made of polyacetals resulted in the clasp models of 3.64 in width and 2.9 mm in height in the clasp-skeleton RPD connection area.

The results of the research show that there are significant differences in the height of optimal profiles used for premolar circumferential RCAs made od different materials (Figure 5). The difference in slope inclination of rapid-flex profiles was lower than the optimal shape of profiles for Co-CrMo alloy and CPTi. Clasp arm made of polyacetals must be 2.5 times bigger than previously mentioned one in order to achieve the retention force of 2.5 N. The research shows that the aspect ratio for clasp modeling should be 10 to 8, while the slope inclination of the profiles for polyacetal clasp arms should be 10°, and 3° for CoCrMo alloy and CPTi (Table 3).

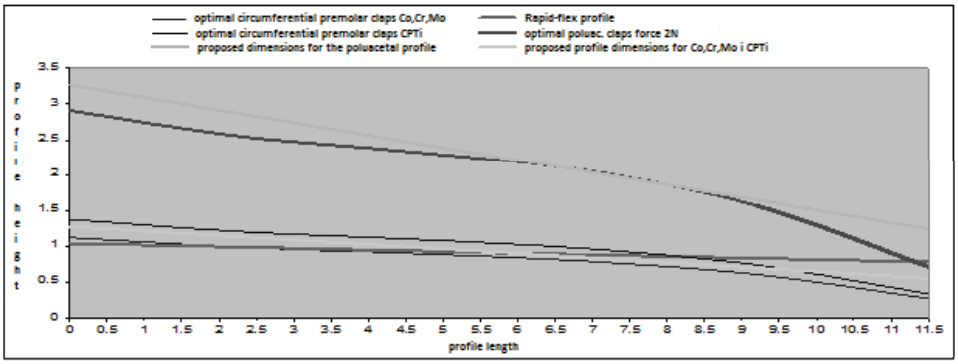

Fig. 5 – The change in height of profiles used for fabricating circumferential premolar clasps.

Table 3

The inclination, height and width (h/w) ratio for the initial, optimized and proposed wax profiles used for RCA fabrication

Wax profiles	I bar and Bonihard		Circumferential premolar	
	inclin. °	h / w ratio	inclin. °	h / w ratio
Rapid-flex wax profiles	1.28	10 / 8	1.28	10 / 8
Optimal profile for CoCrMo	0.8 0.9 3	10 / 5	modifiable	10 / 8
Optimal profile for CPTi	0.9 0.9 3	10 / 5	modifiable	10 / 8
Optimal profile for polyacetal		10 / 5	modifiable	10 / 8
Propos. for CoCrMo and CPTi	0.9 0.9 3	10 / 5	3	10 / 8
Propos. for plyacetal/-can not be done-			10	10 / 8

RCA – retentive clasp ars; CoCrMo – cobalt-chrome-molybdenum; CPTi – commercially pure titan.

Discussion

The finite element method is the most commonly used method for the analysis of retention elements of RPD [28, 29]. The largest contribution to the analysis of RCA using this method was given by Sato et al. [30, 31]. The basis of their research was I-Bar clasp analysis, circumferential retentive clasps and rests of RPD-s [32, 33]. They studied the optimal shape of RCAs with the aspect of stress uniformity and stress distribution. They also simulated the modeling of retentive claps made of CoCrMo alloys, which had the undercut depth of 0.25 mm and 0.50 mm. The results of the research are the proposed dimensions and gradient of RCA claps profile which were very similar to our study.

Mahmoud et al. [35] presented a study with finite element analysis of cast clasps made from Ti–6Al–7Nb, Co–Cr and type IV gold alloys. All objects were loaded in three different directions (outside, inside and outside inclined 30°), and the resulting permanent deformation values were recorded. Nonlinear finite element analysis simulations based on the maximum distortion energy criterion for yielding, were conducted for the clasp models that were reproduced according to the dimensions of each experimental specimen. In their results Ti–6Al–7Nb showed a significantly less permanent deformation followed by type IV gold, while Co–Cr alloy had the greatest permanent deformation. Thes results suggest that the method we used is suitable for predicting different material clasp behavior.

Virtual clasp arms modeled after real clasps made of rapid flex wax profiles by the BIOS concept do not fully meet the reqirements placed upon them. An I-Bar clasp, modeled by the BIOS concept in virtual form, realises excessive deflection, poor stress distribution and excessive safety factor. The horizontal part of the clasp remains inactive, under-utilized respectively, thus the solution to this problem should be sought in the change of slope inclination of the wax profile.

By observing and comparing the illustrations of stress distribution, deflection and safety factors in virtual I-Bar and Bonihard clasp models, it was found that a Bonihard clasp under load acted analogically to the respective I-Bar clasp. The exception being the tip of the Bonihard clasp arm itself, which actually has only a prophylactic effect on retention tooth in terms of stress distribution over a larger tooth area

and has no significant role in RPD retention. Stress distribution on a virtual premolar circumferential RCA BIOS model, is uniform, except for the tip area of the clasp arm which remains inactive. The safety factor is much higher than desired despite the excessive value for deflection, which means that the increase in slope inclination on profiles used for clasp fabrication can provide greater flexibility of RCA and more eaven stress distribution.

BIOS and rapid-flex profiles are provided solely for designing and modeling the retentive clasp arms made of CoCrMo alloy.

I-Bar and Bonihard clasps made of CPTi can be designed by the same concept as the corresponding clasps made of CoCrMo alloy in terms of safety, but it is obvious that clasps made of titanium alloy would be much more flexible. The safety factor with circumferential retentive clasp arms is below plastic deformation limit of the material.

Clasps made of polyacetals must not be modeled by the BIOS concept for CoCrMo alloy, and the proof of it is the safety factor.

By changing the geometry of virtual I-Bar and Bonihard clasp models made of CoCrMo alloy we concluded that those clasps can have an optimal shape in terms of uniform stress distribution and safety factor. The difference between the optimal and other models analyzed is presented in the change of slope inclination on the vertical region of the slope arm. The aspect ratio of the optimal virtual I-Bar and Bonihard clasp models does not match the aspect ratio of rapid flex profiles for the BIOS. Optimal premolar circumferential clasp model made of CoCrMo alloy has the identical aspect ratio to rapid flex profile, but with increased dimensions and changeable inclination slopes.

The optimal shape of all RCAs made of CPTi is geometrically similar to a corresponding shape of enlarged clasp arms made of CoCrMo alloy. I-Bar and Bonihard clasp arms are the simplest RCAs by shape and shape optimization. The optimum ratio of wax profiles used for this clasp fabrication is 5 to 10. Profiles of such dimensions are generally present in the market. The obtained results coincide with the researches by Sato et al. [32, 33]. It should be, however, noted that the results of their study refer to the CoCrMo alloy.

The results of this study suggest that wax profiles fabrication of clearly determined dimensions and slope inclination can be used for RCA modeling out of CoCrMo alloy, as well as of CPTi. It is also established that an optimal I-Bar and Bonihard clasp arm is not possible to be made of polyacetals. Premolar circumferential clasp arms made of polyacetals cannot meet the desired deflection and retention force. Even with unacceptably large-scale cross section dimensions of polycetal clasp arms, stresses exceed the limit of plasticity in clasp arm materials. The clasp arm that would meet the requirements would be over-dimensioned and of poor aesthetic performances. The only way for the tested clasp arms to be presented in practice is to distribute the retention force over more teeth or to plan two retentive clasp arms on the same tooth (vestibular and oral), if it is possible. Another solution is to use polyacetal clasp arms in cases of inserted saddles in visible sector, and to use retentive clasp arms made of metal alloys in the sidewide sector. By conducting an accurate analysis on the optimal shapes of polyacetal RCAs, it is possible to make a wax profile which can be further used for modeling of polyacetal RCAs.

Conclusion

The BIOS concept should be used only for RCA made of CoCrMo alloy. The results showed that the finite element method is a good analysis of virtual retentive clasp arms on the models of premolars, modeled out of various materials by the BIOS concept. This method confirms that the safety factor of virtual retentive clasp arms made by CPTi has a higher value than those made of polyacetale RCA. The finite elements method application offers the possibility of defining the optimal shape and design of virtual retentive clasps on a virtual model of premolars. Polyacetale RCAs have different optimal shape design comparing to RCAs made of CPTi. The case of defining polyacetals RCA shows that it is not possible to design an optimal form in terms of flexibility and security factor level.

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Hepatitis C viral infection among prisoners

Hepatitis C virusna infekcija među zatvorenicima

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Abstract

Background/Aim. Hepatitis C virus (HCV) infection is an important sociomedical problem worldwide because the chronification of the disease is frequent and the occurance of liver cirrhosis and hepatocellular carcinoma can be expected. The aim of this study was to determine the way of infection, pathohistological changes of the liver, virus genotype presence and sustained virological response after pegylated interferon and ribavirin therapy in prison inmates. **Methods.** The study included 52 patients with chronic HCV infection classified in two groups managed during 2008–2010. The first group consisted of prisoners (n = 22) and the second one of "non-prisoners" (n = 30). The patients from both groups underwent diagnostic preparation (biochemical analyses, liver biopsy, hepatitis virus detection and genotypisation using polymerase chain reaction issue). The treatment lasted for 24 weeks for virus genotypes 2 and 3, and 48 weeks for genotypes 1 and 4. **Results.** All the patients were males, approximately the same age (35 ± 4.1 and 31 ± 7.6 years). Virus genotype 1 was significantly more frequent in the prisoners ($p < 0.05$), that demanded longer treatment (48 weeks). At the same time, statistically significant higher number of patients, "non-prisoners", achieved a sustained virological response ($p < 0.01$). **Conclusion.** Intravenous drug abuse and tattoos, separately or together, are the most frequent way of infection in prisoners. The dominant presence of virus genotype 1 resulted in lower number of patients with sustained virological response, probably regardless prison environment and regime.

Key words:
hepatitis C; prisoners; genotype; infection; risk factors; treatment outcome.

Apstrakt

Uvod/Cilj. Hepatitis C virus (HCV) infekcija je važan sociomedicinski problem širom sveta zbog česte hronizacije bolesti kao i pojave ciroze jetre i hepatocelularnog karcinoma. Cilj rada bio je da se utvrdi put širenja infekcije, patohistološke promene jetre, genotipska zastupljenost virusa i stabilni virusološki odgovor na unos pegilovanog interferona i ribavirina kod zatvorenika. **Metode.** Ispitivanjem su bila obuhvaćena 52 bolesnika sa hroničnom HCV infekcijom, lečena u periodu 2008–2010, podeljena u dve grupe. Prvu grupu činili su zatvorenici (n = 22), a drugu „ne-zatvorenici" (n = 30). Svi bolesnici su prethodno bili podvrgnuti dijagnostičkoj pripremi (biohemijska obrada, biopsija jetre, ispitivanje prisustva virusa reakcijom lančane polimerizacije i genotipsko sagledavanje). Lečenje je sprovedeno tokom 24 nedelje za genotip virusa 2 i 3, i tokom 48 nedelja za genotip 1 i 4. **Rezultati.** Svi ispitanici su bili muškog pola, približno iste starosti (35 ± 4.1, odnosno 31 ± 7.6 godina). Dobijeni rezultati pokazuju statistički značajno češću prisutnost genotipa 1 kod zatvorenika ($p < 0.05$), što je zahtevalo duže lečenje (48 nedelja). Istovremeno je zapaženo da je statistički značajno veći broj „ne-zatvorenika", postigao stabilni virusološki odgovor ($p < 0.01$). **Zaključak.** Intravenska narkomanija i tetovaža, pojedinačno ili udruženo, najčešći su način infekcije kod zatvorenika. Dominantna prisutnost genotipa 1 imala je za rezultat niži broj bolesnika sa stabilnim virusološkim odgovorom, verovatno nezavisno od sredine boravka i režima života.

Ključne reči:
hepatitis C; zatvorenici; genotip; infekcija; faktori rizika; lečenje, ishod.

Introduction

Hepatitis C virus (HCV) infection is an important sociomedical problem worldwide. The importance of acute HCV is reflected in a high percentage of the disease chronification (65–80%), or the occurrence of liver cirrhosis (10–20%) and a possible development of hepatocellular carcinoma in 1–5% of people with liver cirrhosis [1, 2]. There are approximately 200 million people with HCV infection today, which is somewhere around 3% of the world population. HCV infection is widespread worldwide, with some specificity in genotypic localization observed. Particularly, North America is characterized by the presence of virus genotype 1a, which predominates, followed by genotypes 2a,

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2b and 3a. The dominant genotype in Europe is 1b, followed by 2a, 2b, 2c and 3a. In Africa dominate genotypes are 4 and 5 [3, 4]. The prevalence of HCV infection among general population is uneven, with higher prevalence in southern Europe [4]. High prevalence of HCV infection was observed in Egypt (20%) and it is explained by the treatment of schistosomiasis [5]. Ways of getting a HCV infection are various. However, studies show that the infection is mostly transmitted by intravenous heroin use, exposure to blood and blood derivatives, nasal drug use, from sexually active persons (promiscuous persons), tattoos, piercing, etc. [1, 6]. Intravenous drug users are at higher risk for blood transmissible diseases (human immunodeficiency virus (HIV), HCV, hepatitis B virus and other). This problem is being significantly potentiated in persons who are housed in prisons. Specifically, among the general population in the United States the prevalence of HCV infection ranges from 1% to 2% and the proportion is significantly higher, up to 80%, in imprisoned adults who are intravenous drug addicts. Studies show that among prisoners 17.6% are intravenous drug users, 56.1% use drugs intranasally, 56% have various tattoos and 39.6% have a history of variety injection equipment use [7]. All of these activities contribute to the significant number of blood transmitted diseases among this population. HCV infection incidence is different in prisons around the world, ranging from 25% to 40% [8–11]. In a certain number of inmates HBV and HIV coinfection can be seen [12].

A relatively small percentage of persons with chronic HCV, in the Correctional Prison (KPZ) in Niš, are submitted to disease examination or treatment by modern standards. A modern therapeutic approach to these patients is identical to general population. The persons with virus genotypes 1 and 4 are treated during 48 weeks with pegylated interferon alpha-2a at a dose of 180 mg once weekly and daily intake of ribavirin at a dose of 1,000–1,200 mg [13]. The patients with HCV genotypes 2 and 3 have shorter treatment, 16 or 24 weeks with the same dose of pegylated interferon alfa-2a and ribavirin in a dose of 800 mg daily [14, 15]. This treatment provides a different percentage of sustained virological response (SVR), in genotypes 2 and 3 the percentage is about 80%, while in the patients with genotypes 1 and 4, SVR achievement is around 50% [16].

The aim of this study was to examine treatment possibilities in this marginalized group of people, to find the most common way of infection in the group, genotypic representation and also to assess therapeutic effects of pegylated interferon alpha-2a combined with ribavirin.

Methods

The study involved 52 patients with chronic HCV infection, during 2008–2010. All the subjects were males, born between 1966 and 1985, and divided into two groups. The first group consisted of imprisoned patients, thus that received their treatment in prisons (n = 22), the second (control) group comprised ”non-prisoners” (n = 30), randomly included patients from the Clinic for Infectious Diseases in Niš. The prisoners were mostly from the KPZ in Niš (n = 15), some were from the KPZ in Leskovac (n = 6), and one (n = 1) was serving his turn in Vranje. A number of imprisoned patients started their treatment in prisons (n = 17), while others had already received their treatment before coming to prison (n = 5). In forming the second group, the approach was discriminant, female patients were eliminated since all the prisoners were male. Also, intentionally both patients groups were approximately the same age.

Using epidemiological analysis we found that some subjects, from both groups, had a history of intravenous drug abuse, so they had to gain psychiatric conformation of being abstinent for more than 6 months. Examining the imprisoned patients we saw extensive tattoos that were made during stay in KPZ. The prisoners had in their files different crimes committed, ranging from theft, robbery and murders. During examination all the prisoners were escorted by the police officers.

All the patients were tested for HIV infection (n = 0) and hepatitis B infection (n = 0). Shortly before starting the antiviral therapy, prisoners underwent hospital preparation: biochemical analyses, upper abdomen ultrasound, liver biopsy, and detection of the virus and its genotype, by the polymerase chain reaction (PCR) assay (Amplicor Monitor Assay; Roche Molecular Systems, Branchburg, NJ 08876, USA). Pathohistological examination of liver biopsy specimens was done at the Pathology Institute, Clinical Center in Niš. PCR and genotypisation were done at the Institute for Infectious and Tropical Diseases in Belgrade. The patients with virus genotypes 1 and 4 received pegylated interferon at a dose of 180 mg subcutaneously, once a week for 48 weeks, plus ribavirin at a daily dose of 1,000–1,200 mg *per os*; while patients with virus genotypes 2 and 3 received the same dose of pegylated interferon but ribavirin in a dose of 800 mg daily for 24 weeks. The prisoners’ therapy was carried out almost without interruption, except in one period of 7 days, when there was a riot in the KPZ Niš. During the therapy the patients were tested for products of heroin degradation, which were found in only one patient (KPZ Leskovac), and so his treatment had to be discontinued after seven months. We should also point out a correct laboratory monitoring of the patients during the therapy, by both prisons’ physicians. Namely, regular monitoring of blood work values was performed, that provided a possibility for medications doses correction. Both groups were followed up on the basis of selected parameters in order to detect some possible features that would be specific for the group.

Data were analyzed by the standard descriptive methods: arithmetic mean and standard deviation ($\bar{x} \pm SD$). To determine a statistical significance the Student’s *t*-test was used; the level of probability (p) < 0.05 was considered statistically significant. Analysis was done using Microsoft Office Excel 2003 software package in Windows XP Professional environment.

Results

The average age of prisoners was 35 ± 4.1 ($\bar{x} \pm SD$) years, while the average age of the patients in the group II was 31 ± 7.6 years. Tables 1–4 show their characteristics.

Table 1

The way of hepatitis C virus infection in the patients

Way of infection	Group of patients		Total [n (%)]
	Prisoners [n (%)]	Non-prisoners [n (%)]	
Intravenous	9 (40.9)	7 (23.3)	16 (30.7)
Unknown	2 (9.1)	10 (33.4)	12 (23.1)
Tatoo	3 (13.6)	4 (13.3)	7 (13.5)
Tatoo + intravenous	6 (27.3)	5 (16.7)	11 (21.2)
Else	2 (9.1)	4 (11.5)	6 (11.5)
Total	22 (100)	30 (100)	52 (100)

Table 2

The genotypes of hepatitis C virus among the patients

Genotypes of the virus	Groups of patients		Total [n (%)]
	Prisoners [n (%)]	Non-prisoners [n (%)]	
1	14 (63.7)	9 (30)	23 (44.2)
1 + 4	3 (13.6)	1 (3.3)	4 (7.8)
2	0 (0)	1 (3.3)	1 (1.9)
3	4 (18.2)	19 (63.4)	23 (44.2)
1 + 2	1 (4.5)	0 (0)	1 (1.9)
Total	22 (100)	30 (100)	52 (100)

Table 3

The liver fibrosis level in the patients

Fibrosis score*	Groups of patients		Total [n (%)]
	Prisoners [n (%)]	Non-prisoners [n (%)]	
F_0	2 (9.1)	3 (10)	5 (9.6)
F_1	11 (50)	10 (33.3)	21 (40.4)
F_2	8 (36.4)	9 (30)	17 (32.7)
F_3	1 (4.5)	7 (23.3)	8 (15.4)
F_4	0 (0)	1 (3.3)	1 (1.9)
Total	22 (100)	30 (100)	52 (100)

***Histopathological findings from liver biopsy; F_0 –no fibrosis (normal liver); F_1 – mild fibrosis; F_2 – moderate fibrosis; F_4 – cirrhosis.**

Table 4

Virological response in the patients after the therapy

Virological response	Groups of patients		Total [n (%)]
	Prisoners [n (%)]	Non-prisoners [n (%)]	
Sustained	13 (59.1)	25 (83.3)	38 (73.1)
Unsustainable	8 (36.4)	5 (16.7)	13 (25.0)
Undefinied*	1 (4.5)	0 (0)	1 (1.9)
Total	22 (100)	30 (100)	52 (100)

***The therapy cessation in one patient.**

Comparison of the viral ribonucleic acid (RNA) copies number in blood showed that the number of patients with less than one million copies of HCV-RNA was significantly higher among prisoners ($p < 0.05$). There were no other significant differences in the number of HCV-RNA copies (Figure 1). Also, we did not find any significant difference in the way of infection between the groups of patients, as well as any significant difference in histopathologic findings of the liver. A significantly higher number of patients in the prisoners' group had HCV genotype 1 ($p < 0.05$), while the greater number of patients in the control group was infected with virus genotype 3 ($p < 0.01$).

We also found a significant difference between the two groups of patients in the treatment duration (48 or 24 weeks). That is, a significantly higher number of prisoners were treated 48 weeks, while in the control group a significantly higher number of patients were treated for 24 weeks ($p < 0.01$).

Also, a significantly higher number of patients in the control group achieved SVR ($p < 0.01$).

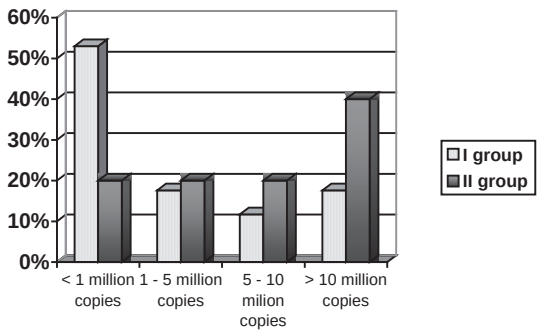

Fig. 1 – The patients groups percentage ratio according to hepatitis C virus ribonucleic acid (HCV-RNA) viral copies in blood.

Discussion

Comparison of the subjects in the groups I and II, based on the obtained statistical data, allowed us to determine some characteristics in the treatment of this marginalized population. The patients of the group I noted in their histories intravenous drug use, alone or associated with tattoos, as the transmission route of HCV infection, but this was encountered even in the patients of the group II in few cases. This observation is consistent with findings obtained by other authors [1, 6]. In the control group a significant number of patients was not able to recall any event that could be the source of infection, and this was labeled as an unknown way of transmission, which is also seen frequently. We also noted significant differences in the distribution of HCV genotypes between the groups. HCV genotype 1 was the most common genotype in the group I subjects, whereas genotype 3 was the most frequent in the control group. Such distribution of genetic background influences the implementation (duration) of the treatment, and there was a significant difference in the treatment duration among the examined groups ($p < 0.01$). At the same time differences in the level of SVR was noted. Namely, it is well- known that patients with chronic HCV genotype 1 achieve SVR in a significantly lower percentage (50%) than patients with other genotypes [16], whereas patients infected with HCV genotype 2 or 3 have a significantly higher percentage of SVR (80%) [16]. This distribution of genotypes explains the difference in treatment length between the groups. Among the subjects of the group I there was not a single registered case of HBV nor HIV coinfection, which can be found in the allegations made by other authors [12]. This may be explained to some extent by a small number of participants in the study. Although, the available knowledge suggests that HBV and HIV co-infection can be encountered among the prisoners sent to the Clinic for Infectious Diseases in Niš, these patients were not present in our study. The treatment was discontinued in one patient because of the presence of heroin traces, after seven months of treatment, during a routine screening of all inmates undergoing antiviral treatment. This method of monitoring for the drug traces in urine allows us, among other things, the elimination of heroin-returnees from the treatment. However, a question remains how this inmate got heroin while being in prison?

Conclusion

Intravenous drug abuse, alone or associated with tattoos, is the most common way of infection in the prisoners. HCV genotype 1 is predominant in the prisoners while HCV genotype 3 is the most frequent in the non-prisoners. There were no significant differences among the patients groups in terms of viral load (HCV-RNA copies number), but more patients with viral load below million copies were in the prisoners group ($p < 0.05$). Significantly greater number of the control group patients achieved SVR due to the viral genotype differences.

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The role of immunohistochemical evaluation in the diagnosis of malignant mesothelioma of the pleura

Uloga imunohistohemijske analize u dijagnozi malignog mezotelioma pleure

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Abstract

Backround/Aim. The final diagnosis of malignant pleural mesothelioma is made exclusively by histopathological examination of biopsy materials that are routinely complemented by the use of immunohistochemical analysis. The aim of this paper was to determine the significance of immunohistochemical analysis and application of certain antibodies in the diagnosis of malignant pleural mesothelioma. **Methods.** This retrospective analysis included clinical data of 32 patients with the histopathological diagnosis of malignant pleural mesothelioma made in the period 2004–2009 at the Institute for Pulmonary Diseases in Sremska Kamenica. The material was processed and analyzed at the Center for Pathology. **Results.** CK5/6 was positive, in 63% of the cases calretinin, in 94% and HBME-1 in 80% of the cases. CK7 was positive in 78%, and EMA in 83% of the cases. All the cases (100%) were negative for TTF-1, CEA, CD20, desmin and MOC31. **Conclusion.** Immunohistochemistry has become an essential diagnostic procedure for the diagnosis and determination of the type of malignant pleural mesothelioma, and due to the lack of individual antibodies a combination of antibody with different sensitivity and specificity is in use today.

Key words:
plural neoplasms; mesothelioma; immunohistochemistry.

Apstrakt

Uvod/Cilj. Konačna dijagnoza malignog mezotelioma pleure postavlja se isključivo patohistološkim pregledom bioptiranog materijala koji se rutinski nadopunjuje i upotrebom imunohistohemijske analize. Cilje rada bio je utvrđivanje značaja imunohistohemijske analize i primene pojedinih antitela u dijagnozi malignog mezotelioma pleure. **Metode.** Retrospektivno je izvršena analiza kliničkih podataka 32 bolesnika kod kojih je patohistološki postavljena dijagnoza malignog mezotelioma pleure u periodu od 2004. do 2009. godine u Institutu za plućne bolesti Vojvodine u Sremskoj Kamenici, a materijal je obrađen i analiziran u Centru za patologiju. **Rezultati.** CK5/6 bio je pozitivan, kod 63% bolesnika kalretinin kod 94%, a HBME-1 kod 80% bolesnika, dok je CK7 bio pozitivan kod 78%, a EMA kod 83% bolesnika. Svi bolesnici (100%) bili su negativni na TTF-1, CEA, CD20, MOC31 i desmin. **Zaključak.** Imunohistohemija postala je značajan dijagnostički postupak u postavljanju dijagnoze i određivanju tipa malignog mezotelioma pleure, a zbog nedostatka samostalnog antitela danas se koristi kombinovanje antitela različite senzitivnosti i specifičnosti.

Ključne reči:
pleura, neoplazme; mezoteliom; imunohistohemija.

Introduction

Immunohistochemistry (IHC) is a highly sensitive laboratory method, used for identification and typization of tissues and origin of cells. It is a procedure of detecting cell surface antigen (Ag). It is based on the principles of an immune response, antigen-antibody reaction (Ag-Ab), in which antibody (Ab) binds only to specific antigen, giving a high specificity to this method. The antibodies, which are used, may be monoclonal or polyclonal. It is highly recommended to use monoclonal antibodies because they are more specific, and visualization of Ag-Ab reaction is performed with fluorescent, enzymatic, chemical or radioactive marked antibodies [1].

The development of immunohistochemistry overcomes many diagnostic problems today and allows adequate differentiation of malignant pleural mesothelioma (MPM). Immunohistochemistry is the most important, highly sensitive and specific assay that allows accurate determination of the type, and even subtype of the tumor. Its major role in establishing the final diagnosis of MPM is to provide a distinction in:

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epithelioid MPM from adenocarcinoma, sarcomatoid MPM from primary or secondary pleural sarcomas and from secondary sarcomatous carcinoma, epithelioid MPM from mesothelial cells hyperplasia and desmoplastic MPM from fibro-hyalinus pleuritis [2].

Currently, there is no tissue-specific antibody with 100% specificity and high sensitivity, which can give certain confirmation or exclusion of MPM. The lack of this tissue-specific antibody induced the production and usage of a large number of mesothelial and epithelial antibodies with different sensitivity and specificity. Therefore, it is highly recommended to use a palette of antibodies that combines two or more positive mesothelial with two or more positive epithelial antibodies [3–5].

Regardless the shortage of tissue-specific antibody, immunohistochemistry is the most significant shift in achieving the differential diagnosis of MPM, and histopathology pattern in general. There is no other method, used in the past 50 years, which had such a strong influence on pathohistology. It is the last diagnostic step before the final diagnosis is made [6, 7].

All modern histopathology laboratories require necessary immunohistochemical capacity for adequate functioning and providing correct diagnoses.

The aim of this study was to determine the significance of immunohistochemical analysis and application of certain antibodies in the diagnosis of MPM.

Methods

The study involved 32 patients, who had been admitted and treated in the Institute for Pulmonary Diseases of Vojvodina in the period from 1st January, 2004 to 31st December, 2009. All the patients had histopathologic confirmation of MPM. Surgery-obtained tissue was also processed and analyzed by immunohistochemical methods at the Pathology Department. Clinical and demographic data included in the study were: age, gender, smoking history, family cases of mesothelioma and symptoms of the disease. Surgical methods, for getting tissue samples were: video-assisted thoracoscopic surgery (VATS; 30 samples) and open lung biopsy (1 sample). In one patient with multiple pulmonary metastases of MPM, tissue sample for patohistological analysis was obtained by transbronchial biopsy. All the samples were fixed in 10% neutral formalin, paraffin-embedded and sectioned in 4 microns thick slices for hematoxylin and eosin (HE) staining.

For IHC analysis the samples were glued to "Superfrost" (Men Glaser), positively charged glass plates already prepared for IHC reactions. After deparaffinization of slices, antigenic determination was demarked by the reaction with citrate buffer (pH 6, high temperature, two times for 10 minutes) and by further cooling in distilled water for 20 minutes. Subsequently, blocking of endogenous peroxidase with 3% hydrogen peroxide (H_2O_2) during 5 minutes was performed.

Beyond, preparations were treated with primary antibodies and then incubated for 30 minutes with biotinylated mouse antibody and then incubated for 30 minutes with streptavidin peroxidase complex system. As a chromogenic substrate diaminobenzidine-tetrahydrochloride (DAB) was used and contrastive analysis was performed with hematoxylin.

The microscopic examination of immunohistochemically processed samples was performed and based on their positivity or negativity to specific antibody, the final diagnosis of malignant pleural mesothelioma of certain type was concluded.

Results

The patients included in our study were 46 to 84 years old, the average age was 59.7 years. The females represented 41% of all the patients (13 patients) while 59% (19 patients) of them were the males. Smoking was reported in 50% of the cases (16 patients). All the patients denied cases of malignant mesothelioma in their family.

The most common clinical manifestations of malignant pleural mesothelioma in our patients were dyspnea, chest pain, cough and symptoms of general infection. Dyspnea was present in 66% of the cases (21 patients), chest pain in 56% (18 patients), cough was noted in 37.5% (12 patients), and symptoms of general infection syndrome were present in 8 patients (25%).

Epithelioid type had 69% of the patients (22 patients), 19% (6 patients) had sarcomatoid, and the remaining 12% (4 patients) had biphasic type of malignant pleural mesothelioma. All the patients had unilateral MPM, in 78% of the cases (25 patients) the tumor was positioned in the right hemithorax, and in 22% (7 patients) in the left.

The antibodies used in the research and the numbers of positive and negative results for each antibody are shown in Table 1. Antibody to cytokeratin 5/6 (CK5/6) was positive in 17 of 27 samples tested (63%), calretinin in 28 of 30 (94%), anti-human mesothelial cell, clone HBME-1 (HBME-1) in 20 of 25 (80%) cases. Antibody to vimentin was positive in all eight treated samples (100%), antibody to epithelial membrane antigen (EMA) in 5 of 6 samples (83%), and antibody to cytokeratin 7 (CK7) in 7 of the 9 treated samples (78%). Antibody to pancytokeratin (PanCK) was performed on one sample only, and it was positive (100%). Antibody to cytokeratin 20 (CK20) was negative in all the 4 (100%) cases. Antibody to thiroid transcription factor 1 (TTF-1) was applied in 9 cases, carcinoembryonic antigen (CEA) in 7, and desmin and epithelial specific antigen Ab-7, clone MOC-31 (MOC-31) in only one sample. These four antibodies showed negativity in each case.

In epithelioid type of MPM (Figure 1) antibody to CK5/6 proved positivity in 13 of 19 (68%) cases, while the antibody to calretinin (Figure 2) was positive in 18 of 20 cases (90%). Antibody to HBME-1 was used in 18 cases and expressed positivity in 15 (83%) cases. Positive findings were found in all the cases (100%) while applying antibodies to: vimentin (3 of 3), EMA (2 of 2) and CK7 (6 of 6). Antibody to TTF-1 was performed in 5 cases (Figure 3), CEA in 6 cases, CK20 in 4, and MOC-31 in one sample. These four antibodies showed negativity (100%) in all the cases.

Tabele 1

The antibodies used in research and the number of positive and negative results for each antibody (Ab)

Ab to	Treated samples (number from a total number of the patients involved in research)	Finding of Ab at all types of MPM (+ / -)	Finding of Ab at epithelioid type of MPM (+ / -)	Finding of Ab at sarcomatoid type of MPM (+ / -)	Finding of Ab at biphasic type of MPM (+ / -)
CK 5/6	27/32	17/10	13/6	1/4	3/0
Calretinin	30/32	28/2	18/2	6/0	4/0
HBME-1	25/32	20/5	15/3	3/2	2/0
Vimentin	8/32	8/0	3/0	3/0	2/0
EMA	6/32	5/1	2/0	1/1	2/0
CK 7	9/32	7/2	6/0	0/2	1/0
PanCK	1/32	1/0	–	1/0	–
CK 20	4/32	0/4	0/4	–	–
TTF-1	9/32	0/9	0/5	0/1	0/3
CEA	7/32	0/7	0/6	–	0/1
Desmin	1/32	0/1	–	0/1	–
MOC 31	1/32	0/1	0/1	–	–

MPM – malignant pleural mesothelioma; CK 5/6 – cytokeratin 5/6; HBME-1 – anti-human mesothelial cell clone HBMEL$_1$; EMA – epithelial membrane antigen; CK-7 – cytokeratin 7; PanCK – pancytokeratin; CK 20 – cytokeratin 20; TTF-1 – thiraid tronsoription factor; CEA – carcinoembriomic antigen; MOC 31 – epithelial-specific antigen Ab-7, Clone MOC-31.

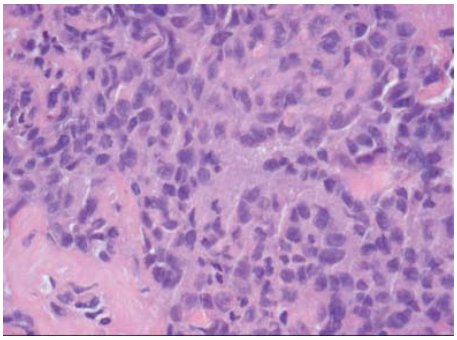

Fig. 1 – Malignant pleural mesothelioma – epithelioid type (H&E, ×400).

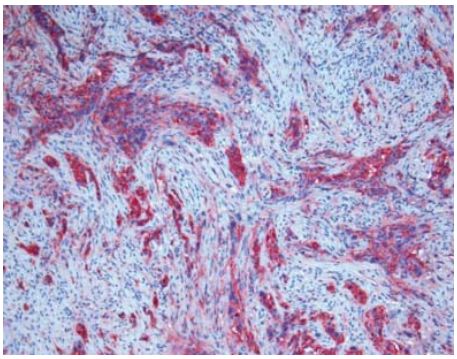

Fig. 2 – Positive staining of tumor cells for calretinin (Calretinin, ×100).

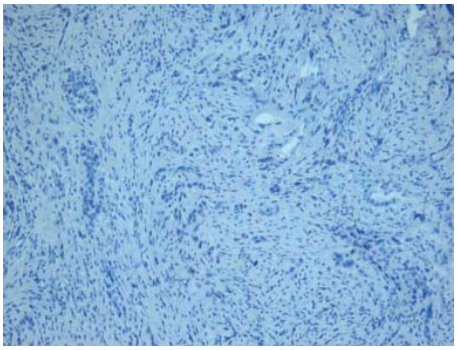

Fig. 3 – Negative staining of tumor cells for TTF-1 (TTF-1, ×100).

In sarcomatoid type of MPM, positivity to calretinin (6 samples), vimentin (3 samples) and PanCK (1 sample) was found in 100% of the cases. Antibody to CK5/6 was positive in 1 of 5 cases (20%) and HBME-1 in 60% (3 of 5 cases). Antibody to EMA showed positivity in 1 of 2 cases (50%). Antibodies to TTF-1 and desmin were performed on one sample with a complete negativity (100%), while the antibody to CK7 showed negativity in both cases done.

In biphasic type of MPM, antibody to CK5/6 was applied in 3 cases and calretinin in 4 cases with 100% positivity in all the samples. Positive findings (100%) were found while applying antibodies to HBME-1, vimentin and EMA (in 2 of 2 cases made). Antibody to CK7 was performed on one sample with absolute positivity. The negative findings (100%) were observed when antibodies TTF-1 (3 of 3 cases) and CEA (1 of 1 case) were used.

The percentages of the results for the applied antibodies in MPM expected to be positive were as follows: antibody to CK5/6 was positive in 68% of the cases with epithelioid, 20% cases of sarcomatoid and 100% of cases in biphasic type. Antibody to Calretinin was positive in 90% of the cases of epithelioid type and in 100% of the cases in sarcomatoid and biphasic type. Antibody to HBME-1 showed positivity in 83% of the cases of epithelioid type, 60% in sarcomatoid and 100% in biphasic type.

The percentage of the results for the applied antibodies in MPM expected to be negative was: antibody to TTF-1 was negative (100%) in all types. Antibody to CEA was performed in epithelioid and biphasic type and in both cases it was negative.

Discussion

The final diagnosis of MPM is performed exclusively by patohistological examination of biopsy-tissue samples, but frequently immunohistochemical analysis is necessary as additional method. Immunohistochemistry is the most reliable method that allows adequate differentiation and overcoming the diagnostic problems in the diagnostic algorithm

of malignant pleural mesothelioma [6, 7]. Due to the lack of tissue-specific positive or negative antibody it is now strongly recommended to use palettes of antibodies that combines two or more mesothelial positive antibodies with two or more epithelial positive antibodies [3–5].

During the last decade, production and usage of a large number of antibodies, with different sensitivity and specificity, placed MPM to serious research activities hoping to discover a winning combination of antibodies.

Keratin antibodies are among the most commonly used antibodies when there is a suspicion on MPM, especially for distinguishing MPM from adenocarcinoma and sarcoma [8]. CK5/6 is the most adequate antibody used for this purpose [9]. This antibody was performed in 27 of our patients and showed positivity in 63% of the cases. The highest positivity was shown in biphasic type (100%), followed by epithelioid (68%) and lowest in sarcomatoid (20%) type of MPM. A research performed by Soomro et al. [6] showed high positivity of this antibody in biphasic (60%) and epithelioid type (100%), and somewhat less in sarcomatoid type (28.6%), as well. Although not showing 100% positivity for all types, antibody to CK5/6 is considered one of the most specific antibodies and most sensitive in the diagnosis of MPM. It is proved to be useful in differentiation of sarcomatoid type of MPM from most sarcoma [10], as well as in differentiation of MPM from lung adenocarcinoma [9]. It is believed that if CK5/6 and anti-mesothelial antibodies are positive, diagnosis of MPM should be made [11]. Antibody to CK7 was positive in 78%, while antibody to panCK was applied only in one tissue sample, and the result was positive. A positive finding to these antibodies is extremely useful concerning the diagnosis of epithelioid type of MPM because it confirms the process of epithelialization in the sample treated [12]. Antibody to CK20 was performed in 4 cases of the epithelioid type of MPM and it was negative in all of them.

Calretinin belongs to a large family of calcium-binding cytoplasmic proteins. Antibody to calretinin was positive in more than 95% of the patients with epithelioid and biphasic type of MPM. Other authors state that the sensitivity of this antibody depends on the clones used and ranges from 73% to 100% [2]. All the authors agree that it provides an excellent positivity in epithelioid and biphasic type, but in terms of sarcomatoid type, there are conflicting data. Kayser [13] confirms good positivity (more than 50%) to calretinin in sarcomatoid type of tumor [13], but there are also data suggesting that this antibody is negative in sarcomatoid type [10]. In our research, we concluded that the antibody to calretinin was positive in 90% of epithelioid and in all the cases (100%) of sarcomatoid and biphasic type, thus agreeing with the majority of authors that it is one of the most useful antibodies in the diagnosis of MPM.

In our study, the HBME-1 antibody was positive in 83% of epithelioid, 60% of sarcomatoid and 100% of biphasic type of MPM. Although this antibody shows a high degree of sensitivity for MPM [14], today it is not considered as a specific antibody for MPM. Recent studies have shown a good reactivity of HBME-1 antibody in tissue with adenocarcinoma of the lung, kidney, thyroid and other tumors of the female genital tract [15]. However, because of its sensitivity, it is still used in the diagnosis of MPM, as one of the most potent antibody.

In our patients, antibody to vimentin showed 100% positivity in all three types of MPM, but concerning the fact that only 8 samples were treated with this antibody, we cannot rely on its sensitivity. Kayser [13] ranged the positivity of vimentin antibody from 8% to 100%, depending on the type of MPM, while other authors listed positivity to vimentin in all samples treated in all three types of MPM [3–5]. Together with antibodies to CK5/6 and/or HMBE-1, antibody to vimentin can be used as a very good indicator for MPM when it is necessary to distinguish it from metastatic carcinoma of the pleura [2].

Epithelioid and biphasic type of MPM in our patients showed 100% positivity for antibody to EMA, while the positivity of sarcomatoid type was 50%. Many papers argue that positivity of this antibody is expressed in MPM and metastatic adenocarcinoma, while the other recorded its negativity in mesothelial hiperplasia [2]. From our point of view, both questions are open for further research and discussion. In everyday laboratory work, antibody to EMA showed excellent results in the diagnosis of MPM, especially in the differentiation of epithelioid type MPM from metastatic adenocarcinoma. It was often used in many laboratories combined with antibody to CEA for making the differential diagnosis of MPM [11, 12].

Antibody to CEA is one of the most commonly used and probably the best antibody in distinguishing MPM from adenocarcinoma [11, 12]. Nevertheless, in our study negative results were obtained in all the cases. There are a few documented cases with MPM positive to CEA, but it is assumed that this positivity is due to the nature and chemical composition of antibody [2]. Our results supported the usage of monoclonal antibody to CEA and agreed with the findings of the majority of authors. This contributes the fact of CEA negativity in diagnosis of MPM.

Antibody to TTF-1 was positive in 75%–85% of lung adenocarcinoma and adenosquamous carcinoma, while in cases with MPM in all histological types negativity is expressed in 100% [2]. In our study, this antibody proved negativity in all the cases and all types of MPM confirming its major role in the differential diagnosis of MPM.

Antibodies to MOC-31 and desmin were performed in only one case showing the expected negativity. Concerning the fact that analysis was done in only one patient, major conclusions about the role of these antibodies cannot be made, but their negativity contribute to differentiation of MPM from other tumors [10, 14].

Conclusion

Immunohistochemistry has become an essential diagnostic procedure in the diagnosis and determining the type of MPM. The deficiency of tissue-specific antibody for MPM ensures the usage of combinations of antibodies with different sensitivity and specificity.

Antibody to CK5/6 (with the expressed positivity) has excellent sensitivity and specificity in differentiating epithelioid type MPM from adenocarcinoma of the lung and sarcomatoid type of MPM from most sarcomas.

Calretinin antibody is one of the most useful and most commonly used antibodies, which provides excellent positivity in epithelioid and biphasic type of MPM, but less in sarcomatoid type.

Antibody to HBME-1 has a low specificity in the diagnosis of MPM, but on the other hand it shows excellent sensitivity and is now widely used as a positive antibody in the diagnostic algorithm of MPM.

Antibodies to TTF-1 and CEA are the most important negative antibodies which are of great confidence in the differential diagnosis of epithelioid type MPM from lung adenocarcinoma.

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Free-end saddle length influence on stress level in unilateral complex partial denture abutment teeth and retention elements

Uticaj promene dužine slobodnog sedla na promenu napona retencionih zuba i spoja jednostrane kompleksne parcijalne proteze

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Abstract

Background/Aim. Different types of dental restorations are used for the therapy of unilateral free-end saddle edentulism. Unilateral complex partial denture is one of the indications for the Kennedy class II partial edentulism. The abscence of major connector and denture plate is an advantage compared to the conventional restorations, because of better comfort and shorter period of adaptation. The aim of the study was to analyse the influence of free-end saddle length change on the behaviour of unilateral complex partial denture supporting structures. **Methods.** Stress levels of the canine and the first premolar as retentional teeth and the attachments were tested under the influence of physiological forces with the loading point shifting distally in relation to the saddle length change. A virtual real size 3D model of the fixed part of the restoration (the canine and the first premolar with milled crowns) was created using the CATIA computer program. It was connected to the mobile part of partial denture with the SD snap in latch attachment. Mobile part of the restoration was designed in the region of 2, 3 and 4 lateral teeth (second premolar, first, second and third molar). By using the finite element method (FEM) stress levels analysis was performed under the load of physiological forces of 150 N in the free-end saddle teeth zone. **Results.** The results of analysis show that physiological forces cause a different stress distribution on the abutment teeth and the attachment, depending on the saddle length. **Conclusion.** The stress level values obtained for the abutment teeth as well as the attachment are far lower than the marginal ones. The behaviour of the system changes under this defined stress, but no plastic deformation occurs.

Key words:
denture, partial; dental abutments; computer simulation.

Apstrakt

Uvod/Cilj. U terapiji jednostrano slobodnog sedla koriste se različiti oblici zubnih nadoknada. Jednostrana kompleksna parcijalna proteza (JKPP) jedna je od indikacija za bezubost klase Kenedi II. Odsustvo velike spojnice i proteze ploče predstavlja prednost u odnosu na konvencionalne nadoknade zbog boljeg komfora i kraćeg perioda adaptacije. Cilj rada bio je analiza uticaja promene dužine slobodnog sedla na ponašanje potpornih struktura JKPP. **Metode.** Ispitivani su naponi očnjaka i prvog premolara kao retencionih zuba i veze (spoja) pod dejstvom fizioloških sila sa pomeranjem tačke opterećenja distalno, u zavisnosti od dužine sedla. Primenom kompjuterskog programa CATIA u realnoj veličini urađen je virtulni 3D model fiksnog dela nadoknade (očnjak i prvi premolar sa namenskim krunama) koji je veza SD *snap in latch* priključena na mobilni deo parcijalne proteze. Mobilni deo nadoknade postavljen je u predelu dva, tri, odnosno četiri bočna zuba (drugi premolar, prvi, drugi i treći molar). Primenom metode konačnih elemenata obavljena je analiza naponskih stanja pri opterećenju fiziološkim silama od 150 N u predelu zuba slobodnog sedla. **Rezultati.** Analiza proračuna pokazala je da pod dejstvom fizioloških sila dolazi do različite raspodele napona na retencione zube i spoj u zavisnosti od dužine sedla. **Zaključak.** Dobijene vrednosti za napone kako na retencionim zubima, tako i na spoju, daleko su manje od graničnih. Pri zadatim naponima dolazi do promene ponašanja, ali ne i do plastične deformacije sistema.

Ključne reči:
zubna proteza, parcijalna; zub, nosač proteze; simulacije, kompjuterske.

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Introduction

Unilateral partial edentulism, present in 1/3 of partially edentulous patients, represents a major challenge to any practitioner. For these cases implant placement is a therapy of choice. If this type of therapy is not possible for any reason, combined complex restorations can be a good alternative. Unilateral complex partial denture (UCPD) is located unilaterally and it is a combination of fixed and mobile restorations which are connected with a special type of extra coronal attachment, whose purpose is to provide retention, stability, denture guidance and axial force transfer on supporting tissues, stated by Ozcelik and Yilmaz [1] and Sherring-Lucas and Martin [2]. Primary part of an attachment is integrated in a milled crown on the abutment tooth, and the secondary part, which is in the unilateral saddle, creates the latch type connection of the whole restoration. Stamenković [3], Phoenix et al. [4] and Henderson and Steffel [5] consider this type of therapy as a restoration with high functional, esthetic and prophylactic values.

With a digital model design it becomes possible to analyze and predict the behavior of supporting tissues and the denture in function. The finite element method (FEM) represents a computerized, mathematically orientated technique. Its purpose is to obtain approximate numeric results of differential equations which describe and predict the behavior of physical systems exposed to different external influences.

FEM is used for the analysis in numerous studies in various areas of dentistry. This unique analysis is irreplaceable for complex geometry of teeth, dentures and restorations, their relationship, as well as for a large number of different materials. All of this complicates finding of analytical solution for stress and deformation values. The use of FEM in dentistry is also important because *in vivo* measuring of restoration or implant loading is very complex, and patients and ethical committees rarely approve it. That is the reason to conduct this testing *in vitro*. The aim of the research was to analyze the influence of free-end saddle length change on stress levels of UCPD abutment teeth and attachments.

Methods

In this study 3 virtual models of the Kennedy class II partially edentulous jaw were designed using the CATIA design computer program with the same abutment teeth – the canine and the first premolar, and the length of the saddle vary from model to model: model 1 – free-end saddle in the area of the second premolar, first, second and third molar; model 2 – free-end saddle in the area of the second premolar, first and second molar; model 3 – free-end saddle in the area of the second premolar and first molar; Virtual model of fixed part of the restoration with appropriate supporting structures (abutment teeth with milled crowns, alveolus, periodontal space); virtual model of the ”SD snap-in-latch” attachment; virtual model of the mobile part is designed as the simulated metal base cowered with acrylate with the number of missing teeth depending on the free-end saddle length.

The components mentioned above are combined to create the real size system for analysis (1 : 1 proportion) compared to natural teeth. The average teeth dimensions were selected to validate the results [6] (Figure 1).

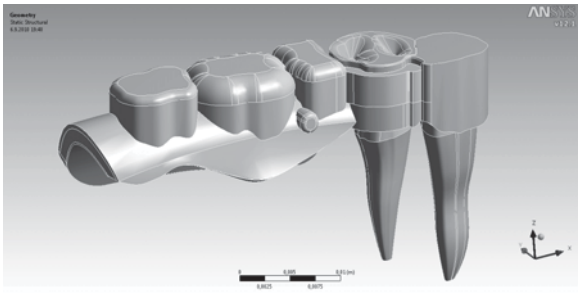

Fig. 1 – A virtual model of complex partial denture.

In order to define a virtual model of the whole system, the average distance between the enamel cementum junction and the crest of the alveolar bone of 2 mm was used, and this is how the length of the root in the bone was specified.

Virtual model analysis of UCPD was performed by using the FEM in the ANSYS Workbench 12 computer package. Stress and deformation analysis of the loaded model of UCPD, using FEM, implies finite elements net forming on a structural model. In this way, a design model is formed.

The net of adequate density is formed for a design model of unilateral complex denture, meaning: model 1 – net of 3320295 nodes and 2179811 elements; model 2 – net of 2433361 nodes and 1681724 elements, and model 3 – net of 2429373 nodes and 1581378 elements.

The 3D 10-nod tetrahedral type of finite elements (the option of 20 nodal, so-called Brick element) was used. Four types of finite elements were used in the model design: SOLID 187, Conta 174, Targe 170 and Surf 154.

All the parameters of the material used in the design process have isotropic properties. Each material has the elastic properties which are usually depicted through elastic modulus, in the field of elastic material behavior (Table 1).

Table 1

Material mechanical characteristics

Material	Young’s modulus of elasticity (MPa)	Poisson’s ratio	Author
Enamel	4.1×10^4	0.30	Rubin et al. [7]
Dentin	1.9×10^4	0.31	Rubin et al. [7]
Cementum	1.37×10^4	0.35	Peters et al. [8]
Pulp	0.000207×10^4	0.45	Rubin et al. [7]
Periodontal ligament	0.00689×10^4	0.45	Reinhardt et al. [9]
Alveolar bone	0.137×10^4	0.30	Güngor et al. [10]
Co-Cr-Mo	23×10^4	0.33	Stamenković [3]
Ceramics	6.9×10^4	0.33	Anusavice [11]

The elastic support unit was used for analysis to simulate the resilience of gum underneath the saddle. When a model is loaded, this base allows certain vertical movement of the saddle, and after that it starts behaving like a solid support. The resilient behavior of the gingiva is, in this way, successfully simulated, and its deflection under pressure allows certain movement of the denture. In this case the value of the elastic coefficient (elastic constant) is 750 N/mm^3, and it was set by comparing the stress values with the model on which the gingiva was created under the saddle during calculation [12]. During analysis the average tooth intrusion of 20 ± 10 μm was adopted.

In order to get the most realistic view and the most accurate calculation, the stress levels as well as the denture and teeth movement were analyzed under the load of the same biting forces at the same points on the model by transferring the applied force into pressure (according to the formula p = F/S, where S stands for the area of the tooth on which the force is applied).

The 150 N reference force was used for loading. On model 1, the individual teeth models, the second premolar, first, second and third molar, in the saddle were loaded. The stress level was monitored on the virtual teeth model. On model 2 the individual teeth models, the second premolar, first and second molar, in the saddle were loaded. The stress level was monitored on the virtual teeth model. On model 3, the individual teeth models, the second premolar and first molar, in the saddle were loaded. The stress level was monitored on the virtual teeth model.

Results

Calculations with preplanned model loading of the unilateral complex Kennedy class II denture were performed using the FEM. The model loading of UCPD was performed on the virtual model of the occlusal surfaces of the free-end saddle artificial teeth. Calculations were performed under the pressure of 150 N force on each free-end saddle tooth, depending on the type of model. The values of the maximal stress for the whole model, for the abutment teeth area and for the attachments area were obtained.

The results of maximal stress for the abutment teeth and the attachments of model 1 for 150 N force applied are shown in Table 2.

The results of maximal stress for the abutment teeth and the attachments of model 2 for 150 N force applied are shown in Table 3.

The results of maximal stress for the abutment teeth and the attachments of model 3 or 150 N force applied are shown in Table 4.

By analyzing all the 3 tables it becomes obvious that the distal shift of the load point results in stress level rise in all the 3 models. When 150 N force is applied on the free-end saddle teeth, maximal stress on the attachment is rising as the focus of the force shifts mesially, which indicates that the attachment absorbs the pressure, therefore providing protection to the abutment teeth.

The comparative graphs of stress level changes on the canine are shown in case when the force acts on the second premolar (Figure 2) and on the first molar (Figure 3). The comparative graphs of stress level changes on the first premolar are shown in case when the force acts on the second premolar (Figure 4) and on the first molar (Figure 5). Figure 6 shows a comparative graph of stress level changes on the abutments depending on the load point. The comparative graphs of stress level changes on the attachment in case when the force acts on the second premolar (Figure 7) and on the first molar (Figure 8). Finally, the comparative graph of the stress level changes on the attachment on the load point can be seen in Figure 9.

Table 2
The results of maximal stress for abutment teeth and attachments (model 1)

Load point	Canine (MPa)	First premolar (MPa)	Attachment (MPa)
Tooth 15	49.04	76.17	339.82
Tooth 16	56.41	91.75	250.63
Tooth 17	61.17	103.86	171.66
Tooth 18	64.50	110.07	160.20

Table 3
The results of maximal stress for abutment teeth and attachments (model 2)

Load point	Canine (MPa)	First premolar (MPa)	Attachment (MPa)
Tooth 15	50.22	56.64	114.67
Tooth 16	55.41	66.83	109.28
Tooth 17	63.08	80.92	115.26

Table 4
The results of maximal stress for abutment teeth and attachments (model 3)

Load point	Canine (MPa)	First premolar (MPa)	Attachment (Mpa)
Tooth 15	50.40	49.11	346.70
Tooth 16	59.22	60.87	187.18

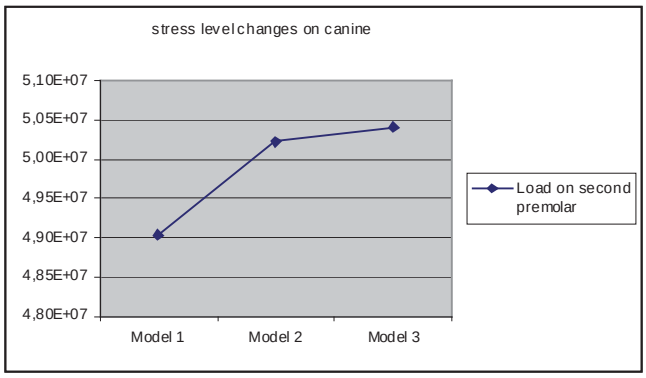

Fig. 2 – Comparative graph of stress level changes on the canine when force acts on the second premolar.

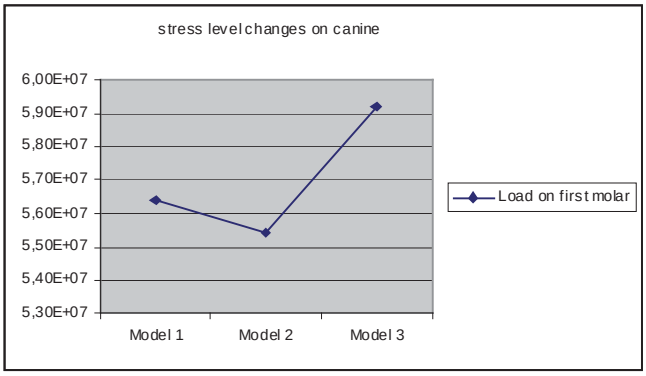

Fig. 3 – Comparative graph of stress level changes on the canine when force acts on the first molar.

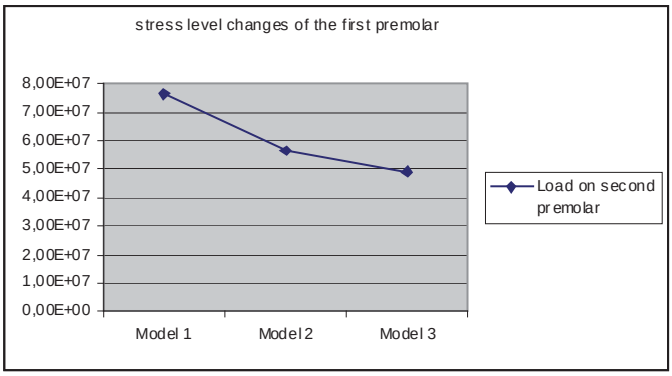

Fig. 4 – Comparative graph of stress level changes on the first premolar when force acts on the second premolar.

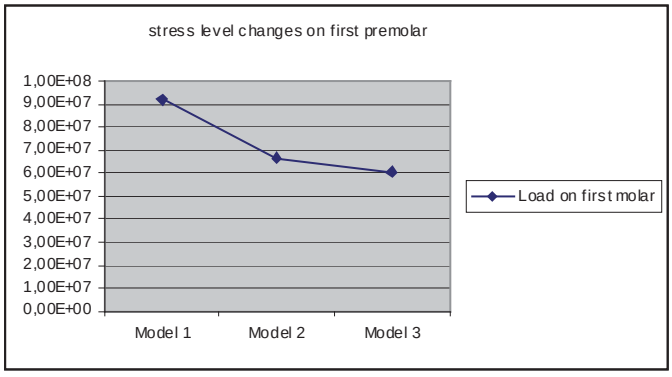

Fig. 5 – Comparative graph of stress level changes on the first premolar when force acts on the first molar.

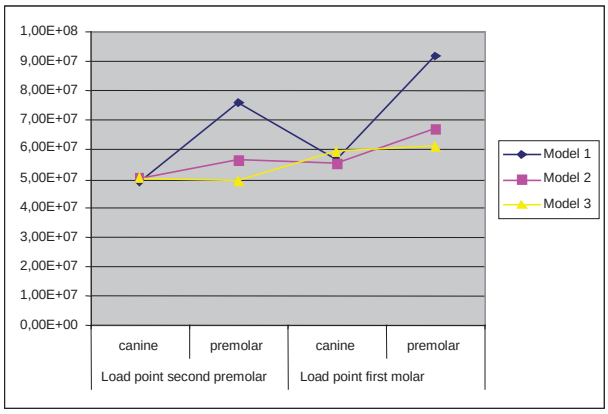

Fig. 6 – Comparative graph of stress level changes on the abutments depending on the load point.

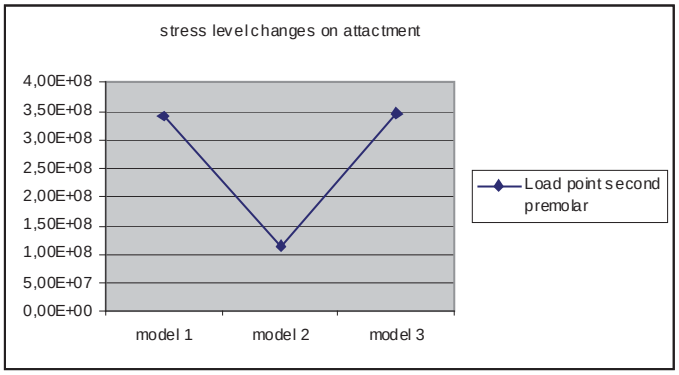

Fig. 7 – Comparative graph of stress level changes on the attachment when force acts on the second premolar.

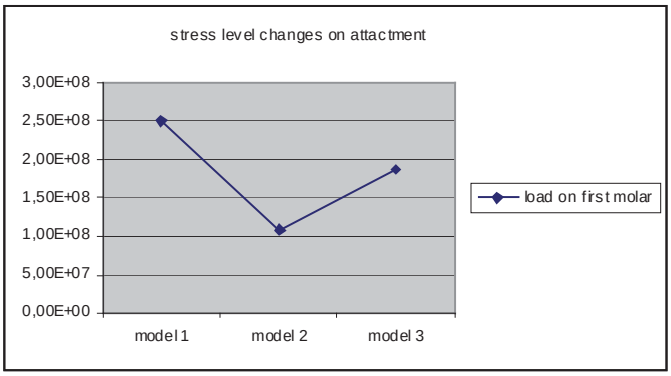

Fig. 8 – Comparative graph of stress level changes on the attachment when force acts on the first molar.

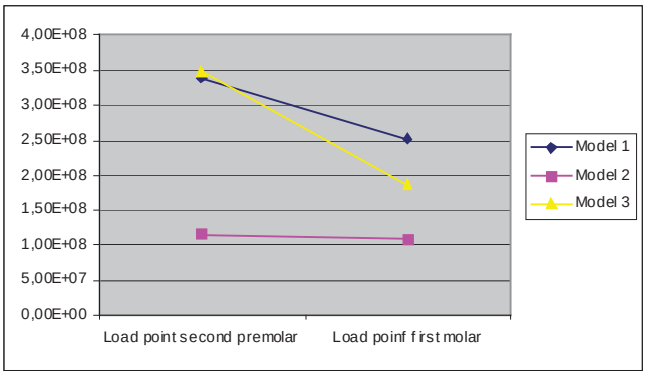

Fig. 9 – Comparative graph of stress level changes on the attachment depending on the load point.

Discussion

UCPD use in dental practice is a controversial subject. Stomatognathic structures which support removable partial denture (RPD) are histologically and anatomically made of different tissues, and therefore RPD has to match specific criteria. RPD building materials demand that all the RPD parts have to be connected, thus providing the necessary stiffness. The practitioner is faced with a problem of equal loading of two biologically different tissues. By comparing the values of mucosal resiliency of 1.5 ± 0.3 mm to the average value of teeth intrusion 20 ± 10 μm, the variable proportion appears. The best scenario is if the patient has fairly large, but still physiological axial movement of the teeth contrary to low mucoperiosteal resiliency. Edentulous jaws do not have the pressure resistant tissues. This research shows that the alveolar mucosa and gingiva can adapt to non-physiological requirements if they are adequately loaded. Periodontal tissues react best to the axially directed pressure. Actions which contribute to unilateral complex denture supporting tissues preservation are: good evaluation of the present status, adequate preparation of the supporting tissues and teeth, appropriate connection selection of the elements supported by teeth and mucosa, future denture base size defining and the restoration of the optimal occlusal relation, as stated by Radović [12].

In unilateral complex denture design, in everyday clinical practice, first and second premolars are most often used as abutment teeth. The canine and first premolar can also be an option. The reasons for this are the attachment manufacturer recommendation and the opinion that the long length saddle acts as the class 1 lever, i.e. rigid pole with a fulcrum on one side, such can damage the RPD supporting structures.

Biting forces vary ranging from minimal to maximal and they represent the reflection of muscle strength and activity. They are limited by the periodontal ligament capacity in the case of people with natural teeth, and by the mucosal sensoric capacity in mobile restoration users. In his study Trenouth [13] states that the biting force intensity ranges between 100 N and 700 N, depending on the intercanine or molar tooth region. Miyaura et al. [14] state that the biting force intensity ranges from 300 N for the patients with removable restorations, to 500 N for the intact dentition. Željković [15] states that the maximal biting force of natural dentition resembles the biting force of patients with fixed prosthetic restorations, but in case of patients with removable restoration it is reduced by 1/3 or 1/4 of its value. Pellizzer et al. [16] studied the behavior of implant supported RPD and UCPD under loads of 150 N, 210 N and 300 N, using FEM, and they concluded that the supporting structures behaved well under those loads. The results of the study by Tumrasvin et al [17] show that the maximal biting force in the upper jaw is around 240 N, and in the lower jaw 300 N, and if there are 3 missing the maximal biting force is 150 N. This is the reason to choose 150 N force to load free-end saddle teeth in this study.

The net of finite elements itself greatly influences the precision of the FEM results. The more complex the net (larger number of nodes and elements for the given model) the more precise problem solution can be expected, because the mathematical model itself resembles the real object more closely. During determination of the necessary net density it is important to bear in mind that there is a certain number of mathematical equations behind every element or nod.

Šćepanović [18] used the FEM to analyse of shape and the safety level check of the RPD retentive clasp arm, while Milić [19] used it for occlusal rest design optimization. Todorović et al. [20] and Radović [12] use the finite element simulation for unilateral complex denture load analysis and therapy possibilities. Aoda et al. [21] also used FEM in their research on unilateral complex dentures. Grbović et al. [22], Darendeliler et al. [23] and Eto et al. [24] used it for the simulation of UCPD behavior under load.

Regularity is found in the effect of 150 N force on the models 1, 2 and 3 free-end saddle teeth. As the load point shifts distally, stress level is rising on the abutment teeth. The explanation for this result lies in the fact that the lengthening of the lever arm results in the stress level rise. The potential solution for this kind of stress level rise is the maximal extension of the denture saddle, directed to reducing the force per unit area. This result is not in accordance with the results of the study by Radović et al. [25]. They found that the stress levels of the model and the abutment teeth decline with the rise of force intensity and distal shift of the load point. The reason for this can be found in the fact that, during this study, the loading force used was considerably lower, resulting in the different behavior of the model and different stress distribution.

UCPD can be considered as a suspended solid structure, as stated by Željković [15], or rigid as stated by Saito et al. [26] in their study. Considering this fact, the change in stress level on the models could be explained with the class 1 lever analysis.

The research shows that the primary abutment tooth, the first premolar, is least loaded in the model 3. This result can be explained by the fact that in this case lever arm is the shortest and the abutment tooth is close to the load point. Contrary to this, the first premolar takes the highest load, regardless the load point, in the model 1. The reason for that is the longer lever arm compared to the other two models. Secondary abutment tooth – canine, takes the highest load in case of the model 3 action force, because here the lever arm is longer than in case of the first premolar, i.e. abutment tooth is further from the load point. The highest tension was found when the force acts on the first molar, and the reason for that is the fact that the length of the lever arms in this case is highest.

Since the second premolar and the first molar were present in the free-end saddle of all the 3 models, the results were compared.

When force acts on the second premolar or the first molar, the loading of the secondary abutment – canine, does not change much with the change in the saddle length. Regularity was found that when the saddle is shortest, the loading of the first abutment is largest. This result can be explained by reduced, i.e. minimal saddle extension compared

with the other two models, so the chewing pressure is in this case transferred dentally to the fullest extent.

By observing the stress distribution on the primary abutment – first premolar, we can see the trend of load decline with the decline of the saddle length, no matter if the load point is positioned on the second premolar or the first molar.

By comparing the models 1, 2 and 3 we can see that the stress level on the first premolar is always higher than the stress level on the canine, which confirms the results of Saito et al. [26]. They found that in case of unilateral removable partial denture retained with attachment, the stress was largely concentrated on the first premolar.

When we compare stress distribution on the attachment, when force acts on the second premolar and the first molar, we can see that the attachment tension is minimal in the model 2, which confirmes that this model is best constructed for the attachment. Worst conditions were found when the load point was on the second premolar on the model with the shortest saddle (model 3). The reason for this result lies in the fact that in this case the load point of the force is very close to the attachment, and the saddle is least extended. The attachment here accepts most of the load and protects both abutments. Although the conditions are unfavorable, these values are under the marginal ones for plastic deformation of the attachment. Very similar situation can be found in the model 1, when the load point is located on the second premolar. Here, the attachment also accepts most of the load, therefore protecting the canine.

Regularity was detected in case when the load point is on a distally positioned tooth, the attachment is less strained. In such conditions the load is more evenly dispersed. This result is in correlation with the study by Todorović et al. [20]. They also conclude that with a distancing load point the stress level on the attachment reduces.

By observing the stress levels in the model 2, we can see the optimal distribution of stress on the abutment teeth, as well as on the attachment, regardless the load point. With the change in saddle length a significant tension of attachment and/or teeth is observed.

Regularity in stress distribution found on the abutment teeth of the models 1 and 3 was also detected. Namely, on the model with the longest saddle, the model 1, the tension on the canine was minimal, regardless the load point position, and the largest part of the load was transferred on the attachment. On the same model we can see the significant tension on the premolar. But still, the attachment accepts most of the loading, therefore protecting the abutment teeth.

Contrary to that, in the model 3, we can see higher tension of the canine than premolar regardless the action force position. Here we can also see the regularity, same as the one in the model 1, where the attachment accepts most of the loading. These results are in accordance with the study of Todorović et al. [20]. They find that when the abutment teeth are exposed to high stress levels, the attachment accepts the stress, therefore protecting the abutment teeth.

Conclusion

This study shows that it is possible to analyze an UCPD with numerical methods. By loading the model of UCPD and by observing the stress distribution for the whole model and for its separate parts we can conclude that models with 2 or 3 teeth in the free-end saddle do not show significant changes in stress levels of abutment teeth with the shift of the load point. A model with 4 teeth in the free-end saddle, shows a significant oscillation depending on the action force position. Also, when the load point is on the second premolar and the first molar, the model with the longest saddle shows the highest stress levels on the primary abutment. It should be noted that the third molar in a free-end saddle is contraindicated in a standard clinical practice and it was created as a part of the virtual model in order to provide adequate analysis of stress distribution in the exsperimental conditions. The stress levels of the abutments of the other two models are similar, under the same loading.

Stress levels found both on abutment teeth and on the attachment are far lower than the marginal ones. Under the preformed stress the system behavior changes, but the plastic deformation of the system does not occur.

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Ultrastructural changes of the peritoneum in a rabbit model of peritoneal dialysis

Ultrastrukturne promene peritoneuma na modelu peritoneumske dijalize kod zečeva

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Abstract

Background/Aim. The number of patients with end-stage renal diseases treated with chronic dialysis is increasing over the last years. Long-term peritoneal dialysis is associated with progressive development of structural and functional alterations of peritoneal membrane. The aim of the study was to analyze ultrastructural alterations of mesothelial monolayer and submesothelial tissue in a modified non-uremic experimental model of peritoneal dialysis in rabbits. **Methods.** The study was performed on 5 healthy Chinchilla rabbits. Surgical procedures of implantation and removal of peritoneal catheter, prevention of catheter clothing, prevention of infection and dialysate instillation were performed according to previously described protocols. Peritoneal tissue samples were collected upon catheter placement and removal after a 5-week follow-up and processed for transmission electron microscopy (TEM) examination. **Results.** The rabbits tolerated anesthesia, surgical procedure and the applied regimen of dialysate instillations well. The animals recovered completely and no adverse effects were noted. In the animals treated with peritoneal dialysis instillations, TEM revealed alterations of the mesothelial monolayer and submesothelial tissue. The mesothelial cells in direct contact with dialysis fluid were prone to shrinking. They lost the typical cobblestone morphology and assumed a flattened shape. The mesothelial cells were often detached from the basement membrane. These cells showed euchromatic nuclei, higher number of microvilli in their apical part and very numerous vesicles. A higher quantity of collagen fibers was noticed in the peritoneal lamina propria in close relation to the basement membrane of mesothelium. The nuclei of the fibroblasts were also euchromatic. Numerous mitochondria, granules and vesicles were present in their cytoplasm. **Conclusion.** The used rabbit model of peritoneal dialysis is simple, practical to perform, reproducible, not expensive and not requiring advanced devices. It is suitable for obtaining peritoneal tissue samples for histological examination and can be used to analyze the effects of dialysis solutions on the rabbit peritoneal membrane.

Key words:
peritoneal dialysis; rabbits; diseases models, animal; peritoneum; microscopy, electron; histology.

Apstrakt

Uvod/Cilj. Poslednjih godina u porastu je broj bolesnika sa terminalnom bubrežnom insuficijencijom koji se leče dijalizom. Dugotrajna primena peritoneumske dijalize praćena je razvojem strukturnih i funkcionalnih promena trbušne maramice. Cilj rada bio je analiza ultrastrukturnih promena mezotelnog prekrivača i submezotelnog vezivnog tkiva trbušne maramice na modifikovanom neuremijskom infuzionom modelu peritoneumske dijalize kod zeca. **Metode.** Istraživanje je izvedeno na pet zdravih zečeva rase Činčila. Hirurške procedure implantacije i vađenja peritoneumskog katetera, prevencija opstrukcije katetera, zaštita životinja od infekcije, instilacije dijalizata, uzimanje i priprema uzoraka peritoneuma za histološku analizu vršeni su prema prethodno opisanim protokolima. **Rezultati.** Eksperimentalne životinje dobro su podnosile aklimatizaciju, hirurške procedure i instilaciju dijalizata, nije bilo komplikacija tokom perioda

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praćenja. Analizom uzoraka tkiva životinja kojima je rađena peritoneumska dijaliza evidentirane su promene u mezotelnom sloju i u vezivnom tkivu submezotelnog sloja korišćenjem transmisione elektronske mikroskopije (TAM). Mezotelne ćelije koje su u direktnom kontaktu sa dijaliznom tečnosti podležu deskvamaciji, niske su, ljuspaste, sa proširenjem u predelu perinukleusnog dela; imaju brojne mikrovile u apikalnom delu i mnogobrojne transportne vezikule. Ispod mezotelnih ćelija, uočavaju se prošireni limfni sudovi i snopovi kolagenih vlakana koji se pružaju u različitim pravcima i okružuju tela i razgranate produžetke fibroblasta. Nukleusi fibroblasta su pretežno euhromatski, a u citoplazmi se uočavaju brojne sekretorne granule, vezikule, kao i mitohondrije. **Zaključak.** Modifikovani model peritoneumske dijalize kod zečeva koji smo koristili u ovoj studiji je praktičan, reproducibilan i ne zahteva sofisticiranu tehnologiju, te je pogodan za dalja histološka istraživanja dugotrajnih efekata dijaliznih rastvora na membranu peritoneuma zečeva.

Ključne reči:
dijaliza, peritoneumska; zečevi; bolest, modeli na životinjama; peritoneum; mikroskopija, elektronska; histologija.

Introduction

The number of patients with end-stage renal diseases treated with chronic dialysis methods has been increasing over the past years [1, 2]. In spite to significant scientific and technological advances in dialysis treatment, it is still associated with high morbidity and mortality rates [3]. Peritoneal dialysis (PD) has been an established form of renal replacement therapy for patients with end-stage renal disease for three decades now and it is currently performed in about 15% of uremic patients around the world [4].

The peritoneal membrane is a live biological membrane, structurally similar to other mesothelial membranes in the body [5]. It is affected by uremia *per se*, but also by chronic exposure to dialysis fluid during PD [6]. Water and solvents exchange during PD is performed through the peritoneal membrane. The failure of peritoneal membrane to provide adequate dialysis correlates with structural changes in the peritoneal membrane [7]. The mesothelial layer of the peritoneal membrane is stimulated and suffers injury in long-term PD patients. Furthermore, structural changes are also observed in the submesothelial layer in the peritoneum of PD patients [5, 8–11]. These ultrastructural changes of the mesothelium and submesothelial tissue alter the transport characteristics of the peritoneum as dialysis membrane and can ultimately cause ultrafiltration failure [12].

A limitation of peritoneal alterations study resulting from PD are technical and ethical difficulties to obtain biopsies of the peritoneum. Therefore, various uremic and non-uremic, acute and chronic animal models of peritoneal dialysis enable investigation of structural and functional changes of peritoneum exposed to PD.

The aim of this study was to analyze ultrastructural alterations of mesothelial monolayer and submesothelial tissue in a modified non-uremic infusion experimental model of peritoneal dialysis in rabbits.

Methods

The study was performed on five healthy Chinchillas rabbits (3 males and 2 females), weighing 2,932 ± 504 g at the beginning of the experiment. The rabbits were housed at room temperature (22 ± 2°C) and 12 hours light cycles, in individual cages, which were cleaned daily. The animals were given standard pellet rabbit food (Veterinary Institute, Subotica, Serbia) and water *ad libitum*. All the rabbits were allowed to adapt to new living conditions for at least five days prior to catheter insertion. During the study period of five weeks (one week for recovery following catheter placement and four weeks of dialysis) a diary of animal behavior was kept, including daily measurements of body mass, body temperature, food and water intake and defecation, antibiotics administration, other therapy and interventions (wound toilette, catheter suturing etc.).

Animals were anesthetized with tiopental BP 1G (Rotexmedica, Trittau, Germany; 0.5 mL/kg administered thorugh ear vein) for catheter placement at the beginning of experiment and for catheter removal, at the end of experiment. The peritoneal catheter in this study was made from the Tro-soluset infusion system (Troge Medical GMBH, Hamburg, Germany). The catheter was inserted in the abdominal cavity through a small incision on the front abdominal wall, below the left costal arc and parallel to the median abdominal line. It was then led through a subcutaneous tunnel to the exit site on the neck, according to a previously described procedure [13–15].

To prevent infection cefuroxime (Nilacef®, Hemofarm AD, Serbia) was administered intramuscularly daily three days before catheter placement and three days following catheter removal. The same antibiotic was administered daily through the peritoneal catheter during the four weeks instillation period. Antibiotic doses were calculated according to body mass.

To prevent clothing, the catheter was infused with 10 IU of heparin-sodium (Heparin®, Galenika a.d, Belgrade, Serbia) every day.

Following a 7-day recovery period after catheter placement, the animals were instilled with dialysis solution (Dianeal PD4 Glucose, with 3.86% glucose; Baxter Vertriebs GmbH, Vienna, Austria), previously warmed at 37°C, once a day for 28 days [13–15]. To prevent dyspnea, the initial dose of dialysis solution of 60 mL was gradually increased by 10 mL/day until a total dose of 40 mL/kg was reached.

Peritoneal tissue samples for histological analysis were taken upon peritoneal catheter insertion and catheter removal. This tissue is extremely fragile and susceptible to mechanical irritation and environmental factors. Therefore, oval tissue samples, 18 × 3 mm, were taken with extreme caution, immediately after opening the abdominal cavity, to avoid any damage.

For transmission electron microscopic (TEM) analysis tissue samples were fixed for 24 h in 4% glutaraldehyde diluted in Sorensen's phosphate buffer 0.1M (pH 7.4), then rinsed in Sorensen and cacodylate buffer. The samples were then postfixed in 1% osmium tetroxide in 0.1M cacodylate buffer and left over night in 4% uranyl acetate. After dehydratation in ethanol and propylene-oxide, the samples were embedded in Epon. Fine sections were contrasted with uranyl acetate and lead-citrate and analyzed with a transmission electron microscope Morgagni 268D.

All experimental procedures were performed according to the European Council Directive (86/609/EEC) and with the permission from The Committee for Animal Care, University of Belgrade.

The results were statistically analyzed with Microsoft Office Excel 2006 and shown as mean values and standard deviations.

Results

All the animals tolerated aclimatization, surgical procedure and dilaysate instillations well. No peritonitis, nor high temperature or other signs of infections were noted.

During a 5-week study period the animals showed steady mass increase (Figure 1), while body temperature remained in physiological range (Figure 2).

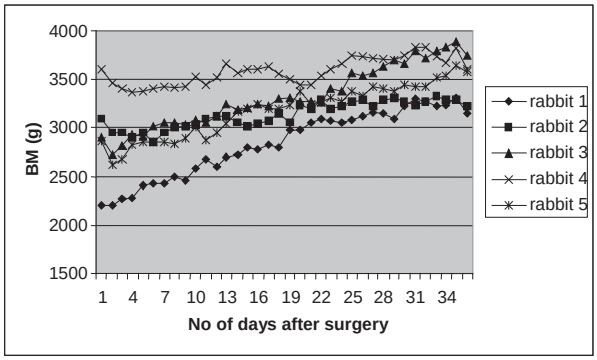

Fig. 1 – The rabbits body mass (BM) during the study period.

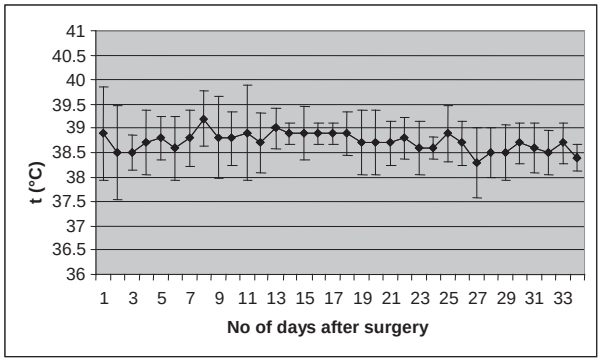

Fig. 2 – The rabbits body temperature (t) during the study period ($\bar{x} \pm SD$).

The animals had neither infection of the surgical wound nor peritonitis episode, and no catheter clothing, thanks to preventive use of antibiotic and heparin.

TEM analysis of the control samples of peritoneal tissue, taken before dialysate instillations, revealed flat or cubic irregular mesothelial cells. Adjacent mesothelial cells were connected with intercellular connections, mostly desmosomes (Figure 3). The apical plasmalema formed numerous cytoplasmic extensions – microvilli. Pinocytotic vesicles were observed in the apical and other parts of plasmalemma. The submesothelial connective tissue showed fibroblasts, collagen and elastic fibers. The fibroblasts were large and heavily branched, so only their parts of different shapes and sizes could be seen on individual sections.

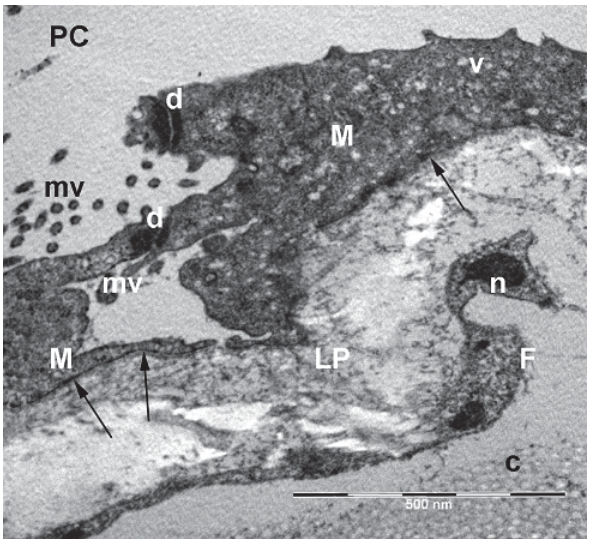

Fig. 3 – Transmision electron microscopic analysis of the rabbit peritoneum before instillation of dialysis fluid.
PC – peritoneal cavity; M – mesothelial cell; LP – lamina propria; F – fibroblast; n – nucleus; mv – microvilli; v – vesicles; d – desmosome; c – collagen. Arrows – basement membrane.

TEM analysis of peritoneal tissue samples taken after dialysate instillations showed alterations in both mesothelial and submesothelial layers.

Mesothelial cells exposed to dialysis fluid were flat, with widening of the perinuclear space (Figures 4 and 5).

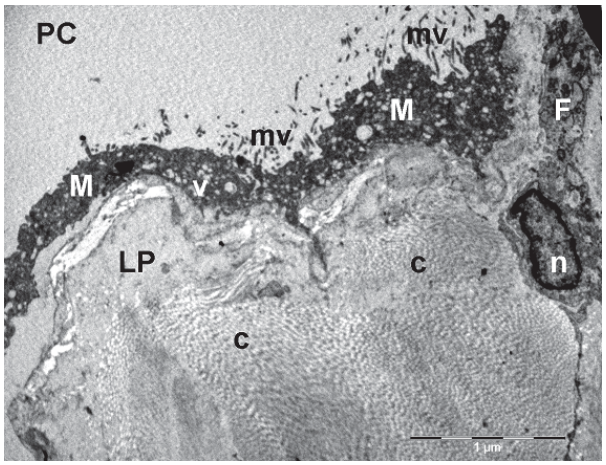

Fig. 4 – Transmision electron microscopic analysis of the rabbit peritoneum after instillation of dialysis fluid.
PC – peritoneal cavity; M – mesothelial cell; LP – lamina propria; F – fibroblast; n – nucleus; mv – microvilli; v – vesicles; c – collagen.

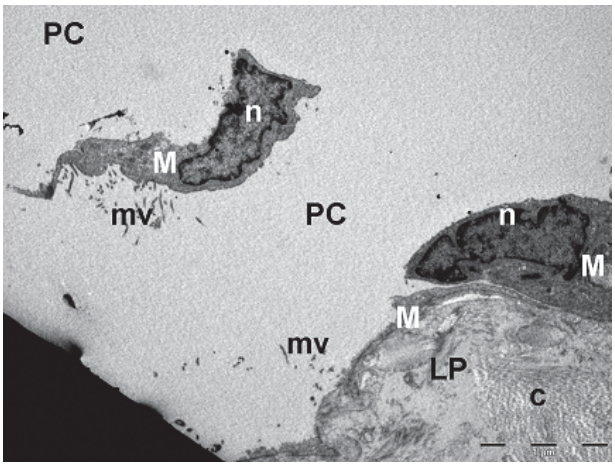

Fig. 5 – Transmision electron microscopic analysis of the peritoneal mesothelium after exposure to dialysis fluid.
PC – peritoneal cavity; M – mesothelial cell; LP – lamina propria; F – fibroblast; n – nucleus; mv – microvilli; v – vesicles; c – collagen.

Their nuclei were euchromatic, with prominent nucleoli. Numerous transport vesicles were observed throughout cytoplasm, as well as increased number of microvilli on the apical plasmalemma of the mesothelial cells. Patches of peritoneum devoid of mesothelium were seen even at lower magnification.

The submesothelial layer was dominated by the bundles of cross-striated collagen fibers with a different course, surrounding fibroblasts (Figures 4 and 6). Fibroblasts had mostly euchromatic nuclei and numerous secretory granules, vesicles and mytochondria in their cytoplasm (Figure 6).

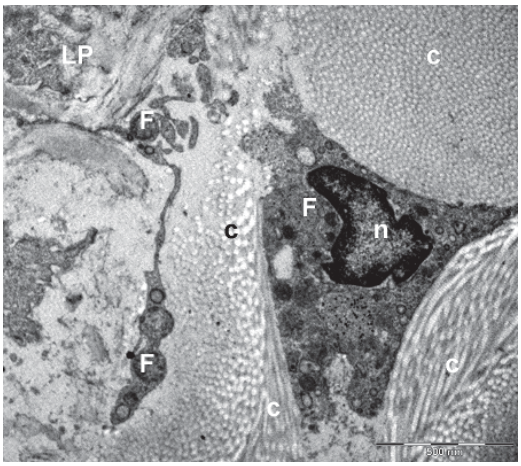

Fig. 6 – Transmision electron microscopic analysis of the peritoneal lamina propria after exposure to dialysis fluid.
LP – lamina propria; F – fibroblast; n – nucleus; c – collagen.

Discussion

Animal models of PD have significantly contributed to better understanding of structural and functional changes of peritoneum exposed to PD. The modified rabbit PD model used in our study is practical, reproducible and did not require sophisticated technology. The animals tolerated the procedure well and no complications, such as peritonitis or catheter obstruction, were noted. This model is, therefore, adequate for further investigation of the long-term effects of dialysis solutions on the peritoneal membrane in rabbits [13–15].

Long-term PD results in peritoneal injury with structural changes and functional decline [16]. The so-called conventional solutions have electrolyte content similar to serum and acidic reaction in order to prevent caramelization of glucose during heat sterilization. Glucose is widely used as osmotic agent in these solutions in concentrations 15 to 40 times higher than in physiological fluids. Therefore, PD patients are exposed to large quantities of glucose, even up to 100 kg per year [17]. High acidity and high glucose concentration, the presence of lactate as buffering agent and numerous glucose degradation products (GDP) formed during sterilization and storage, contibute to peritoneal injury. Typical structural alterations of peritoneum exposed to long-term PD include the loss of mesothelial cells, thickening of submesothelial tissue and various vascular changes [9, 13, 15, 17, 18].

A number of *in vitro* and *in vivo* studies have shown adverse effects of GDP on the peritoneum. Accumulation of these products (especially toxic are 3,4-dideoxyglucosone and methylglyoxal) in the peritoneum cause structural changes in the tissue, either by direct action or by inducing the formation of advanced glycosilated end products (AGE) [19, 20]. The adverse effects of GDPs include: inhibition of cell proliferation and reparation of lesions; decreased IL6, fibronectin and collagen type 1 synthesis; promotion of apoptosis; increasing reactive oxygen species synthesis and carbonyle stress; increasing expression of receptors for AGEs, adhesion molecules, growth factors, vascular endothelial growth factor and IL8; cellular gluthatione depletion; reduced expression of intercellular tight-junctions; upregulation of HLA antigen expression on mesothelial cells and induction of epithelial-mesenchymal transition [6, 21, 22].

The mesothelial cells are flat or cubic specialized epithelial cells lining the internal organs and peritoneal, pleural, pericardial and synovial cavities [5]. The shape of mesothelial cells in rabbits from our study, prior to exposure to dialysis solution, corresponds to these data (Figure 3). The luminal surface of mesothelial cells has numerous finger-like cytoplasmic extensions – microvilli, increasing the functional mesothelial surface for exchange between mesothelial cells and peritoneal cavity. These microvilli protect the delicate mesothelial surface from frictional injury by entrapping water and serous exudates, which act as lubricants for the cells. Microvilli remain present even during PD, thus increasing the surface for water and solutes exchange between peritoneal cavity and the cytoplasm of mesothelial cells (Figure 4).

The number of pinocytotic vesicles, which are normally present in the apical region of mesothelial cells, multiplies during PD due to intensive exchange processes (Figure 4). The apical suface of mesothelial cells also shows a single microcilia and cytosceleton from intermediar vimentin and

cytokeratin filaments. A well developed Golgi apparatus, numerous perinuclear mytochondria, granulated endoplasmic reticulum and ribosomes, as well as euchromatic nuclei in mesothelial cells are indicative of their dynamic biosynthetic activity. This activity becomes even more significant during PD (Figure 5).

A submesothelial layer of peritoneum consists of extracellular matrix made up of glycosaminoclycans (hyaluronic acid and chondroitin sulfate) and proteoglycans, collagen fiber bundles (mostly type I and III) and elastic fibers, with fibroblasts and free cells (macrophages, mastocytes, leukocytes and multipotent cells). Vascular structures and lymphatics are found in the subserous place. This tissue functions as a molecular filter regulating transition of various molecules and cells through peritoneum. The fibroblasts become activated during PD and enhance synthesis of collagen, which can be found in thick bundles in the submesothelial layer (Figure 4 and 6).

The mesothelial cells isolated from drained dialysate change their epithelial-like morphology into fibroblast-like cells. This mesothelial-fibroblast transformation is characterized by the loss of cadherin and cytokeratin markers, which are typical for the epithelial phenotype, and by appearance of alpha-smooth muscle actin. These transformed mesothelial cells are usually found in dialysate from PD patients treated with conventional dialysis solutions [6, 23].

Conclusion

The modified rabbit PD model used in our study is practical, reproducible and does not require sophisticated technology. The animals tolerated the procedure well and no complications, such as peritonitis or catheter obstruction, were noted. The studied experimental model is suitable for obtaining peritoneal tissue samples for histological examination. This model is, therefore, adequate for further investigation of long-term effects of dialysis solutions on the peritoneal membrane in rabbits.

Aknowlegments

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Use of intravenous immunoglobulin in neonates with haemolytic disease and immune thrombocytopenia

Primena intravenskih imunoglobulina kod novorođenčadi sa hemoliznom bolesti i imunskom trombocitopenijom

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Abstract

Background/Aim. Intravenous immunoglobulin is a blood product made of human polyclonal immunoglobulin G. The mode of action of intravenous immunoglobulin is very complex. It is indicated in treatment of neonatal immune thrombocytopenia and haemolytic disease of the newborn. The aim of the study was to present our experience in the use of intravenous immunoglobulin in a group of term neonates. **Methods.** We analysed all relevant clinical and laboratory data of 23 neonates who recieved intravenous immunoglobulin during their hospitalization in Neonatal Intensive Care Unit of Mother and Child Health Care Institute over a five year period, from 2006. to 2010. **Results.** There were 11 patients with haemolytic disease of the newborn and 12 neonates with immune thrombocytopenia. All of them recieved 1–2 g/kg intravenous immunoglobulin in the course of their treatment. There was no adverse effects of intravenous immunoglobulin use. The use of intravenous immunoglobulin led to an increase in platelet number in thrombocytopenic patients, whereas in those with haemolytic disease serum bilirubin level decreased significantly, so that some patients whose bilirubin level was very close to the exchange transfusion criterion, avoided this procedure. **Conclusion.** The use of intravenous immunoglobulin was shown to be an effective treatment in reducing the need for exchange transfusion, duration of phototherapy and the length of hospital stay in neonates with haemolytic disease. When used in treatment of neonatal immune thrombocytopenia, it leads to an increase in the platelet number, thus decreasing the risk of serious complications of thrombocytopenia.

Key words:
thrombocytopenia, neonatal alloimmune; anemia, hemolytic; infant, newborn; immunoglobulins, intravenous.

Apstrakt

Uvod/Cilj. Intravenski imunoglobulini su preparat humanih imunoglobulina G dobijenih iz plazme zdravih davalaca. Mehanizam delovanja intravenskih imunoglobulina veoma je složen. Njihova primena je indikovana u lečenju hemolizne bolesti i imunskoj trombocitopeniji novorođenčeta. Cilj rada bio je prikaz sopstvenog iskustva u primeni intravenskih imunoglobulina u grupi terminske novorođenčadi. **Metode.** Analizirani su anamnestički podaci, klinički nalazi i laboratorijski rezultati 23 novorođenčeta koji su u periodu od 2006. do 2010. dobijali intravenske imunoglobuline tokom hospitalizacije u neonatalnoj intenzivnoj nezi Instituta za majku i dete Srbije „Dr Vukan Čupić". **Rezultati.** Kod 11 novorođenčadi indikacija za primenu intravenskih imunoglobulina bila je hemolizna bolest novorođenčeta, dok je 12 novorođenčadi imalo imunsku trombocitopeniju. Kod svih bolesnika primenjena je doza od 1 do 2 g/kg telesne mase intravenskih imunoglobulina. Nisu registrovane komplikacije primenjene terapije. Kod novorođenčadi sa hemoliznom bolešću došlo je do znatnog sniženja nivoa bilirubina, što je omogućilo da najveći broj bolesnika izbegne eksangvinotransfuziju. Primena intravenskih imunoglobulina dovela je do značajnog porasta broja trombocita kod bolesnika sa imunskom trombocitopenijom. **Zaključak.** Primena intravenskih imunoglobulina vrlo je efikasna za snižavanje potrebe za eksagvinotransfuzijom, za skraćenje trajanja fototerapije i skraćenju trajanja hospitalizacije kod novorođenčadi sa hemoliznom bolešću. Kod novorođenčadi sa imunskom trombocitopenijom primena intravenskih imunoglobulina dovodi do značajnog porasta broja trombocita, snižavajući time rizik od potencijalno ozbiljnih komplikacija trombocitopenije.

Ključne reči:
trombocitomenija, neonatalna, aloimunska; anemija, hemolitička; novorođenče; imunoglobulini, intravenski.

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Introduction

Intravenous immunoglobulin (IVIG) is a blood product made of human polyclonal immunoglobulin G (IgG). It is obtained from plasma of thousands of healthy blood donors, therefore providing a great variety of antibodies. It contains more than 95% of unmodified immunoglobulin G and some traces of immunoglobulin A and immunoglobulin M. It has been first used in treatment of primary immunodeficiency and nowadays is used in treatment of many hematological, neurological, rheumatic and dermatological diseases [1]. The mode of action of immunoglobulin is complex and it includes modulation of Fc-receptor mediated phagocytosis, complement binding and prevention of membrane attack complex formation, inhibition of some cytokines, down-regulation of antibody production etc. [1, 2]. According to well-established recommendations, the indications for use of IVIG in neonates are: haemolytic disease of the newborn (HDN), immune thrombocytopenia and fetal hydrops caused by PARVO B19 virus [3]. The use of IVIG in prevention and treatment of neonatal sepsis, especially in preterm neonates, is still controversial [4, 5].

The aim of this study was to present our experience in the use of IVIG in a group of term neonates.

To our knowledge, this is the first presentation of national experience in the use of IVIG in neonates with hemolytic disease of the newborn and neonates with immune thrombocytopenia.

Methods

Over a five year period, from 2006 to 2010, intravenous immunoglobulin was administered in the Neonatal Intensive Care Unit (NICU) of Mother and Child Health Care Institute of Serbia "Dr. Vukan Čupić" in the treatment of 23 term neonates. This presentation encompasses two groups of patients: neonates with haemolytic disease of the newborn and neonates with immune thrombocytopenia. All the data concerning pregnancy, perinatal history, management in immediate postnatal period as well the treatment received prior to admission to the NICU, were collected from medical records. We obtained written consent from the parents for blood sample collection of the neonates.

The group I of patients included of neonates with isoimmune haemolytic disease (HD) due to Rh or ABO incompatibility between the blood group of the mother and the newborn, proven by the positive direct Coombs test, indirect hypebilirubinemia and increased reticulocyte count. Hyperbilirubinemia was considered significant if phototherapy and/or exchange transfusion (ET) was required [6]. Laboratory investigations included: serum bilirubin level (total and direct), direct Coombs test, full blood count with reticulocyte count. Each neonate was treated with continuous intensive phototherapy. All neonates received 1–2 g/kg body weight of IVIG in intravenous infusion, over no less than 6 hours. Estimation of serum bilirubin was done 6 hours after termination of IVIG infusion and every 12 hours in the next two days.

The group II of patients included neonates with immune thrombocytopenia. Laboratory investigations included: full blood count, standard biochemical analyses, sepsis workup with determination of C-reactive protein. Abdominal and cranial ultrasonography was performed in each patient. Indication for platelet transfusion was platelet count of less than 20×10^9/L. All the patients in this group received 1–2 g/kg body weight of IVIG in intravenous infusion. Platelet count was repeated 12 and 24 hours after IVIG infusion, and later during hospitalization as needed, according to the latest platelet number. Platelet antigen typization could not be done since it is not available in our country.

Results

During a 5-year period, 23 neonates received IVIG during their hospitalization in the NICU. Haemolytic disease of the newborn was an indication for IVIG treatment in 11 (47.8%) patients, while 12 (52.2%) patients had immune thrombocytopenia.

In the group of patients with haemolytic disease, 7 (63.6%) patients had OA incompatibility, two (18.2%) OB incompatibility and two (18.2%) patients had Rh incompatibility. The average age on admission was 1.2 days. All the patients had received phototherapy and one patient had had ET prior to admission to our NICU. All the neonates had normal physical examination apart from jaundice. Bilirubin level on admission ranged from 218 to 347 μmol/L. All the patients received IVIG (dose 1–2 g/kg body weight), with no side effects noted. They were all receiving intensive phototherapy concomitantly. The average duration of phototherapy was 40 hours. Two patients needed ET according to their bilirubin level on admission. When plotted on nomogram for prediction of risk for exaggerated jaundice [6], all our patients were in high risk zone on admission. Twenty-four hours after IVIG infusion 8 (72.8%) of them were in low intermediate risk zone, while 2 (18.2%) of them were in high intermediate risk zone and one (9.0%) patient was in low risk zone, as shown in Figure 1. Four (33.3%) of the patients received red blood cell transfusion because of severe anemia. The average length of hospitalization in this group of patients was 9.0 days.

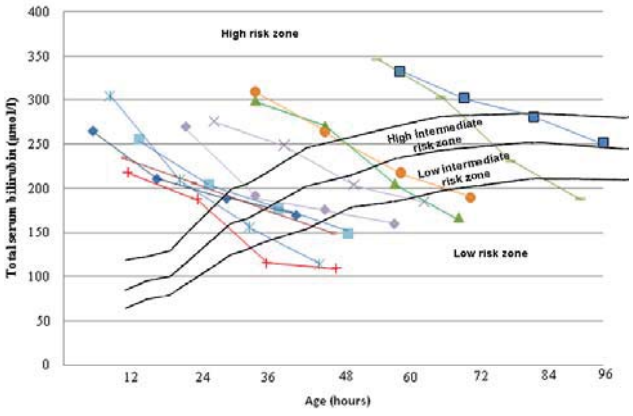

Fig. 1 – Bilirubin level trend in neonates with haemolytic disease.

In the group of patients with immune thrombocytopenia, two (16.7%) neonates were born to mothers with thrombocytopenia due to immune thrombocytopenic purpura (ITP), whereas 10 (83.3%) patients were born to mothers with normal platelet count. The average age in this group of patients was 1.8 days. The platelet count on admission ranged from 7 to 45 × 10^9/L, with an average being 20.6 × 10^9/L. All the patients had normal physical examination apart from 8 (66.6%) patients in whom petechiae and/or ecchymoses were noted. Abdominal and cranial ultrasonography performed in each patient showed no signs of bleeding. All patients were treated with 1g/kg body weight of IVIG for up to two consecutive days, depending on the response in platelet count. The administration of IVIG was not associated with any complications. Three of the patients (25,0%) were put on corticosteroid treatment for prolonged thrombocytopenia, which did not resolve after IVIG administration. The average length of hospitalization was 13.3 days. The average period until the platelet count reached ≥ 100 × 10^9/L was 2, 4 days. All patients had normal platelet count on their discharge from hospital, with an average platelet count of 166.9 × 10^9/L.

Discussion

Intravenous immunoglobulin has been successfully used in treatment of isoimmune haemolytic anemia. Isoimmune haemolytic anemia of the newborn is a cause of neonatal hyperbilirubinemia due to haemolysis of fetal red blood cells, caused by transplacentally transmitted maternal antibodies active against antigens present on fetal erythocytes. It leads to an increased risk of bilirubin encephalopathy and kernicterus. The therapy is aimed at lowering the serum concentration of bilirubin or keeping it from further increase, so avoiding the levels at which kernicterus may occur. Neonatal isoimmune haemolytic disease is conventionally treated by phototherapy and ET [6]. The exact mechanism of action of IVIG in HDN is still not precisely explained. Intravenous immunoglobulin is believed to occupy the Fc receptors of reticuloendothelial cells and prevent further lysis of antibody-coated erythrocytes. It is found to decrease hemolysis leading to reduction in serum bilirubin level [7–12]. There is subsequently an important decrease in need for exchange transfusion [6, 7, 9].

Due to widely used preventive administration of anti-D prophylaxis in Rhesus-negative women, there is a decrease in Rhesus sensitization and subsequent haemolytic disease of the newborn. That is why a high proportion of haemolytic disease of newborn is nowadays caused by antibodies to other red blood cell antigens (anti-A, anti-B etc.) [7]. In our group of patients with haemolytic disease, Rh incompatibility was the cause of haemolysis in only two (18.2%) patients, whereas the major cause was OA incompatibility (63.6%).

Many studies have shown that the use of IVIG leads to a significant reduction in the need for ET [8–15]. Our data also confirmed that administration of IVIG reduced bilirubin level, decreasing it beneath the threshold for ET in 8 (66.6%) patients. Twenty-four hours following IVIG infusion an important decrease in bilirubin level was observed in most of our patients (72.8%), diminishing their risk from high to low intermediate. An infant whose bilirubin level is in low intermediate or low risk zone is at a very low risk to develop severe hyperbilirubinemia [6]. This result goes along with results of many authors who showed a significant reduction in bilirubin level after IVIG use [8–11]. Early administration of IVIG as soon as the diagnosis of haemolytic anemia is made is therefore recommended by many authors [8–11, 13]. Monpoux et al. [11] suggest that after an initial period of 4 hours of intensive phototherapy, IVIG might be used even in jaundice with negative Coombs test, mostly caused by ABO incompatibility.

The average duration of phototherapy in our patients was 40 hours and the average length of hospitalization was 9.0 days. Although we did not have a control group, the average length of hospitalization in our HD group is similar to that of HD group presented by Voto et al. [16]. In a systemic review by Gottstein and Coone [8], a reduction in the duration of phototherapy and hospital stay, when IVIG is used along with phototherapy for haemolytic disease, is emphasized. However, there is a great heterogeneity between the studies [8]. Shortened hospitalization and duration of phototherapy make a financial benefit which exceeds the cost of IVIG. It is considered to be a relatively safe product with rarely seen serious side effects [7–9, 13]. We did not note any immediate adverse effects either.

It is postulated that there is an icreased rate of late transfusions required in patients treated with IVIG for HDN 8. It is probably secondary to further haemolysis after the effect of IVIG has expired, so the Fc sites on reticuloendothelial cells become free again to bind antibody sensitized neonatal erythrocytes [7, 8, 11, 13]. One of the drawbacks of our analysis might be the lack of data showing the incidence of late transfusion rate in HDN group of patients.

Although we realize that the absolute number of our HDN group is limited, one may notice that our results do not differ from those of other similar studies [9, 13].

There are two types of neonatal immune thrombocytopenia. Neonatal autoimmune thrombocytopenia is secondary to transplacental passage of maternal platelet autoantibodies. It can be seen in neonates born to mother with autoimune disease, most commonly idiopathic thrombocytopenic purpura (ITP) or systemic lupus eritematodes (SLE). Neonatal alloimmune thrombocytopenia (NAIT) is caused by maternal alloimmunisation against fetal platelet antigens inherited from the father but absent on mother's platelets. Maternal anti-platelet antibodies then cross the placenta and destroy fetal platelets. The majority of casese are caused by antibodies against Human Platelet Antigen 1a – HPA-1a [17, 18]. The greatest risk of severe thrombocytopenia is intracranial haemorrhage (ICH), which may cause death or lead to neurological sequelae. Treatment of a neonate with NAIT is aimed at preventing or stoping thrombocytopenic bleeding [19, 20]. The first therapeutic choise is platelet transfusion [21]. It is often combined with IVIG, whose administration is associated with an increase in platelet count, after a period of 24–48h [22].

In our group of patients with thrombocytopenia, neonatal autoimmune thrombocytopenia was present in 2 (16.7%) patients. It is known that about 10% of neonates of mothers affected with some autoimmune disease, mostly ITP and SLE, develop thrombocytopenia [19]. It is a less common cause of thrombocytopenia than NAIT, as is shown in our group of patients. Neonatal autoimmune thrombocytopenia is usually mild. Both of our patients had severe thrombocytopenia ($\leq 20 \times 10^9$/L), which is uncommon, but they had no signs of visceral bleeding and recovered their platelet count after IVIG administration.

Fetomaternal incompatibility for HPA-1a causes about 75% of all cases of NAIT [21, 23–26]. It is typically diagnosed in an otherwise well neonate, who develops petechiae and/or purpura shortly after birth. Thrombocytopenia may also be noted when a full blood count is checked for sepsis workup or some other clinical reasons [17]. In our group of patients, 8 (66.6%) of them had petechiae and/or purpura with otherwise normal physical examination. When there is no possibility to screen blood of the mother and the father, which is the case in our country, it is of great importance to exclude other possible causes of thrombocytopenia (TORCH screening, "blueberry muffin" rash, intrauterine growth restriction, thrombosis, sepsis, etc). The testing to confirm NAIT would be performed in order to discover an antibody in the mother's plasma directed against a platelet-specific antigen present in the father, but not in the mother. This is a complex testing which should be done only in a laboratory with experience in this field [17].

If a neonate has low platelet count with no other explanation, so the clinical diagnosis of NAIT is confirmed, the first therapeutic choice in a bleeding newborn would be a random donor platelet transfusion if the platelet count is less than 30×10^9/L [18, 21]. Platelet number which presents an indication for transfusion slightly differs according to the literature source [20]. Neonates with no signs of bleeding and platelet count of more than 30×10^9/L should be closely monitored (including repeated platelet count and ultrasound examination). Many authors believe that in case of severe thrombocytopenia, the best treatment modality would be to give random donor platelet transfusion first and then to infuse IVIG and closely follow the platelet count [17, 20–22]. This is also the strategy accepted by our team. After transfusion of platelets, when it was indicated, all neonates in our presentation were infused with IVIG, which was not associated with any side effects.

Although there is no strong evidence to support the use of corticosteroids in the treatment of NAIT, some authors propose the use of methylprednisolone for neonates with prolonged thrombocytopenia if it persists after IVIG treatment [17]. Three (25%) of our patients were put on a short course of corticosteroids until their platelet count reached normal range.

Conclusion

Indications for the use of IVIG in neonates in our group of patients were haemolytic disease and immune thrombocytopenia of the newborn. Its use in addition to phototherapy in treatment of haemolytic disease of the newborn led to the reduction in the degree of haemolysis and therefore the need for exchange transfusion. It was also shown to be effective in increasing the platelet count in case of immune thrombocytopenia of the newborn. No side effects of IVIG were noted, so it can be considered a safe treatment for the newborn.

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Posterior breast cancer – mammographic and ultrasonographic features

Prepektoralni karcinom dojke – mamografske i ultrazvučne osobine

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Abstract

Background/Aim. Posterior breast cancers are located in the prepectoral region of the breast. Owing to this distinctive anatomical localization, physical examination and mammographic or ultrasonographic evaluation can be difficult. The purpose of the study was to assess possibilities of diagnostic mammography and breast ultrasonography in detection and differentiation of posterior breast cancers. **Methods.** The study included 40 women with palpable, histopathological confirmed posterior breast cancer. Mammographic and ultrasonographic features were defined according to Breast Imaging Reporting and Data System (BI-RADS) lexicon. **Results.** Based on standard two-view mammography 87.5%, of the cases were classified as BI-RADS 4 and 5 categories, while after additional mammographic views all the cases were defined as BI-RADS 4 and 5 categories. Among 96 mammographic descriptors, the most frequent were: spiculated mass (24.0%), architectural distortion (16.7%), clustered microcalcifications (12.6%) and focal asymmetric density (12.6%). The differentiation of the spiculated mass was significantly associated with the possibility to visualize the lesion at two-view mammography ($p = 0.009$), without the association with lesion diameter ($p = 0.083$) or histopathological type ($p = 0.055$). Mammographic signs of invasive lobular carcinoma were significantly different from other histopathological types (architectural distortion, $p = 0.003$; focal asymmetric density, $p = 0.019$; association of four or five subtle signs of malignancy, $p = 0.006$). All cancers were detectable by ultrasonography. Mass lesions were found in 82.0% of the cases. Among 153 ultrasonographic descriptors, the most frequent were: irregular mass (15.7%), lobulated mass (7.2%), abnormal color Doppler signals (20.3%), posterior acoustic attenuation (18.3%). Ultrasonographic BI-RADS 4 and 5 categories were defined in 72.5% of the cases, without a significant difference among various histopathological types ($p = 0.109$). **Conclusion.** Standard two-view mammography followed by additional mammographic projections is an effective way to demonstrate the spiculated mass and to classify the prepectoral lesion as category BI-RADS 4 or 5. Additional ultrasonography can overcome the mimicry of invasive lobular breast carcinoma at mammography.

Key words:
breast neoplasms; mammography; ultrasonography; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Prepektoralni karcinom dojke odnosi se na lokalizaciju tumora u posteriornim delovima dojke, u blizini zida grudnog koša, što ometa njegovo otkrivanje kliničkim pregledom, mamografijom ili ultrazvukom. Cilj rada bio je analiza dijagnostičke mamografije i ultrazvuka dojki u detekciji i diferencijaciji prepektoralnog karcinoma dojke. **Metode.** Ispitano je 40 žena sa patohistološki verifikovanim karcinomom. Analiza mamografskih i ultrazvučnih osobina sprovedena je u skladu sa leksikonom *Breast Imaging Reporting and Data System* (BI-RADS). **Rezultati.** Standardnom mamografijom iz dva pravca promene su definisane kao BI-RADS 4 i 5 kod 87,5% ispitanica, dok su posle primene dopunskih mamografskih projekcija sve promene bile BI-RADS kategorije 4 i 5. Od 96 mamografskih deskriptora, najveća učestalost bila je: stelatnih senki (24,0%), narušene arhitektonike (16,7%), grupisanih mikrokalcifikacija (12,6%) i fokalne asimetrije parenhima (12,6%). Diferentovanje stelatne senke bilo je povezano sa vizualizacijom promene iz dve mamografske projekcije ($p = 0,009$), bez uticaja veličine ($p = 0,083$) ili patohistološkog tipa karcinoma ($p = 0,055$). Mamografski znaci invazivnog lobularnog karcinoma razlikovali su se u odnosu na ostale patohistološke tipove karcinoma (narušena arihitektonika, $p = 0,003$; fokalna asimetrija parenhima, $p = 0,019$; udruženost četiri ili pet indirektnih znakova maligniteta, $p = 0,006$). Ultrazvučnim pregledom bilo je moguće otkrivanje svih promena, od kojih su kod 82,0% ispitanica diferentovane morfološke karakteristike prema tipu tumora. Od 153 ultrazvučnih deskriptora, najče-

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šći su bili: tumor nepravilnog oblika (15,7%), lobularni tumor (7,2%), patološki kolor dopler signali (20,3%) i posteriorno slabljenje akustičnog signala (18,3%). BI-RADS kategorije 4 i 5 su definisane ultrazvukom kod 72,5% bolesnica, bez značajne razlike između pojedinih patohistoloških tipova karcinoma ($p = 0{,}109$). **Zaključak.** Standardna mamografija iz dva pravca i dopunske mamografske projekcije omogućavaju diferentovanje stelatne senke i definisanje BI-RADS kategorije 4 i 5 za tumore prepektoralne lokalizacije. Dodatnim ultrazvučnim pregledom prevazilazi se mimikrija u mamografskom ispoljavanju invazivnog lobularnog karcinoma.

Ključne reči:
dojka, neoplazme; mamografija; ultrasonografija; osetljivost i specifičnost.

Introduction

Cancer is one of the major public health problems. Based on the global cancer epidemiology data, there are 12.7 million new cancer cases estimated per year and 7.6 million deaths from cancer per year, with 28 million cancer survivors within five years from the initial diagnosis. According to the South Eastern European Research Oncology Group (SEEROG) data, the most frequent type of cancer in women in Serbia is breast cancer; age-standardized incidence rate is 57.9 and age-standardized mortality rate is 19.3 [1].

Mammography and breast ultrasonography have defined and different roles in diagnosis of palpable lesions and screening of occult cancer. Specific diagnostic challenges for breast imaging methods are designated as "difficult cases". The posterior breast tumors (also known as the prepectoral tumors) are the lesions located in the posterior aspect of breast, close to the anterior chest wall. As the consequence of deep localization within the breast and the morphology of anterior chest wall, the evaluation of posterior breast tumors with conventional diagnostic methods can be difficult: physical examination is a subjective method of limited sensitivity, while mammography often visualizes only part of the lesion, even in the cases of large, palpable tumors [2]. Owing to this distinctive anatomical localization, the posterior breast tumors belong to the group of difficult breast imaging cases.

The purpose of this study was to evaluate possibilities of diagnostic mammography and breast ultrasonography in detection and differentiation of posterior breast cancer.

Methods

A total of 40 women diagnosed with a first primary invasive breast cancer were included in the study. Inclusion criterion was a palpable breast mass, located in the posterior third of the breast at mammography (Figure 1), based on the definition of Breast Imaging Reporting and Data System (BI-RADS) lexicon [3].

The women ranged in age from 28 to 83 years (mean age, 60.2 years), and 26 were postmenopausal, and 14 premenopausal. In 23 women the masses were in the right breast and in 17 women in the left breast. The localization of masses was as follows: the upper outer quadrant in 33 cases, the upper inner and lower outer quadrant in 6 cases, equally, and the lower inner quadrant in one case. The mean mass size was 3.3 ± 1.4 cm. All the patients underwent standard two-view mammography (CC, cranio-caudal and MLO, medio-lateral oblique), either with the model SC Diagnost, Phillips mammography system (36 women, Clinical Hospital "Bežanijska kosa", Belgrade, Serbia), or with the model Selenia, Hologic mammography system (four women, Institute of Oncology and Radiology of Serbia, Belgrade, Serbia). Targeted breast ultrasonography (Hitachi EUB HV 7500) after mammography was performed using a high-frequency 13 MHz transducer in all patients in the Primary Health Care Center "Serbian Railways" Belgrade, Serbia. Histopathologic diagnoses of breast masses were 22 invasive ductal carcinomas (IDC), 13 invasive lobular carcinomas (ILC), four ductal/lobular carcinomas and one medullary carcinoma. After the surgery, the multifocality was confirmed in three patients. The infiltration of pectoralis major muscle was not found. To test the significance of differences, χ^2 test was used. Statistical significance was accepted at the level of $p < 0.05$.

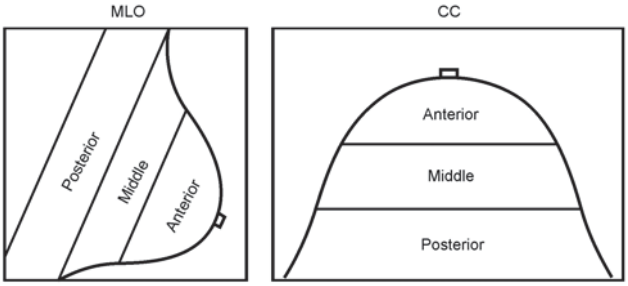

Fig. 1 – The depth of the lesion is assigned to anterior, middle, or posterior third of the breast and determined by two lines parallel to the edge of *pectoralis* major muscle at medio-lateral oblique (MLO) view, or to the edge of image at cranio-caudal (CC) view.

Results

Breast pattern at mammography was fatty (ACR1 and ACR2) in 32 cases (80.0%) and dense (ACR3 and ACR4) in 18 cases. Mammography detected the lesion at both, crenio-caudal (CC) and mediolateral oblique (MLO) views in 33 patients (82.0%), while in seven patients the lesion was detected at one standard mammographic view only. In these seven patients, the additional mammographic projections were performed in concordance with the lesion localization (Figure 2).

Based on standard MLO and CC projections 87.5% of the cases were classified as BI-RADS 4 and BI-RADS 5 categories, while in the conjunction with additional mam-

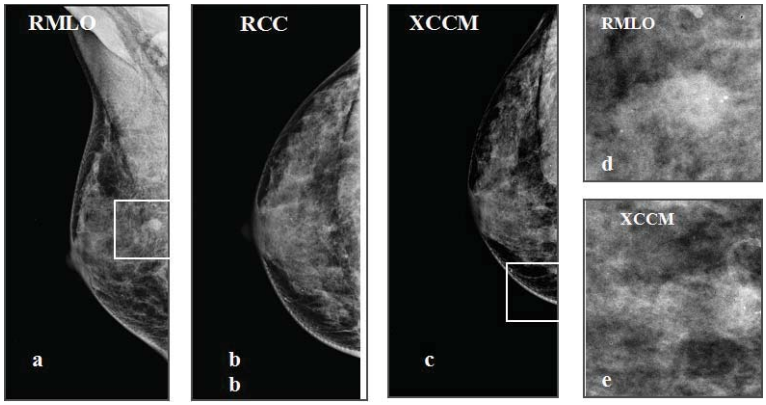

Fig. 2 – Work-up of a palpable, hard mass in the lower inner quadrant of the right breast. Spiculated mass with microcalcifications is visible in posterior third of the breast at RMLO view (a, d), and is not detectable at RCC view (b). Additional mammographic view (XCCM – medial extended cranio-caudal view) confirms the morfology and localization of the mass (c, e).

mographic projections all the cases were defined as BI-RADS 4 and BI-RADS 5 categories. A total number of mammographic BI-RADS descriptors were 96 (Table 1). Among them, the most frequent were: 23 of 96 (24.0%) spiculated masses, 16 (16.7%) architectural distortions, 12 (12.6%) clustered microcalcifications and 12 (12.6%) focal asymmetric densities.

The differentiation of the spiculated mass was significantly associated with the possibility to visualize the lesion at two-view mammography ($p = 0.009$), without the association with lesion diameter ($p = 0.083$) or histopathological type ($p = 0.055$). The mammographic features of ILC were significantly different from other histopathological types, due to the most frequent following signs: architectural distortion ($p = 0.003$) and focal asymmetric density ($p = 0.019$), as well as to the association of four or five subtle signs of malignancy, such as non-spiculated masses, architectural distortions and focal asymmetric densities ($p = 0.006$).

All the lesions were detectable by ultrasonography: the mass lesions in 33 of 40 (82.0%) patients and the non-mass, hypoechoic lesion in 7 patients. The mass lesions were present in all 22 cases of IDC and in 8 of 13 cases of ILC ($p = 0.010$). Ultrasonographic examination detected a total of 153 signs (Table 2). Predominant abnormal ultrasonographic findings included: 24 of 153 (15.7%) irregular masses, 11 (7.2%) lobulated masses, 31 (20.3%) lesions with abnormal

Table 1

Posterior breast cancer – mammographic findings according to the BI-RADS lexicon

Mammographic descriptors*	Number (n)	Percent (%)
Spiculated mass	23	24.0
Lobulated mass	7	7.3
Ill defined mass	6	6.2
Architectural distortion	16	16.7
Clustered microcalcifications	12	12.6
Focal asymmetric density	12	12.6
Skin retraction	6	6.2
Skin thickening	5	5.2
Increased vascular markings	3	3.1
Marked peripheral ducts	2	2.0
Nipple retraction	1	1.0
Pathological axillary lymph nodes	3	3.1

***Total number of descriptors in 40 patients (n = 96).**

Table 2

Posterior breast cancer – ultrasonographic findings according to the BI-RADS lexicon

Mammographic descriptors*	Number (n)	Percent (%)
Irregular mass	24	15.7
Lobulated mass	11	7.2
Oval-shaped mass	3	1.9
Non-mass lesion	7	4.7
Hyperechoic peripheral zone	10	6.5
Posterior acoustic attenuation	28	18.3
Absent/mixed posterior acoustic attenuation	12	7.8
Pathological vascularization	31	20.3
Pectoralis fascia discontinuity	8	5.2
Pathological axillary lymph nodes	19	12.4

***Total number of descriptors in 40 patients (n = 153).**

color Doppler signals, 28 (18.3%) lesions with posterior acoustic attenuation.

Using the combination of all ultrasonographic signs listed in Table 2, ultrasonographic BI-RADS 4 and BI-RADS 5 categories were defined in 29 of the 40 patients (72.5%), while for 11 patients BI-RADS classification was non-conclusive. Ultrasonographic BI-RADS classification did not differ among various histopathological types of breast cancer ($p = 0.109$). The frequency of mammographic and ultrasonographic BI-RADS 4 and BI-RADS 5 categories was significantly different ($p = 0.013$).

Additional mammographic projections are standardized and rely on the localization of tumor (Table 3, modified from Tucker AK [9]).

Table 3

Posterior breast tumors – recommendations for additional mammographic projections

Localization of the lesion at mammography	Additional projection
CC, lateral quadrants	XCCL, LO, L
CC, medial quadrants	XCCM, LO, L
MLO, axillary tail	XCCL, L
MLO, upper quadrants	XCCL, CC, L
MLO, central region	XCCL or XCCM
MLO, lower quadrants	XCCM, CC, L
MLO, inframammary sulcus	CC, L

CC – cranio-caudal; MLO – medio-lateral oblique; XCCL – lateral extended cranio-caudal, LO – lateral oblique, L – lateral, XCCM – medial extended cranio-caudal.

Discussion

We performed the analysis of mammographic and ultrasonographic features of palpable posterior breast cancers as a model of this distinctive cancer localization.

Localization of breast tumors in the posterior aspect of breast tissue, near the retromammary fat and the anterior chest wall, influences the line of pathophysiological, clinical and radiological specificities. According to the data published by Kopans [4] more than 70% of breast cancers develop in the parenchyma in the zone 1 cm wide, that lies immediately beneath the subcutaneous fat, or anterior to the retromammary fat. This is likely due to the fact that this is, geometrically, the volume where the most of breast tissue is found. Furthermore, fat acts as a reservoir for carcinogens, because of circulating androgens conversion to estrogens. Hence, there is the propensity for cancers to develop adjacent to these fat areas, including posterior breast tissue.

Brown et al. [5] confirmed this hypothesis in a clinical study of the 200 screening detected cancers, 354 true interval cancers and 122 false negative interval cancers. They concluded that the distribution of cancers across the breast is uneven, with clusters in the posterior breast tissue.

According to Tabar et al. [6] the majority of breast cancer will be found in one of the following four regions of the mammogram, so-called "forbidden areas" [5]. These regions require special attention of the radiologist and include: the area parallel with the edge of the pectoralis major muscle at the MLO view (a so-called "milky way"); the retroglandular, clear space at the CC view ("no man's land"); the medial half of the breast, best seen at the CC projection and the retroareolar area. In Tabar's classification, the terms "milky way" and "no man's land" belong to the localization of posterior breast tumors at mammography.

Our analysis of posterior breast tumors emphasizes the importance of additional mammographic projections. Additional projections provide increased diagnostic information on routine two-view examinations when the results are inconclusive, or when a lesion is seen only in one view. Some posterior breast tumors may be visible at one of standard views, or non-detectable, even in the case of clinically obvious mass. In these cases, further work-up and additional mammographic projections are necessary, with the goal to visualize the lesion on at least two mammographic views. In our study, the differentiation of a spiculated mass depended on the visualization of the lesion at two mammographic projections, without influence of tumor size and histopathological type. It is crucial to detect spiculated masses, since they have a much higher risk of malignancy than other, indirect signs of malignancy, such as non-spiculated masses, calcifications, architectural distortions or focal asymmetric densities [7]. According to that, after additional mammographic projections, we obtained the correction of BI-RADS 4 and BI-RADS 5 mammographic categories from initially 87.5% to 100%. In comparison to mammography, ultrasonographic BI-RADS 4 and BI-RADS 5 categories were defined in a significantly lower percentage (72.5%). Consequently, standard two-view mammography is not a *cul-de-sac* of breast radiological exploration. Therefore, the diagnostic mammography followed by additional mammographic projections appear superior to ultrasonography in differentiation of palpable posterior breast tumors [8, 9]. Nevertheless, the case-by-case, skillful and inventive approach to the selection of additional projections and patient positioning is imperative. This teamwork of radiologist and radiologic technologist in effort to solve the problem of posterior breast tumors detection and differentiation is an art of radiology.

The same principles of work-up are applicable to screening mammography. According to data of Majid et al. [10] even though mammography is the gold standard for the detection of occult breast carcinoma, 10–30% of breast cancers may be missed at screening mammography. Authors recommend a number of steps that will significantly enhance the accuracy of image interpretation at screening mammography: do not rely on standard views alone to diagnose a detected abnormality and complete the evaluation with additional

mammographic projections; review clinical data and use ultrasonography to help assess a palpable or mammographically detected mass; be strict about positioning and technical requirements to optimize image quality; be alert to subtle features of breast cancer; compare current images with multiple prior studies to look for subtle increases in lesion size; look for other lesions when one abnormality is seen; judge a lesion by its most malignant features.

Additional diagnostic challenge in the group of posterior breast tumors is ILC, because of the subtle and atypical mammographic and ultrasonographic features. ILC is the second most common breast malignancy after IDC [11]. ILC is derived from small, uniform tumor cells with round nuclei and narrow cytoplasm. ILC infiltrates the stroma in single-file cell strands along ductuli (a so-called "Indian-file" pattern) and has a tendency to spread diffusely or between the collagen fibers of the breast, without a significant desmoplastic reaction. The explanation of these pathological features at molecular level is the absence of a cell adhesion molecule, E-cadherin, in 84%–100% of ILCs [12]. Characteristic pathological growth pattern of infiltrative linear columns of discohesive cells, rather than the discrete mass of cohesive cell, influences the appearance of ILC at imaging, with often reduced conspicuity at both mammography and ultrasonography [13]. In our group of patients the indirect mammographic signs of malignancy as well as the association of 4 or 5 indirect mammographic signs more frequently appeared in cases of ILC than in other histopathological types of cancer. We also found nonspecific, focal hypoechoic areas to be a more frequent ultrasonographic feature of ILC than IDC. Owing to the combination of numerous ultrasonographic BI-RADS lexicon descriptors, ultrasonographic BI-RADS classification of ILC and IDC was not significantly different. According to the references, the sensitivity of ultrasonography for ILC detection ranges from 68% to 85.7% and the additional use of ultrasonography increases the overall ILC detection [14].

Discontinuity of pectoralis major muscle fascia was detected by ultrasonography in 8 patients. This finding, suggestive of pectoralis major muscle infiltration, was not confirmed after the surgery. According to literature data, a dynamic contrast-enhanced magnetic resonance imaging (MRI) has specific advantage in detection of the posterior breast tumor extension in underlying musculature [15]. Highly suspicious MRI sign of pectoralis muscle involvement is an abnormal enhancement in these structures, while violation of the fat plane between the tumor and the muscle, without other findings, does not indicate tumor involvement of these deep structures. The routine use of breast MRI should be considered for preoperative surgical planning in women with posterior breast tumors [2].

Conclusion

Our study shows that diagnostic mammography and breast ultrasonography offer specific benefits in the evaluation of palpable posterior tumors. Standard two-view mammography followed by additional mammographic projections is an effective way to demonstrate the spiculated mass and to classify the lesion as category BI-RADS 4 or BI-RADS 5. Meticulous ultrasonographic examination using multiple descriptors can overcome the mimicry of invasive lobular breast carcinoma at mammography.

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Causes of rhabdomyolysis in acute poisonings

Uzročnici rabdomiolize u akutnim trovanjima

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Abstract

Background/Aim. Rhabdomyolysis (RM) is potentially lethal syndrome, but there are no enough published data on its frequency and characteristics in acute poisonings. The aim of this study was to determine the causes and severity of RM in acute poisonings. **Methods.** Patients hospital charts were retrospectively screened during a one-year period in order to identify patients with RM among 656 patients treated due to acute poisonings with different agents. All the patients with RM were selected. Entrance criterion was the value of creatine kinase (CK) over 250 U/L. The severity of RM was assessed according to the Poison Severity Score. The patients were divided into three groups: the first one with mild RM (CK from 250 to 1,500 U/L), the second with moderate RM (CK from 1,500 to 10,000 U/L) and the third with severe RM (CK greater than 10,000 U/L). **Results.** RM occurred in 125 (19%) of the patients with acute poisonings. It was mainly mild (61%), or moderate (36%), and only in 3% of the patients was severe RM. The incidence of RM was the highest in poisonings with opiates (41%), pesticides (38%), neuroleptics (26%), anticonvulsants (26%), ethyl alcohol (20%), and gases (19%). Psychotropic agents were the most common causes of poisoning, and consequently of RM. Fatal outcomes were registered in 32 (25.60%) of all RM patients. The incidence of fatal outcomes in poisonings with mild, moderate and severe RM was 19.73%, 31.11% and 75%, respectively. **Conclusion.** RM syndrome occurs at a relatively high rate in acute poisonings. Although agent's toxicity is crucial for the outcome, severe RM and its complications may significantly influence the clinical course and prognosis of poisoning. Routine analysis of CK, as a relevant marker for RM may indicate the development of RM in acute poisoning and initiate prompt therapeutic measures in preventing acute renal failure as the most frequent consequence of extensive rhabdomyolysis.

Key words:
rhabdomyolysis; poisoning; creatine kinase; diagnosis; pharmaceutical preparations; opiate alkaloids; pesticides; coma.

Apstrakt

Uvod/Cilj. Rabdomioliza (RM) predstavlja potencijalno letalan sindrom, o čijoj učestalosti i karakteristikama u akutnim trovanjima nema mnogo podataka. Cilj rada bio je da se odrede uzročnici i težina RM u ovim stanjima. **Metode.** Retrospektivno su analizirane istorije bolesti 656 bolnički lečenih bolesnika zbog akutnog trovanja različitim agensima tokom jedne godine. Izdvojeni su bolesnici sa RM, a kriterijum je bio da su imali aktivnost kreatin kinaze (CK) u serumu višu od 250 U/L. Težina RM procenjivana je na osnovu skale težine trovanja (PSS). Bolesnici su bili podeljeni u tri grupe: prvu grupu sa blagom RM (CK od 250 do 1 500 U/L), drugu grupu sa srednje teškom RM (CK od 1 500 do 10 000 U/L) i treću grupu sa teškom RM (CK viša od 10 000 U/L). **Rezultati.** RM je nađena kod 125 (19%) bolesnika sa akutnim trovanjima, pri čemu je uglavnom bila blaga (61%) ili umerena (36%), a samo kod 3% bolesnika teška. Učestalost pojave RM bila je najveća kod akutnih trovanja opijatima (41%), pesticidima (38%), neurolepticima (26%), antikonvulzivima (26%), etil alkoholom (20%) i gasovima (19%). Psihotropni lekovi bili su najzastupljeniji uzročnici trovanja, a samim tim i RM. Smrtni ishod je zabeležen kod 25,6% bolesnika sa RM, pri čemu je letalitet iznosio 19,73% u trovanjima sa blagom RM, 31,11% sa srednje teškom i 75% u trovanjima sa teškom RM. **Zaključak.** Sindrom RM pojavljuje se relativno često u akutnim trovanjima. Za ishod trovanja od presudnog značaja je toksični agens, ali teška RM i njene komplikacije mogu značajno da utiču na tok i prognozu trovanja. Rutinska analiza CK, kao relevantnog pokazatelja RM kod akutnih trovanja, može ukazati na razvoj ovog sindroma i doprineti pravovremenom preduzimanju terapijskih mera za sprečavanje nastanka akutne bubrežne insuficijencije kao najčešće posledice ekstenzivne RM.

Ključne reči:
rabdomioliza; trovanje; kreatin kinaza; dijagnoza; lekovi; narkotici; pesticidi; koma.

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Introduction

Rhabdomyolysis (RM) is a common and potentially lethal clinical syndrome that results from acute necrosis of myocytes and fibber content releasing into circulation [1]. The first description of RM originates from the Bible – an episode of mass poisoning of Jews who ate quail during their journey from Egypt. Hemlock was eaten by quail, and the poison from this plant, cicutoxin, causes convulsions and RM followed by acute renal failure (ARF) in humans [2]. However, the true significance of RM was first pointed out by Bywaters and Beall [3], who described ARF in crush syndrome among the victims of bombing of London in World War II. The effects of RM on human health and finances can be illustrated by the fact that approximately 26,000 cases of RM have been reported during only one year in the United States [4].

RM occurs in a variety of diseases and disorders, including toxic effect of chemicals, malignant hyperthermia, drug-induced polymyositis and dermatomyositis, muscle ischemia (crush syndrome, compartment syndrome, shock and coma, occlusive arterial disease), extensive muscle stress (marathon, military training, epileptic status, prolonged myoclonus or dystonia, agitation, delirium), the influence of physical factors (heat shock, burns), viral and bacterial infections, water-salt balance disorders (hypokalemia, hyponatremia, hypophosphatemia), hyperosmolar states and endocrine dysfunctions, some genetic diseases, and neuropathies (polyneuropathy, central motor neuron disease) [5–7]. RM can be classified as traumatic and nontraumatic. The most frequent causes of RM are crush syndrome, strenuous exercise, alcohol abuse and some drugs and toxic substances [8]. Therefore, RM can be expected as common in toxicological practice.

In acute poisoning, RM can be the result of multiple causing mechanisms [9]. For instance, alcohol, cocaine and possibly heroin act directly myotoxically [10]. This mechanism should be distinguished from RM that develops secondarily, due to muscle ischemia during seizures or local muscle compression in comatose states [11]. An increasing energy consumption can contribute to RM in some cases like hyperpyrexia in cocaine-induced RM.

Classic symptoms of RM involve the presence of a muscle pain, weakness, and red-to-brown urine. However, there are variations in the clinical presentation of the disease, so the classic triad occurs in only about 10% of patients. Gabow et al. [12] showed that as much as 50% of patients with RM had no muscle pain and only 5% of patients had verified muscle swelling on admission. Therefore, the absence of muscle symptoms and signs on admission does not exclude the diagnosis of RM. In a great number of cases, the clinical picture of RM is covered by the clinical picture of acute poisoning and the diagnosis of RM can easily be missed. To make the diagnosis of RM is more difficult in patients with altered states of consciousness, who cannot point to their problems. For the diagnosis of RM the coma itself may be more important than the cause of coma [13]. Laboratory tests that confirm the presence of RM include elevated creatine kinase (CK) in blood (normally 24 U/L – 195 U/L)), typically more than 5 times higher than the upper limit of normal, as well as the presence of myoglobin in urine [14, 15]. However, some authors [16] state lower levels of CK (etc. > 500 U/L) as appropriate for the diagnosis of RM. RM cannot be definitely excluded on the basis of negative myoglobin in urine because it has a very short half-life (2–3 hours) [17].

Major complications of RM are ARF, compartment syndrome, arrhythmias and cardiac arrest, disseminated intravascular coagulation, hepatic dysfunction [18]. An aggressive rehydration is considered to be the standard therapeutic measure in preventing ARF in patients with RM. The role of mannitol and bicarbonate is controversial [18, 19].

The aim of this study was to determine the causes and the severity of RM in acute poisonings.

Methods

Hospital charts of patients were retrospectively screened during a one-year period in order to identify patients with RM among 656 patients treated due to acute poisonings with different agents in the Clinic of Emergency and Clinical Toxicology, Military Medical Academy, Belgrade. We selected all patients with elevated CK (higher than 250 U/L). In poisoning caused by multiple agents, the leading agent was declared as a cause of RM. The severity of RM was assessed according to the Poison Severity Score [20]. The patients were divided into 3 groups: with mild RM (mild pain and tenderness, CK level from 250 U/L to 1,500 U/L); with moderate RM (pain, rigidity, cramping and fasciculation, CK level from 1,500 to 10,000 U/L) and with severe RM (intense pain, extreme rigidity, extensive cramping and fasciculation, RM with complications, CK greater than 10,000 U/L, compartment syndrome). In assessing the increase of CK, the maximum value of each patient during the hospital stay was taken into account.

Statistical analysis

The results of variables are expressed as the mean value ± standard deviation or as the frequency (%) from groups total. In order to determine the difference in frequency of appearance of RM in various types of poisoning, $\chi 2$ test was performed. Differences were considered to be significantly important if the null hypothesis could be rejected with > 95% confidence. The SPSS 17.0 statistical software package was used for all calculations.

Results

Elevated level of CK was registered in 125 (19%) among 656 patients hospitalized due to acute poisonings. There were 52.8% males and 47.2% females with the mean age of 40.84 ± 7.53 years. The demographic characteristics of RM patients according to causative agents are shown in Table 1.

Severity of rhabdomyolysis

The peak CK values in poisonings caused by different toxic agents are shown in Table 2. In relation to the severity of RM (according to PSS), considering CK as a basic pa-

Table 1

Demographic characteristics of the rhabdomyolysis (RM) patients according to causative toxic agents

Agents of poisoning	Age (years)		Gender	Total patients
	$\bar{x} \pm SD$	Range	m/f, n (%)	(n)
Psychoactive drugs				
benzodiazepines	47.3 ± 16.0	19–74	7/12 (36.8/63.2)	19
neuroleptics	34.9 ± 10.5	22–55	9/9 (50.0/50.0)	18
anticonvulsants	37.2 ± 13.8	15–64	8/8 (50.0/50.0)	16
antidepressants	46.7 ± 11.9	29–54	2/2 (50.0/50.0)	4
antiparkinsons	39.0 ± 0.0	39–39	0/1 (0.0/100.0)	1
Other drugs	25.2 ± 12.3	15–43	3/1 (75.0/25.0)	4
Pesticides	46.8 ± 18.4	23–79	8/12 (40.0/60.0)	20
Corrosives	59.0 ± 17.1	26–78	4/10 (28.6/71.4)	14
Opiates	24.2 ± 7.5	17–47	11/3 (78.6/21.4)	14
Ethyl alcohol	29.6 ± 22.1	19–69	5/0 (100.0/0.0)	5
Mushrooms	50.2 ± 17.9	21–68	4/1 (80.0/20.0)	5
Gases	31.0 ± 6.4	22–37	5/0 (100.0/0.0)	5
All groups	40.8 ± 17.5	15–79	66/59	125

$\bar{x}$ – mean; SD – standard deviation; m-male; f-female; n – number of patients.

Table 2

Creatine kinase (CK) serum level in the rhabdomyolysis patients in acute poisonings

Agents of poisoning	CK (U/L)	
	$\bar{x} \pm SD$	Range
Psychoactive drugs		
benzodiazepines	2151.3 ± 2968.1	261–10608
neuroleptics	3627.4 ± 2973.9	302–11420
anticonvulsants	1249.4 ± 1124.9	315–3602
antidepressants	2382.0 ± 2670.9	347–6270
antiparkinsons	865.0 ± 0.0	865–865
Other drugs	2880.2 ± 2435.6	362–5986
Pesticides	1633.9 ± 1811.7	276–7314
Corrosives	907.8 ± 793.7	258–2843
Opiates	3115.4 ± 3949.5	277–10306
Ethyl alcohol	888.6 ± 1022.3	275–2665
Mushrooms	1460.8 ± 1593.6	447–4160
Gases	2516.8 ± 3229.4	520–8180
All groups	2091.3 ± 2545.2	258–11420

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

rameter, RM was classified as mild, moderate and severe, including 76 (61%), 45 (36%) and 4 (3%) of the patients, respectively. The severity on RM depending on the causes of acute poisonings is shown in Table 3.

Mild rhabdomyolysis

In the group of 76 patients with mild RM, the most common toxic agents were drugs – in 33 of the patients. Among the drugs, specific agents included benzodiazepines (diazepam, midazolam, bromazepam, lorazepam and prazepam), anticonvulsants (carbamazepine and phenobarbitone), neuroleptics (haloperidol, chlorpromazine, thioridazine), antidepressant maprotiline, antiparkinson biperiden and antihypertensive drug moxonidine.

Mild RM was caused by pesticides in 12 of the patients. Organophosphorus insecticides (OPI) malathion, diazinone and dimethoate were the causes of RM in 10 patiens. Paraquat and amitraz were toxic agents in single cases.

Ingestion of corrosive agents, hydrochloric or acetic acid, caused mild RM in 12 of the patients. Among the agents producing mild RM were heroin (9 of the patients) and ethyl alcohol (4 of the patients). Mushrooms and gases from fire caused RM each per 3 of the patients.

Moderate rhabdomyolysis

Drugs (27 of the patients) and pesticides (8 of the patients) were the most common causes of poisonings in the group of 45 patients with moderate RM. Neuroleptics (chlorpromazine - 4, thioridazine - 3, haloperidol - 2, clozapine – 2 and fluphenazine 1) were the most frequent among the drugs – in 12 of the patients; then benzodiazepines (diazepam – 4, bromazepam 1) and anticonvulsants (carbamazepine – 4, phenobarbitone 1) each per 5 of the patients. Antidepressants (amitriptyline) caused moderate RM in 2 of the patients. Single cases with beta blocker (propranolol), antihistamine (promethasine) and anticholinergic agent (atropine) poisoning were recorded. Among pesticides, the most common causes were OPI with malathion in 4 of the patients, and diazinone in 1 of the patient. Single cases of poisoning with dinitro-ortho-cresol, paraquat and an unidentified organochlorine pesticide were found.

Table 3

Severity of rhabdomyolysis (RM) in relation to the causes of acute poisonings

Agents of poisoning	Severity of RM, n (%)			Total patients
	Mild	Moderate	Severe	(n)
Psychoactive drugs				
benzodiazepines	13 (68.4)	5 (26.3)	1 (5.3)	19
neuroleptics	5 (27.8)	12 (66.7)	1 (5.6)	18
anticonvulsants	11 (68.7)	5 (31.2)	0 (0.00)	16
antidepressants	2 (50.0)	2 (50.0)	0 (0.0)	4
antiparkinsons	1 (100.0)	0 (0.0)	0 (0.0)	1
Other drugs	1 (25.0)	3 (75.0)	0 (0.0)	4
Pesticides	12 (60.0)	8 (40.0)	0 (0.0)	20
Corrosives	12 (85.7)	2 (14.3)	0 (0.0)	14
Opiates	9 (64.3)	3 (21.4)	2 (14.3)	14
Ethyl alcohol	4 (80.0)	1 (20.0)	0 (0.0)	5
Mushrooms	3 (60.0)	2 (40.0)	0 (0.0)	5
Gases	3 (60.0)	2 (40.0)	0 (0.0)	5
All groups	76	45	4	125

n – number of patients.

The rest of the causes of moderate RM were opiates (heroine) – 3 of the patients, corrosives (acetic acid) 2 of the patients, *Amanita phaloides* 2 of the patients, gases (carbon monoxide and intoxication from inhaled fumes in fire – each per one of the patients), and ethyl alcohol –1 patient.

Severe rhabdomyolysis

In four cases of severe RM, heroin overdose and combined drug poisonings were present, each per two patients. In one drug-overdosed patient, the causes of poisoning were benzodiazepines diazepam and alprazolam, but the clinical course included prolonged coma before admission, pneumonia and sepsis with multiorgan failure. The second case was multi - drug poisoning by neuroleptics (chlorpromazine and haloperidol), antiparkinson drug (trihexyphenidyl) and anticonvulsant drug (carbamazepine).

The frequency of rhabdomyolysis

A total of 370 acute drug poisonings were registered in the observed period; 301 were caused by psychotropic drugs and 69 by the other drugs. Overall, psychotropic drugs were the most common causes of RM. RM was present in 58 (19.27%) of the cases out of all psychotropic drug poisonings. Out of a totally 73 acute pesticides poisonings, RM was registered in 20 (27.39%). RM was recorded in 14 (18.42%) out of 76 patients with corrosive ingestion and in 14 (41.17%) among 34 opiate overdosed patients. Out of 25 patients hospitalized due to acute ethyl alcohol intoxication, 5 (20%) had RM. RM occurred in 5 (18%) out of 28 mushrooms poisoning. Out of totally 27 patients hospitalized for acute poisoning with harmful gases, RM was noted in 5 (18.51%). Two patients had carbon monoxide or toxic fumes in fire as the causative agent, while a single had chlorine.

The highest frequency of RM was found in patients poisoned with opiates, then pesticides, neuroleptics and anticonvulsants (Figure 1).

There was a significantly higher rate of RM occurrence in patients with acute opiate poisoning than in patients poisoned by corrosives, benzodiazepines and antidepressants (Table 4). The frequency of RM was significantly higher in patients with pesticide poisoning in relation to the corrosives, benzodiazepines and antidepressants. Among the other analysed agents, there were no significant differences in RM incidence.

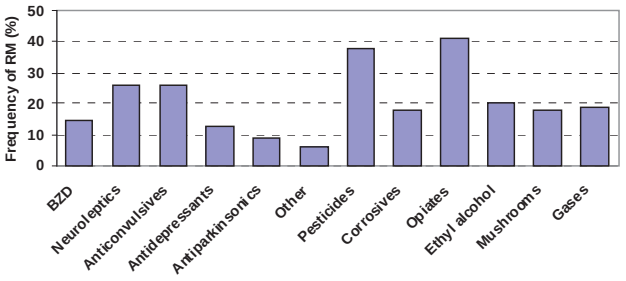

Fig. 1 – The frequency of rhabdomyolysis (RM) in various types of poisonings.
BZD – benzodiazepines.

Table 4

Most common causes of rhabdomyolyses (mutual comparison)

Agents of poisoning	Opiates (χ^2 value)	*p*
Antidepressants	5.46	< 0.05
Benzodiazepines	10.39	< 0.05
Corrosives	5.26	< 0.05
	Pesticides (χ^2 value)	
Corrosives	5.37	< 0.05
Benzodiazepines	11.35	< 0.001
Antidepressants	5.33	< 0.05

We also analysed CK values in the patients with disorder of consciousness in terms of coma and in those with increased motor activity in terms of agitation. There were 47 (37.6%) patients with coma, 12 (9.6%) agitated patients, while the rest, 66 (52.8%), were conscious or with mild level of CNS depression (drowsiness).

Clinical course and outcome of rhabdomyolysis

A total of 21 (16.8%) of the patients with RM developed ARF. In cases with mildly and moderately elevated CK, nephrotoxicity of agents such as acetic acid, *Amanita*

phalloides and paraquat, and sepsis or shock caused ARF rather than RM. Out of 4 cases with severe RM, ARF developed in 2, both with typical acute tubular necrosis caused by heroin overdose.

Fatal outcomes were registered in 32 (25.60%) of all the RM patients. The incidence of fatal outcomes in poisonings with mild, moderate and severe RM was 19.73%, 31.11% and 75%, respectively.

The causes of death in patients with mild or moderate RM were corrosives, pesticides, drugs, opiates and *Amanita phalloides*. Fatal outcome was the consequence of toxic effects typical for these agents, and severe disturbances such as shock, gastrointestinal bleeding, respiratory or hepatic failure. RM was just a sign that had no significant effect on the outcome. In the group of 4 patients with severe RM, fatal outcome developed in 3 patients. The causes of death were heroin (in 2 of the patients), and complications (pneumonia, sepsis, shock) of poisoning with benzodiazepines in one patient.

Discussion

In this study CK was chosen for assessment of RM severity in acute poisonings because it is a reliable biological parameter. High CK concentrations suggest the presence and damage of myocytes in a proper way [21]. CK is slowly and totally degraded and removed from circulation, so concentrations in serum remain elevated much longer than myoglobin concentration in urine.

Data on the incidence of RM in acute poisonings are unreliable, as it often goes unnoticed [22]. CK elevation was noted in many of our patients – even 19% of the total number of those hospitalized for acute poisoning. Because of predominant clinical manifestations of acute poisonings and without the presence of the usual characteristics of RM, its subclinical course may be overlooked [17]. According to Elzadi-Mool et al. [23], the most frequent grade of RM in patients poisoned with various agents presenting in coma, was the moderate one (55%). Our observation included all poisoned patients, and in the majority of patients with RM, it was mild (60.80%). This disorder was diagnosed only based on biochemical indicator (CK), without clinical signs and symptoms. It was easier to recognize the cases of moderate to severe RM, which were manifested by clinical disorders (pain in the muscles, rigidity or swelling, changes in colour and the amount of urine, the development of renal failure). In such cases, RM contributed to the severity of poisoning.

More than 150 medications and toxins have been described as a cause of RM [1], but drugs and alcohol are the most common [24]. Underlying mechanisms are different, such as immobilization, increased psychomotor activity, or direct toxic effects [25, 26] which may act via altering myocyte function by inhibition of calcium metabolism, due to impairment of adenosine phosphate production, or alterations in carbohydrate metabolism.

Psychotropic drugs were the most prevalent causes of poisoning, and consequently of RM, in our study, as well as in a report of Mousavi et al. [27]. Though the incidence of RM in acute benzodiazepine poisonings is not high (14.5%) benzodiazepines were the most frequent cause of RM in our patients, simply because they were the most common cause of poisoning. These drugs were involved in 13 patients with mild, 5 with moderate, and 1 with severe RM and fatal outcome due to septic and cardiocirculatory complications. Benzodiazepines primarily cause RM due to secondary mechanism, mainly because of local muscle compression and ischemia during prolonged immobilization in prolonged consciousness depression.

Neuroleptics were the second among the drugs causing RM in our patients. Considering the incidence (26%) and severity (mainly moderate to severe), RM was the most pronounced in poisonings with these prescription drugs. Phenothiazines haloperidol and flufenazin, were the causes of neuroleptic malignant syndrome (NMS) in one patient, with agitation, hyperthermia and fatal outcome.

RM is a manifestation, as well as one of diagnostic criteria for NMS. It could be subclinical, only with increased activity of CK, or could cause massive myoglobinuria and ARF. However, haloperidol can cause RM even without NMS [28].

Convulsions caused by cyclic antidepressants are very frequent causes of RM [27]. In this study, maprotiline and amitriptyline caused mild to moderate RM in 4 of the patients in the absence of manifested convulsions.

In this study, RM developed most frequently (41%) in opiates overdoses. Heroin was the most common opiate. Two of heroin overdosed patients (one combined with "ecstasy") with severe RM developed ARF and other complications resulting in fatal outcomes. Severe RM is reported as very frequent complication of opiate intoxication – even in 22 of 188 consecutive patients in a study of Larpin et al. [29]. Except for heroin, RM with extremely high CK level, up to 100,000 U/L, or higher, may occur in other opiates overdoses, like methadone or morphine [30–33]. Opiate-induced RM ensued secondary, by muscle compression in coma and consciousness disorders in general, but a short period before severe RM manifestation indicates that direct myotoxic effect probably has the most important role in these cases [34].

The patient who except for heroin allegedly ingested only two "ecstasy" tablets developed severe clinical picture including also fulminate hyperthermia. Very serious complications after ingestion of relatively small amounts of 3,4-methylenedioxymethamphetamine (MDMA) may indicate to a direct pharmacological interaction effects (disturbances that are characteristic of serotonin syndrome) and individual susceptibility [35, 36].

Even severe acute poisonings with ethyl alcohol rarely need admitting to the hospital or do not require long hospitalization [37]. In our series of 5 patients which had to be admitted for hospital treatment due to prolonged coma or complications like aspiration pneumonia, all had mildly to moderately elevated CK.

Underlying mechanisms of RM caused by ethyl alcohol include a combination of ischemia due to immobilization, or agitation and other movement disorders, hypokalemia, hypophosphatemia and direct myotoxicity [26]. Different levels of

CK in RM due to acute ethyl alcohol intoxications can be found in data published [38] and except for cases of heavy ethyl alcohol abuses with coma, muscular swelling, myoglobinuria and ARF, there are also reports on chronic alcoholics with a high level of CK [39, 40] not connected with compression and ischemia.

RM is not rare in acute pesticide poisonings [41]. We noted elevated CK in 38% of the patients admitted due to pesticide ingestion. Acute poisonings with OPI were most frequent in our patients. Their manifestations include coma and/or convulsions as a central toxic phenomena. OPI also lead to disturbances in the neuromuscular junction, causing muscle fasciculation and fibrillation, which is another cause of RM in poisoning with these substances. However, only a few cases of OPI poisoning complicated by severe RM have been reported [42, 43]. We noted mild RM in one patient with paraquat poisoning and moderate RM in paraquat and dinitro-orthocresol poisonings, per one patient each. In addition to the effects on the kidney, liver, adrenal, and, in the case of paraquat, the subsequent effects on the lungs, dipyridyl compounds paraquat and diquat lead to local caustic action on the exposed skin and mucous membranes. However, Park et al. [41] reported that among 1,420 patients with acute paraquat intoxication, none had rhabdomyolysis. Dinitrophenol ingestion leads to disruption of the process of oxidative metabolism and overproduction of heat to which the CNS is particularly sensitive. For this reasons, tonic-clonic convulsions and coma quickly perform as severe poisoning manifestations [44].

We noticed elevated CK, mainly of mild level, in acute poisonings with corrosive substances. This is probably due to the releasing of this enzyme from the damaged muscle of the digestive tract, and restlessness of these patients.

In this study, mild to moderate elevation of CK was recorded in 5/28 patients with clinical picture of poisoning by hepatotoxic mushrooms, probably *Amanita phaloides*. Some humans edible wild mushrooms like *Russula subnigricans* can cause severe RM due to mycotoxin effects [45]. Mushrooms *Tricholoma equestre or Tricholoma flavovirens* can cause RM, even with fatal outcomes [46, 47]. *Amanita phaloides* is not reported to be myotoxic, so elevation of CK in blood may be due to co-ingestion of other mushrooms or the consequence of severe clinical disturbances including hypotension and prolonged inactivity.

RM due to carbon monoxide poisoning is not reported frequently [48, 49] though hypoxia causes cerebral and muscle metabolism disorders, manifesting as coma and convulsions in severe cases. In addition to myocardial necrosis, a manifestation of poisoning is necrosis of skeletal muscle. One patient from our series had mildly elevated CK, with MB fraction within normal value.

Burns and heat stroke are known physical factors that induce RM [6]. Because of their thermal effect, fumes in fire lead to burn of the upper respiratory tract mucosa, and therefore may cause RM, like in our patients. In such cases, one should bear in mind an increased muscle activity of the participants in fire.

Conclusion

In this study, RM occurred at a relatively high incidence in acute poisonings – in even 19% of all the cases. The majority of the patients had mild or moderate RM, while severe RM occurred in only 0.6% of the total number of patients. Psychotropic drugs were the most frequent causative agents of poisoning, and therefore of RM. Among the prescription drugs, we noticed the highest incidence and severity of RM in poisoning with neuroleptis. Though limited by a small number of patients, the results of this study show the highest frequency of RM in opiate (heroin) and OPI poisonings. Although agent's toxicity is crucial for the outcome, severe RM and its complications may significantly influence the clinical course and prognosis of poisoning. Routine analysis of CK, which is a relevant marker for RM, may indicate the development of RM in acute poisoning and initiate prompt therapeutic measures for preventing ARF as the most frequent consequence of extensive RM.

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Adaptive mechanisms of mitochondria in response to exercise

Adaptivni mehaniyam mitohondija kod napornog vežbanja

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Ključne reči:
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Introduction

It is known that mitochondria are unique organelles capable of, depending on the physiological stimuli, changing their number and size [1, 2]. Physical exercise has proved to be a powerful stimulus to mitochondrial biogenesis in skeletal muscle, which involves the orchestrated expression of the mitochondrial genome and the nuclear genes that encode mitochondrial proteins [3]. The human mitochondrial genome consists of approximately 1,500 genes, 37 are encoded mitochondrial DNA (mtDNA), and the rest of the nuclear DNA (nDNK). Mitochondrial DNA (mtDNA) is a small double-stranded circular molecule containing 16,569 pairs of nucleotides. It encodes 13 subunits of complexes involved in oxidative phosphorylation, and components necessary for its own mRNA translation: large and small rRNA and 22 tRNA. The process of oxidative phosphorylation (OXPHOS) is necessary for formation of ATP, which is used for work, heat to maintain body temperature and membrane potential required for ion transport. Mitochondria also generate most of the reactive oxygen species (ROS) and electrons involved in their formation are usually derived from the reduced electron carriers of the respiratory chain. If not neutralized (damaged mitochondria are removed by apoptosis), ROS can damage mitochondrial proteins, lipids and nucleic acids which inhibit oxidative phosphorylation [4, 5]. A large number of disorders of oxidative phosphorylation are attributed to mutations, which are more common in mitochondrial DNA (mtDNA) than in the DNA of chromosomes. These mutations are inherited maternally. However, not all mtDNA mutations and variations are deleterious. About 25% of all mtDNA variations are referred to as adaptive, and in some cases may be an important factor in the individual's predisposition to a better physical condition [6].

This paper is an overview of recent research mitochondrial biogenesis and its adaptive effects to the potential impact of an increase in athlete s endurance.

Exercise-induced mitochondrial biogenesis in skeletal muscle

Skeletal muscles show significant metabolic and morphological adaptations in response to a number of physiological and pathophysiological conditions. One of the major phenotypic changes occurs in mitochondria in response to exercise or chronic contractile activity. In fact, intense exercise leads to significant metabolic changes that may impair mitochondrial function: the formation of reactive oxygen species due to higher rates of oxygen uptake during intense work [7, 8], hydrolysis of creatine phosphate leads to elevated levels of phosphate, which may affect the permeability of mitochondria, increased Ca^{2+} activates pyruvate dehydrogenase, alpha (α)-ketoglutarate dehydrogenase and NAD-linked isocitrate dehydrogenase, and the maximum permeability of the pores can lead to swelling and rupture of the outer membrane of mitochondria leading to autophagia of mitochondria and apoptosis or necrosis of the cells [9]. This distortion function of mitochondria in strenuous exercise can cause not only fatigue, but muscle damage. Just under these physiological conditions highly dynamic structure of mitochondria and the appearance of mitochondrial adaptation are expressed. Exercise not only increases mitochondrial ATP synthesis through oxidative phosphorylation but also affects its morphology, increased gene expression of enzymes and proteins and changes the dynamics of fusion and fission, opposing processes that are in balance and are responsible for remodeling mitochondrial network [10, 11]. These adaptation changes are most noticeable in low-oxidative white muscle

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fibers, whose initial mitochondrial content ranging from 1–3% of the total cellular volume [12].

Mitochondrial adaptations in muscle are highly specific and dependent on the type of exercise, its frequency, intensity and duration. Prolonged and strenuous training can produce an increase in mitochondrial content of 50–100% for a period of 6 weeks [13]. Experiments in animal models (8 weeks of training on the treadmill, 80% VO_2, 5 days per week) showed an increased mitochondrial function, reflected by an increased activity of mitochondrial enzymes and the maximum speed of ATP synthesis in isolated mitochondria [14]. The physiological meaning of mitochondrial adaptation in muscle is reflected in metabolic changes, which are expressed more in the metabolism of lipids compared to carbohydrates. For example, the formation of lactic acid is reduced, glycogen loss is smaller, the utilization of high-energy phosphates is reduced, as well as muscle fatigue [15]. These mitochondrial adaptations in response to exercise are generally referred to as mitochondrial biogenesis, as a synonym for metabolic plasticity. It is a complex process that involves increasing in the mitochondrial content per gram of tissue and changes in the mitochondrial composition, with an alteration in mitochondrial protein-to-lipid ratio. This sequence of molecular events that initiate mitochondrial biogenesis begins with an increase in intracellular Ca^{2+}, which is a mediator of interaction actin and myosin, which then activates the kinase, for example, Ca^{2+} calmodulin kinase (CaMK) and phosphatase, which trigger a signaling cascade and increase gene expression of transcription factors. Specifically, muscle contraction leads to an increase in the maximum capacity of muscle to generate ATP *via* oxidative phosphorylation. Repeated muscle contractions lead to reducing the concentration of ATP and increasing the concentration of free ADP, thus causing activation of creatine phosphokinase (CPK), formation of ATP and creatine. ADP is also a substrate and allosteric activator of the glycolytic pathway and control mitochondrial respiration. These adaptations, along with increased activities of mitochondrial β-oxidation enzymes, lead to a greater lipid and less carbohydrate oxidation during exercise and enhance endurance performance. As a result of increased mitochondria, oxygen consumption and ATP production per mitochondrion are less at the same submaximal work rate in trained compared to untrained muscle. This means that with more mitochondrial respiratory chains, the rate of electron transport per respiratory chain will be "turned on" to a lower level to achieve the same rates of oxygen utilization and ATP production per gram of muscle at the same work rate in the trained compared to the untrained state. Consequently, the concentration of ATP and PC decreases less, and ADP, AMP and inorganic P increase to lower "steady state" levels, while glycogenolysis and glycolysis are turned on to a lower degree in the trained compared to the untrained state in response to the same submaximal work [16].

The literature supports the fact that adaptive responses to exercise are manifested during the recovery phase that follows the exercise period [17]. This happens because stoppage the exercise, reduces the energy required for the processes such as gene expression and protein synthesis from serving contractile activity purposes to those that are more anabolic. Holoszy and Winder [18] showed that Δ-aminolevulinic acid synthase (ALAs), enzyme involved in determining the functional content of mitochondrial cytochromes of respiratory chain, was increased several hours after the exercise bout. Similar results were observed in heart muscle postexercise [19]. It suggests that the recovery period is an important component of the adaptation phase of the genes necessary for the proliferation of mitochondria in muscle. However, research shows that chronic muscle disuse, as limb immobilization, denervation or bed rest, decreases mitochondrial content and the whole oxidative capacity. Chronic muscle inactivity disrupts the expression of both nuclear and mitochondrial genomes and inhibits mitochondrial biogenesis, increases apoptotic susceptibility contributing to a greater degree of apoptosis and a resultant increase in muscle atrophy [20].

Mitochondrial dynamic structure is also reflected in the ability to constantly fuse and divide in response to various physiology and pathological stimuli. They are able to change their shape through fission and fusion events, opposing processes that exist in equilibrium, leading to continuous remodeling of the mitochondrial network. If fusion predominates, mitochondria become more interconnected and networked [21]. In contrast, excessive fission leads to mitochondrial network breakdown, the loss of mtDNA, an increase in ROS production and respiratory defects [22]. Recent studies show that these processes have important consequences for the morphology, function and distribution of mitochondria. First, fusion and fission control the shape, length and number of mitochondria. Second, fusion and fission allow mitochondria to exchange lipid membranes and intramitochondrial content. Third, the shape of mitochondria affects the ability of cells to distribute their mitochondria to specific subcellular locations, and finally, mitochondrial fission facilitates apoptosis, which has consequences for development and disease [23]. Despite the fact that the exact mechanisms responsible for mitochondrial fission and fusion events have not been identified, a significant progress has been made in recognizing some genes and proteins involved in this process – mitofusin 1 and 2 (Mfn1 and Mfn2) and dynamin-related GTPase (OPA). The mechanisms of mitochondrial fission are still poorly understood, but there are dynamin-related protein 1(Drp1) and mitochondrial fission protein (Fis1), who regulates this process. A recent study has demonstrated an increase in Mfn1 and Mfn2 mRNA levels in human skeletal muscle 24 h post-exercise [24], but the regulation of the expression of these mitofusin izoforms have not yet been investigated. This remains an important area for future investigation in the study of mitochondrial structure and function in muscle.

Nuclear receptor peroxisome proliferator-activated receptor delta (PPAR-Δ) and coactivator peroxisome proliferator-activated receptor-γ coactivator1-γ (PGC1γ) are considered as important regulators of many metabolic processes, including mitochondrial biogenesis in muscle and heart [25, 26]. PGC-1γ binds and coactivate DNA binding transcription factors and increases their activity, or binding for many nu-

clear receptors increases transcriptional activity of their target genes. In addition to increased mitochondrial content, this coactivator induces other adaptations related to the strenuous training, including an increased proportion of muscle type I fibers and an increase in resistance to fatigue [27, 28].

Expression of PGC-1γ is dynamically regulated by altered types of physical activity. In response to a single bout of exercise, PGC1γ mRNA and protein are significantly elevated in mice, rats and humans [29]. This increase in gene expression is present as early as two hours after exercise. The same increase is also present in the repeated exercise [30], which indicates that the contractile activity is a main stimulus for exercise-induced PGC-1γ upregulation. It is evident that this coactivator plays an important role in the maintenance of mitochondrial content and function in muscle, but the literature data show [31] that its absence does not abolish the effect of endurance exercise on mitochondrial biogenesis, which was confirmed by increasing the protein markers, and concludes that there is a substitution of alternative transcription factors in the coordination of increased mitochondrial content [32].

Thus, it is clear that exercise can lead to changes in the expression of numerous transcription factors involved in mitochondrial biogenesis. The progressive increase in the accumulation of these proteins and coactivating factors in response to exercise indicates their important role in the mediation of mitochondrial adaptation to exercise, but the mechanisms by which this expression is regulated remain unclear.

Mitochondrial mutations and physical performance

Since the majority of mitochondrial proteins are encoded by nuclear genes, inheritance of mitochondrial disorder is autosomal recessive. In contrast, the disorders caused by mutations in mtDNA show great variability due to the phenomenon of heteroplasmy (intracellular mixture of mutant and normal mtDNA), because when the heteroplasmic cell divides, it is just a matter of coincidence which mitochondria and thus mtDNA will be distributed into the daughter cells. There is a combination of neurological and myopatic symptoms (MELAS, Leigh disease, Barth syndrome, Leber hereditary neuropathy of opticus etc.). On the other hand in many mitochondrial mutations declines of the energy output or energy deficit are present [33]. All mtDNA variations are usually classified into deleterious mutations present in maternally inherited disease, ancient polymorphisms, the characteristic of our ancestors to adapt to new environmental conditions and somatic, that occur with aging (they provide the aging clock) [34]. However, some variations of mtDNA appeared to have a positive effect and led to a functional mitochondrial adaptation. For example, the mtDNA variant of adaptation to warm climates results in more tightly coupled oxidative phosphorylation, with maximum ATP output and minimizing heat production. These changes in mtDNA permit maximum muscle performance, but these people are predisposed to obesity, diabetes, excessive ROS production, degenerative diseases and premature aging. Partially uncoupled mitochondria generate more heat, but at the expense of efficiency in ATP production. Individuals with these variants are more tolerant to cold, and less susceptible to obesity, they generate less ROS and are more resistant to aging and degenerative diseases, but have reduced endurance.

Based on the fact that the mitochondrial genome has 37 genes, alleles in some places define nine haplogroups [35]. The different versions of mtDNA within a population can be defined by distinct sets of polymorphisms called as haplogroups. Haplogroups serve as markers of genetic as well as geographic clusters. Castro et al. [36] were among the first to study the correlation of each haplogroup with elite athletic performance. Analysis of the Spanish long-distance runners, professional cyclists and rowers, revealed that the haplogroup T is less frequent in these athletes compared with the control, and athletes carrying this haplotype are clearly at a genetic disadvantage for performance in endurance sports. Scott et al. [37] compared the frequencies of mtDNA haplogroups found in elite Kenyan endurance athletes with those in the general Kenyan population. National Kenyan athletes, international Kenyan athletes and members of the general population of Kenya were compared and results showed that the haplogroup distribution of national and international athletes differed significantly from controls, and mtDNA haplogroup of international athletes were different from the general Kenyan population. The definitive conclusions of other studies are not relevant because of a small number of athletes, and because this one points out the complexity of comparing results from athletes of different ethnic groups [38].

Since mitochondrial metabolic and genetic therapies used to treat mitochondrial disease, it may become the subject of use by healthy people who want to change their energetic phenotype changing their mtDNA genotype and enhancing their physical performance. For example, changing a single mtDNA nucleotide of elite athletes to increase mitochondrial ATP production through altered oxidative phosphorylation coupling could increase physical performance by several percent [4]. Such a substitution could not be detected by standard anti-doping tests.

Mitochondrial nutrient supplementation

Mitochondrial nutrients are a group of micronutrients that are either mitochondrial components or those which metabolites influence the structure and function of mitochondria [39]. They protect mitochondria from oxidative damage and eliminate oxidative stress, increase the antioxidant defense, enhance mitochondrial metabolism by repairing of mitochondria or by increasing mitochondrial biogenesis, protects mitochondrial enzymes and stimulate mitochondrial enzyme activity by elevating substrate and cofactor levels [40]. Well-known mitochondrial nutrients or prosthetic groups are: R-alpha lipoic acid, acetyl-L-carnitine, coenzyme Q10, B vitamins, creatine, resveratrol, vitamin E, etc. Their individual effects in reducing oxidative stress and tissue damage and improved mitochondrial function in strenuous exercise have been demonstrated both in animal and human studies [41],

but more positive effects of combined supplements have been pointed out because of their synergistic action [42]. Special attention is payed to their role in stimulating transcription of genes involved not only in mitochondrial biogenesis but also in mitochondrial fusion in skeletal muscle, resulting in the increase in mitochondria function and better antioxidant defense, and thus leading to enhancement of physical performance and of fatigue recovery. Mitochondrial nutrients are selected based on their characteristics, the target of action and possible synergistic interactions, such as a group of antioxidants (coenzyme Q10, lipoic acid and glutathione), the energy enhancers (creatine, pyruvate, choline) or their precursors and cofactors (lipoic acid, coenzyme Q10, B vitamins). Some nutrients may have multiple functions, and some combinations may possess unique functions, quite different from their individual effects. B vitamins (riboflavin, piridoxin, biotin and nicotinamide) are used for cellular repair and production, and are particularly important for the protection of mitochondrial and other enzymes, because they are their precursors and cofactors. It is found that athletes with a lack of vitamin B have a reduced high-intensity exercise performance and are less able to repair damaged muscles [43]. Lipoic acid is a coenzyme involved in mitochondrial metabolism, it recycles vitamins C and E, raises intracellular glutathione and chelates iron and copper, and in coadministration with creatine and acetyl-L-carnitine shows synergistic effects in improving mitochondrial function [44]. Coenzyme Q10 affects the synthesis of ATP, thus increasing mitochondrial activity, delaying fatigue, reducing oxidative stress and damage to muscle tissue during exercise [45]. Resveratrol (RSV) is a natural polyphenolic compound mainly found in the skin of grapes and is well known for its phytoestrogenic and antioxidant properties. Research data shows that of the effects RSVs are in association with an induction of genes for oxidative phosphorylation and mitochondrial biogenesis by mediated roles in increasing PGC1γ activity [46].

Conclusion

Exercise is a powerful stimulus to mitochondrial biogenesis in skeletal muscle. The results of mitochondrial biogenesis are increased mitochondrial content, improved aerobic capacity and better ATP output, thus improving muscular endurance, reducing the predisposition to fatigue and increasing the effectiveness of physical exercise. The purpose of these changes in mitochondria is not only the process of energy supplying for muscle work, but they are part of a well-tailored mechanism of metabolic adaptation that reduces exercise-induced stress and maintains the physiological balance. Individual effects of numerous transcription factors that are part of mitochondrial biogenesis cascade and mitochondrial network remodeling, are not exactly specified, as well as their interactions, and they need further study and definition of sites, roles and possible activation outside in the form of natural, dietary or pharmacological activators. Better understanding of mitochondrial variation can contribute to more detailed introduction with the differences in aerobic capacity and defining the phenotype of elite athletes.

Mitochondrial nutrient supplementation enhances the physical performance of endurance exercise, decreases oxidative stress and fatigue and stimulates mitochondrial biogenesis. Future directions include their identifications and investigation useing modern technology of nutrigenomics for optimal effects and combinations.

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Premature ovarian failure: immunological aspects and therapeutic strategies

Prevremeno otkazivanje funkcije jajnika: imunološki aspekti i terapijske strategije

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ovarian failure, premature; autoimmunity; drug therapy.

Ključne reči:
jajnik, prevremeno otkazivanje funkcije; autoimunitet; lečenje lekovima.

Introduction

Premature ovarian failure (POF) is one of the most enigmatic and challenging conditions in reproductive medicine, that requires multidisciplinary approach and management. POF generally describes a syndrome consisting of amenorrhea, sex steroid deficiency, and elevated levels of gonadotropins in a woman aged more than two standard deviations below the mean age at menopause estimated for the reference population [1]. Infertility and psychological stress are common consequences of this entity, the prevalence of which is 0.9–3%. It is estimated to affect about 1% of women younger than 40, 0.1% of under 30 and 0.01% of women under the age of 20 [2, 3]. POF, premature ovarian insufficiency (POI), premature menopause, premature dysfunction (POD), or hypergonadotropic hypogonadism is one of the most enigmatic disorders. This condition is not irreversible and permanent due to the presence of residual oocytes capable of recruitment and fertilization. Therefore a more appropriate term for this condition might be POD, sygnifying a premature decline, rather than a failure in ovarian function, below the limit associated with fertility and steroidogenesis [4].

Classically, ovarian failure can be considered under the headings of genetic (X-chromosome anomalies; specific genetic mutations referred to oocyte, enzymes, or hormone receptors), autoimmune and environmental causes (viral infection, chemotherapy, radiotherapy, and pelvic surgery). In most cases, however, no precise cause can be identified, and these forms are referred to as idiopathic [5]. Numerous evidence, including association with multiple autoimmune endocrine disorders, clinical reversibility, transitory estrogen deficiency, histological and immunological features and the demonstration of circulating ovarian antibodies in serum samples from women with POF, have suggested its immunological origin. Between 10 and 30% of women with POF have a concurrent autoimmune disease, the most commonly reported being hypothyroidism, and the most clinically important hypoadrenalism, as well as association with myasthenia gravis, systemic lupus erythematosus, rheumatoid arthritis and Crohn's disease has been reported. For many women in whom the cause of ovarian failure is unknown, autoimmunity may be the pathogenic mechanism, as a primary or secondary immune dysfunction process against the ovaries [6, 7].

Autoimmune involvement in premature ovarian failure

The main function of the immune system is to distinguish between self and non-self. Malfunction of down-regulating controlling mechanisms may result in an excessive autoimmune response against self, i.e. an autoimmune disease [8]. Premature ovarian failure can occur as a result of primitive reduced pool of oocytes accelerated follicular atresia; or impaired folliculogenesis. An exaggerated autoimmune reaction involved in atretic acceleration, oocyte wastage or impaired folliculogenesis first described an association between an autoimmune adrenal deficiency and POF [9]. Autoimmune attack might be general or in most instances, partial, reversible, and responsible for, in many cases, fluctu-

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ating course of POF [6, 10]. However, the reality of such autoimmune process, its exact role in ovarian failure, the antigen determinant(s) of ovarian antibodies and cellular immunity, and the efficiency of immunosuppressive therapy should be further clarified.

Physiological role of the immune system in ovarian function

Unlike the testis, the ovary is not an immunologically privileged site. Several immune cells are recruited by the ovaries during the menstrual cycle such as macrophages, lymphocytes and polymorphonuclear granulocytes. These cells are able to secrete cytokynes, which participate in the paracrine regulation of follicular development, ovulation and luteal function. Cytokines modulate gonadotropin-mediated control of ovarian function and generally act in an inhibitory fashion. Tumor necrosis factor-α (TNF-α) secreted by ovarian macrophages is an inhibitor of steroidogenesis, and with proinflammatory cytokine, interleukine 2 (IL-2) decreases corpus luteum function and acts in a cytotoxic manner [11]. Ovulation has been considered an inflammatory process including both leukocytes and cytokines. Therefore, it is logical to consider that abnormalities of this process could perturb ovarian function and be involved in POF by accelerating follicular atresia or by disturbing folliculogenesis.

Clinical aspects of premature ovarian failure

It has long been recognized that POF could be associated with nearly all organ-specific autoimmune diseases, as well as with several autoimmune diseases in the same patients, referred to as autoimmune polyglandular syndrome (APS) [6, 7, 12]. APS-I, also called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare autosomal recessive disease caused by mutation in the autoimmune regulator (AIRE) gene, and without any association with a specific human leukocyte antigen (HLA) haplotype [13, 14]. It mainly affects children, and is associated with mucocutaneous candidiasis, ectodermal defects, hypoparathyroidism, Addison's disease, and POF that occurs in 40-60% of cases [14, 15]. APS-II, also called Schmidt-Carpenter syndrome, an autosomal dominant disease, linked to chromosome 6, and associated with HLA B8DR3DR4, comprises Addison's disease, insulin-dependent-diabetes, hypothyroidism and POF with the prevalence of which is 10-25% [7, 16]. APS-III is quite similar to APS-II, except there is no adrenal deficiency, but other autoimmune diseases, such as anemia perniciosa or vitiligo are often associated [17].

Because of a particular association with Addison's' disease, three different situations have to be distinguished: POF associated with adrenal autoimmunity; POF associated with non-adrenal immunity (most frequently associated with thyroid autoimmunity); and isolated, idiopathic POF, the latter which cannot exclude an autoimmune mechanism, possible provoced by environmental factors [18]. Transitory estrogen deficiency, higher anti-Mullerian hormone and inhibin levels (useful ovarian peptides in the assessment of follicular reserve); higher spontaneous recovery of ovarian cycles; and/or spontaneous pregnancy, under hormone replacement therapy (HRT), or without any treatment, suggest a partial, reversible autoimmune attack, particularly in idiopathic POF with a variable degree of ovarian function preservation [5, 19, 20]. Cases of POF associated with antiadrenal autoimmunity represent a homogeneous and well-characterized subgroup of ovarian failure, whereas in other forms of this disease, there is a large diversity in clinical, immunological and histological features.

Immunological features of autoimmune premature ovarian failure

The detection of autoantibodies directed against various ovarian targets strongly supports the hypothesis of an autoimmune aetiology of POF. Different autoantibodies were found in different clinical features of autoimmune POF. POF patients associated with adrenal autoimmunity commonly presented with autoantibodies that recognize several types of steroid-producing cells of the adrenal cortex, testis, placenta and ovary, therefore called steroid cell antibodies (SCA), with the prevalence of which is ~ 60% in APS-I patients; 25–40% in APS-II patients; and almost 78–100% in patients with both Addison's disease and POF [6, 10, 21]. These findings support the idea of a shared autoimmune response in ovarian and adrenal autoimmunity. In POF patients not associated with adrenal autoimmunity, as well as in isolated, or idiopathic POF, the prevalence of SCA remains < 10%. In those patients other autoantibodies could be found, divided into non-ovarian, and ovarian autoantibodies. Thyroid autoimmunity is the most prevalent (25–60%) associated endocrine autoimmune abnormality reported in POF patients without an adrenal autoimmune involvement, and with the presence of high levels of non-ovarian, thyroid peroxidase antibodies, leading to clinical/subclinical hypothyroidism development [1, 22, 23]. Antiovarian autoantibodies (AOA) are usually considered to be a suitable, and independent marker of autoimmune ovarian disease, although their specificity and pathogenic role is questionable. Evidenced data that AOA have been detected in ~ 30–60% of POF patients (particularly idiopathic POF patient), often appearing before the onset of clinical symptoms, with a possibility of prediction future ovarian failure in women with unexplained infertility, support its possible role as a marker either, of a primary, or secondary immune dysfunction process against the ovaries [15, 19, 24, 25]. There are several other autoantibodies towards specific ovarian targets potentially mediated autoimmune damage in POF: 3β-hydroxysteroid dehydrogenase autoantibodies, particularly found in isolated idiopathic POF; gonadotropin receptors autoantibodies; zona pellucida autoantibodies; as well as anti-oocyte cytoplasm antibodies towards maternal antigen that embryos require (MATER) [6, 13, 19].

Recently, authors pointed to the concept of functional autoantibodies (stimulating and/or suppressive) control in autoimmune diseases, particularly those comprising "sister-organs", such as the ovary, thyroid and adrenal glands [26]. Abnormalities of the cellular immunity, i.e. T lymphocytes

(especially effector helper, CD4-positive T cells), macrophages and dendritic cells, also play an important role in autoimmune reactions, particularly in the development of autoimmune lesions, described also in POF, and thus support the autoimmune mechanism of this disease [27, 28].

Histological findings in premature ovarian failure

In those cases where POF is associated with adrenal autoimmunity, histological examination almost always confirms the presence of ovarian follicles with characteristic signs of an autoimmune oophoritis: follicles are infiltrated by inflammatory cells, including lymphocytes, plasma cells, and macrophages; the steroid producing cells being the main target of the immune attack. Only a few patients whose POF is not associated with adrenal autoimmunity presented with typical oophoritis. The rarity of inflammatory infiltrates in these patients does not exclude the possibility of an autoimmune mechanism. Follicular depletion might be the final stage of primary or secondary autoimmune process directed against ovarian structures. However, systematic histological screening of POF revealed detectable follicles varying from few to numerous in 40% of cases [6, 14]. Hypothetically, autoantibodies to the ovary may have been present in the ovary without reaching detectable levels in the serum or inducing local inflammation.

Management of premature ovarian failure

POF is a delicate condition and a difficult diagnosis for women to accept. Women with POF have unique needs that require special attention. The loss of reproductive capabilities requires multidisciplinary management which includes the provision of proper counseling, nutrition supplement advice, HRT, possible immunosuppressive therapy, and reproductive health care, including contraception and fertility issues [29]. Although in most of the cases POF is idiopathic there is the need for further tests, looking for a specific aetiology, such as autoimmune, and genetic studies, the latter especially important in familial POF. The strong association of POF with APS makes screening for this condition essential [24, 30]. In idiopathic POF full attention must given to the investigation of indirect autoimmune signs, such as association with autoimmune disease (clinical aspect, hormone levels, and antibodies). The recovery of ovarian function may occur after regression of the autoimmune status and control of coexistent endocrine disease. Although ovarian biopsy is gold standard for detecting autoimmune cause of immune ovarian destruction, it is questionable whether it accurately represents the follicular density of the whole ovary, particularly in idiopathic POF, characterized with variable degree of ovarian function preservation [12, 31].

After confirming the diagnosis of POF and optimal assessment of ovarian reserve, including endocrine and ultrasonography markers for evaluation of ovarian volume and follicular pool, the urgent need to determine the optimum therapeutic hormonal regimens is required, both in terms of immediate menopausal symptoms relief and also for the protection against the long-term sequelae of estrogen deficiency, such as osteoporosis [30, 32–34]. In POF patients estrogen expresses dual useful action: the treatment of estrogen deficiency consequences; and the recovery of ovarian function, by restoration of receptor sensitivity to gonadotropins with salutary effect on folliculogenesis and conception, especially important in women seeking fertility [12, 35, 36]. Estrogen expresses crucial role in modulation of neuroendocrine environment to improve reproductive functioning. Dose, type and route of HRT are also very important. Dose should be higher than that used in an older age group, and the most suitable progesterone preparation should be combined with estrogen [35]. Estrogens are generally considered to enhance autoimmunity, through activating effector helper T lymphocytes and macrophages, potentially facilitating the maturation of pathogenic autoreactive B cells and diminishing the production of potentially protective B cells [37]. Oral estrogens appear to increase coagulation activation through procoagulant factors activation and reduction in anticoagulant factors such as antithrombin [38]. Thus transdermal HRT may be preferred in women with coagulant disturbances and more prone to thrombosis, such as patients with thrombophilias. As an essential prohormone in ovarian follicular steroidogenesis dehydroepiandrosterone (DHEA) could be an effective first step treatment of POF in the duration of 2–6 months before starting HRT [39]. Androgen replacement is useful in some instances with clinical signs and symptoms of androgen insufficiency, i.e. hypoactive sexual desire disorder (HSDD) [35, 40].

Immunosuppressive therapy, using different dose and term of glucocorticoids should be considered in a selected population of well-defined autoimmune POF patients, as well as in idioptahic POF patients, in whom the resumption of ovarian activity is possible, spontaneously, under HRT, and/or under immunomodulating treatment [14, 41, 42]. The combination of corticosteroids with pituitary suppression followed by ovarian stimulation with gonadotropins may be also benefitial in restoring ovarian function in patients with idiopathic POF [43, 44]. Other fertility issues should be considered in POF patients seeking fertility, including different regimes of ovulation induction and assisted conception techniques, such as *in vitro* fertilization (IVF) using donor gamets or embryos, or *in vitro* maturation (IVM) of oocytes derived from stem cells or primordial follicles [12, 30, 45].

Conclusion

The major aim in future research is to determine the "unknown etiology group", which represents the majority of POF patients. The pursuit of an autoimmune link offers an exciting research opportunity, with the possibility that some cases of POF might be temporarily reversible with immune suppression. Accepting the concept that POF is a heterogenous disorder in which some of the idiopathic forms are based on an abnormal self-recognition by the immune system will lead to novel approaches in the treatment of infertility in these patients. The ideal treatment strategy for young women with POF poses a clear challeng. Treatment should be multi-

disciplinary and individualized which includes the provision of proper counseling, nutrition supplement advice, HRT, immunosuppressive therapy in a selected population who may benefit from immunomodulatory therapy and possibly recover ovarian function, and reproductive health care including fertility issues. The choice, different needs of these women and individual risk factors must be taken into consideration.

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CASE REPORTS

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Recurrent longitudinally extensive transversal myelitis in a patient with Sjögren's syndrome

Rekurentni longitudinalno ekstenzivni transverzalni mijelitis kod bolesnika sa Sjegrenovim sindromom

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Abstract

Introduction. Longitudinally extensive transverse myelitis (LETM) is a transversal myelitis that extends through three or more vertebral segments in length. **Case report.** A 52-year-old woman was hospitalized due to pain in the lumbar region, difficulty in walking, hypoesthesia of the anogenital area and urinary retention. In the past medical history, two years earlier, the patient had been diagnosed with transversal myelitis confirmed by MRI of the cervical spine and six months earlier, the patient was diagnosed with primary Sjögren's syndrome (SS). During the current hospitalization MRI of the spinal cord revealed extensive inflammatory lesions of almost the whole spinal cord. Lumbar puncture (LP) revealed mild pleocytosis and slightly increased protein level. Isoelectric focusing of cerebrospinal fluid (CSF) and serum proteins was normal. Visual evoked potentials were normal. Serological testing excluded acute viral infections. Corticosteroid therapy was applied with good therapeutic response. Control MRI revealed regression of pathological changes in the spinal cord. **Conclusion.** A wide range of disorders can cause LETM, but usually the first line diagnosis is neuromyelitis optica (NMO). Based on the detection of NMO immunoglobulin G in the serum of affected patients, a variety of allied disorders were grouped under the name of NMO spectrum disorders, including recurrent myelitis associated with LETM and myelitis associated with autoimmune disorders such as SS. There have been only a few cases reported in the literature with recurrent LETM associated with non-organ specific autoimmune disorder.

Key words:
myelitis, transverse; sjorgen's syndrome; neuromyelitis optica; diagnosis; drug therapy; treatment outcome.

Apstrakt

Uvod. Longitudinalno ekstenzivni transverzalni mijelitis (LETM) je transverzalni mijelitis koji zahvata tri ili više susednih segmenata kičmene moždine. **Prikaz bolesnice.** Bolesnica, stara 52 godine, hospitalizovana je zbog bolova u lumbalnom delu kičme, otežanog hoda, hipestezije anogenitalne regije i retencije urina. Pre dve godine, magnetnom rezonancom (MR) cervikalne kičme dijagnostikovan joj je transverzalni mijelitis, a pre šest meseci primarni Sjegrenov sindrom (SS). Tokom sadašnje hospitalizacije MR kičmenog stuba verifikovana je ekstenzivna inflamatorna lezija koja je zahvatala kičmenu moždinu skoro celom dužinom. Analizom likvora utvrđena je blaga pleocitoza i blaga hiperproteinorahija. Nalaz izoelektričnog fokusiranja proteina likvora i seruma bio je uredan, kao i nalaz vizuelnih evociranih potencijala. Serološkim testovima isključena je akutna virusna infekcija. Ordinirana je kortikosteroidna terapija uz dobar terapijski odgovor. Kontrolnim nalazom MR potvrđena je regresija inflamatorne promene kičmene moždine. **Zaključak.** Brojne bolesti i poremećaji mogu uzrokovati LETM, ali na prvom mestu neuromijelitis optika (NMO). Na osnovu detekcije NMO imunoglobulina G u serumu bolesnika, brojne srodne bolesti grupisane su pod imenom NMO spektar bolesti, koji uključuje rekurentni mijelitis povezan sa LETM, kao i transverzalni mijelitis u sklopu autoimunih oboljenja kao što je SS. U literaturi je opisano samo nekoliko slučajeva rekurentnog LETM povezanog sa organ nespecifičnim autoimunim bolestima.

Ključne reči:
mijelitis, transverzalni; sjegrenov sindrom; neuromijelitis optika; dijagnoza; lečenje lekovima; lečenje, ishod.

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Introduction

Longitudinally extensive transverse myelitis (LETM) is a relatively recent term designating a transversal myelitis (TM) that extends through three or more vertebral segments in length [1]. It is much rarer and has more severe prognosis than other types of TM. LETM is usually associated with neuromyelitis optica (NMO), but as a possible diagnosis systemic autoimmune diseases [Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), antiphospholipid syndrome], neuroinflammatory conditions (Behcet's disease, sarcoidosis), multiple sclerosis (MS), and infectious diseases should always be considered [2].

Case report

A 52-year-old woman suddenly felt severe pain in the right lumbar region without irradiation. Two days later, the patient noticed difficulty in walking and hypoesthesia of the anogenital area accompanied with urinary retention. Since the patient had had similar problems before, she immediately contacted the physician who referred her to our hospital.

Regarding the past medical history from other hospital center, the patient had been diagnosed with TM confirmed by magnetic resonance imaging (MRI) of the cervical spine that showed intramedullary lesion extending from medulla oblongata downwards to Th3 level of the spinal cord (Figure 1A), two years earlier. At that time, computed tomography (CT) scan of the brain revealed no abnormalities. Lumbar puncture (LP) showed increased cell count with lymphocyte predomination. Serological laboratory tests of serum and cerebrospinal fluid (CSF) for common viruses and *Borrelia burgdorferi* were negative. The deficit that remained after the rehabilitation treatment was discrete muscular weakness of the left arm and the right leg. Control MRI of the cervical spine, performed tree times in a 2-year period, revealed regression of pathological changes in the spinal cord. Meanwhile, after some period of pain, swelling and stiffness in both hands and feet, in the Institute for Rheumatology the patient was diagnosed as primary SS based on: clinical findings (xerophtalmia, xerostomia) laboratory findings [positive antinuclear antibodies (ANA), anti Ro/SS-A antibodies, rheumatoid factor (RF)], ultrasound of salivary glands (focal areas of inflammation)]. It is important to emphasize the fact that patient did not use the prescribed therapy – hydroxychloroquine.

On current admission to our neurological department, the patient was alert, orientated, with all vital signs within normal limits. Neurological findings were as follows: deviation of tongue to the right, mild to moderate quadriparesis with increased tendon reflexes, hypoesthesia of all extremities with a level of decreased sensibility on Th4 dermatome and below, urinary retention and obstipation.

Standard laboratory tests showed elevated sedimentation rate, and mild anemia. C-reactive protein (CRP) level was normal and lactate dehydrogenase (LDH) elevated. MRI of the spinal cord revealed extensive inflammatory lesions of almost the whole spinal cord (Figure 1 B). MRI of the brain showed a few confluent T2W and fluid attenuated inversion recovery (FLAIR) hyperintense lesions of brainstem and cerebellum. Lumbar puncture revealed pleocytosis (101/mm³) with lymphocyte predomination (82%) and increased protein level (0.82 g/L). Isoelectric focusing of CSF and serum proteins was normal. Visual evoked potentials (VEP) were normal. Abdominal ultrasound showed only liver hemangioma. Chest x-ray was without pathological changes. Serological testing for viruses, in consultation with an immunologist and infectologist, excluded acute viral infections.

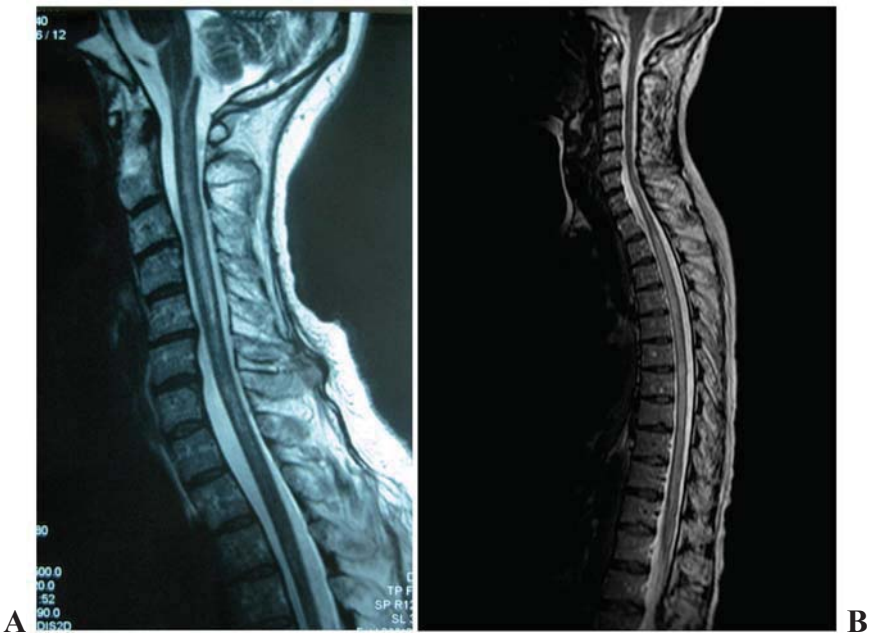

Fig. 1 – Magnetic resonance (MR) T2-weighted sagittal image from 2009.
A – The area of increased signal intensity accompanied with swelling (smooth expansion) of the medulla oblongata, the entire cervical part and the first three levels of the thoracic part of the spinal cord, that could be attributed to acute longitudinal myelitis. The same sequence on the magnetic resonance imaging (MRI) of the spine from 2011. B – Newly formed intramedullary lesions of the same MR characteristics, from Th3 downwards making a conus that correlates with actual clinical findings. Note the chronic atrophic changes of the previously affected medulla oblongata and cervical spine.

Based on immunological findings – increased RF, positive ANA, decreased levels of C3 and C4, normal lupus anticoagulant (LA) and anti double stranded (anti-ds) DNA antibodies, and positive Schirmer's test, regarding all previous and new findings, an immunologist confirmed the diagnosis of SS.

We applied corticosteroid therapy with intravenous methylprednosolone 1 g daily during six days, switched to prednisolone 1 mg/kg daily, with good therapeutic response and gradual recovery of symptoms.

Control MRI of the spine, performed two weeks later, revealed initial regression of pathological changes in spinal cord. Control LP findings were within normal limits.

The patient was discharged after 18 days on prednisolon therapy, with her previous deficit (mild hemiparesis and discrete hemihypesthaesia of the left limbs), but incontinent.

Discussion

A wide range of disorders can cause LETM, but usually the first line diagnosis is NMO [1]. Since the discovery of a specific serum biomarker, neuromyelitis optica immunoglobulin G (NMO-IgG), the concept of understanding and definition NMO has changed [3]. This biomarker distinguishes NMO from other demyelinating disorders. Based on the detection of this biomarker in the serum of affected patients, a variety of allied disorders are grouped under the name of NMO spectrum disorders (NMOSD), also including recurrent myelitis associated with LETM and optic neuritis or myelitis associated with autoimmune disorders such as SLE and SS [4]. Recurrent TM associated with longitudinal spinal cord lesions appear to be rather NMO-IgG seropositive. NMO-IgG seropositivity after the first attack predicts a relapsing course in most of the patients within three years of the first attack [5].

Sjögren's syndrome can be complicated with neurological problems, and frequently neurological signs are the first manifestations of SS. According to the study of Delalande et al. [6], neurological complications are the first symptom of SS in 81% patients, about 35% have spinal cord involvement and one third of these have acute myelopathy. In some patients, NMO and non-organ specific autoimmune disorders, particularly SS or SLE, coexist. It is the result of the presence of NMO-IgG in some patients with SS or SLE who have neurological involvement [7].

In the presented case, the first attack of LETM was initially considered idiopathic, but after confirming the diagnosis of SS it was assumed as first manifestation of SS. Current clinical findings and diagnostic procedures excluded sarcoidosis, Behcet's disease, SLE, metastatic tumors, viral infection as possible diagnosis. The patient did not fulfill diagnostic criteria for MS. Devic's disease was considered unlikely due to the absence of absolute diagnostic criteria – optic nerve lesions on MRI, also negative VEP, but subclinical NMO or NMOSD could not be excluded because we were not able to test NMO-IgG (not available in our laboratories). In the context of current findings, recurrent LETM in the presented case, even without testing NMO-IgG, made us to conclude that the presented patient probably had NMOSD.

There have been a few cases in the literature on LETM and recurrent LETM associated with non-organ specific autoimmune disorder [8–10], but, to our knowledge, there have been no reports on recurrent LETM associated with SS. Moreover, we found the presented case interesting because inspite of the fact that LETM extended to practically entire spinal cord, the patient had good outcome.

Conclusion

A wide range of disorders can cause LETM, but usually the first line diagnosis is NMO. Based on the detection of NMO immunoglobulin G in the serum of affected patients, a variety of allied disorders were grouped under the name of NMO spectrum disorders, including recurrent myelitis associated with LETM and myelitis associated with autoimmune disorders such as SS. There have been only a few cases reported in the literature with recurrent LETM associated with non-organ specific autoimmune disorder.

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CASE REPORT

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Von Meyenburg complex (hamartoma of the bile duct) mimicking liver metastases

Fon Majenburgov kompleks (hamartom žučnog kanala) sličan metastazama u jetri

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Abstract

Introduction. Hamartomas of the bile duct (von Meyenburg complex) are benign lesions of the liver that may mimic liver metastases. Histologically, they consist of cystic dilatations of the bile duct, encompassed by fibrous stroma. **Case report.** We reported a 68-year-old male patient in who gross, ultrasonic and MSCT appearance suggest multiple liver metastases. The diagnosis of von Meyenburg complex was made after histopathologic examination of liver biopsy. **Conclusion.** Von Meyenburg complex in an uncommon entity which should be taken into consideration as a differential diagnosis of liver metastases.

Key words:
hamarthoma; bile ducts; diagnosis, differential; diagnostic techniques and procedures; biopsy.

Apstrakt

Uvod. Hamartom žučnih vodova (von Meyenburg-ov kompleks) je benigna promena koja može imitirati metastaze u jetri. Histološki ga grade dilatirani žučni duktusi okruženi fibroziranom stromom. **Prikaz slučaja.** U radu je prikazan bolesnik, star 68 godina, kod koga su ultrazvuk, multislajsna kompjuterizovana tomografija (MSCT) i makroskopski izgled promena na jetri ukazivali na multipli metastatski depozit. Dijagnoza von Meyenburg-ovog kompleksa postavljena je nakon histološkog pregleda promena sa jetre. **Zaključak.** Von Meyenburg-ovog kompleks je redak entitet i treba ga uzeti u razmatranje kao diferencijalnu dijagnozu metastaza u jetri.

Ključne reči:
hamartom; žučni putevi; dijagnoza, diferencijalna; dijagnostičke tehnike i procedure; biopsija.

Introduction

Von Meyenburg complexes (VMCs) is a benign liver lesion. This lesions consists of bile ducts which are incorporated in the connective stroma. Some of billiary ducts are cystically dilated. The incidence of VMCs is age-dependent. Redston et al. [1] reported that this lesion is found in approximately 1% in children and in about 5–6% in adults. Patients often, have no symptoms and the lesion is usually discovered accidentally. Röcken et al. [2] and Jain et al. [3] suggest to possible malignant transformation of these lesion. Von Meyenburg complexes are small lesions, and they are often not detected on radiological examinations, including ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) [4–7]. Its clinical importance is that it may mimic malignant liver disease. We presented a case of VMCs which preoperatively suggested hepatic metastases.

Case report

A 68-year-old male patient with multiple hepatic lesion which ultrasonographic and multisliced computed tomography (MSCT) appearance suggested multiple liver metastases was accepted for surgical exploration and liver biopsy. The patient vomited for a month and lost weight. During surgery numerous whitish irregular lesions of various sizes scattered in the hepatic surface imitating metastatic deposits were noted through both liver lobe and all liver quadrants. Exploration of the abdominal cavity showed no pathological changes nor peritoneal carcinomatosis. Liver biopsy was done and three samples taken for analysis. Tissue was of brown-yellow-gray color and medium-firm consistency. Histological analysis demonstrated multiple lesions (Figure 1) composed of biliary ducts incorporated in fibrotic tissue (Figure 2), with cystic dilatations of the same intrahepatic biliary ducts (Figure 3) suggestive hamartomas of the bile duct or von Meyenburg complex.

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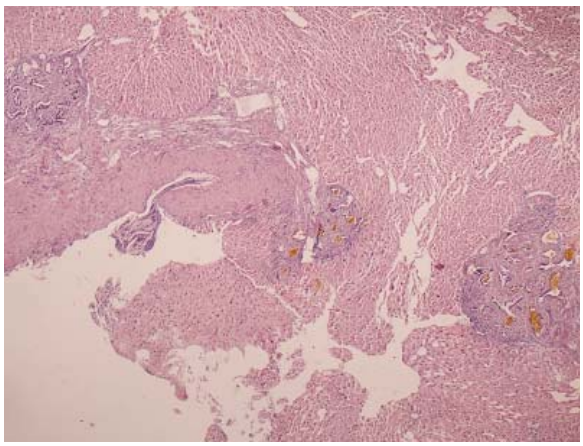

Fig. 1 – Multiple von Meyenburg complex in liver tissue (HE, 40×).

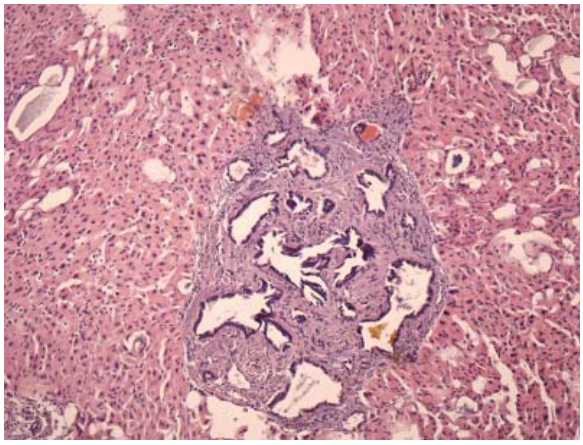

Fig. 2 – Von Meyenburg complex – intrahepatic biliary ducts surrounded by fibrotic tissue (HE, 100×).

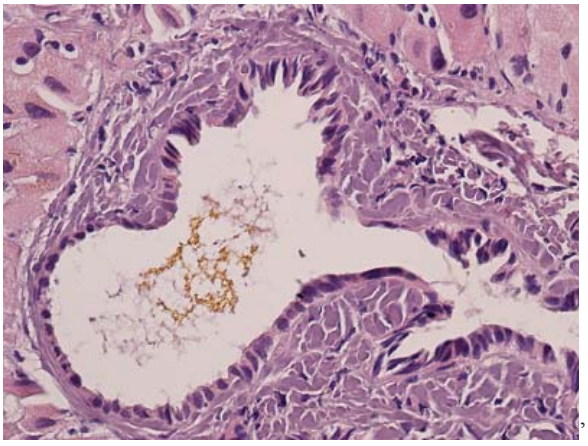

Fig. 3 – Von Meyenburg complex – dilatations of intrahepatic biliary duct (HE, 400×).

Discussion

Hamartomas of the bile duct named von Meyenburg complex are rare lesions, usually characterized by multiple small nodules located near the Glisson's capsule. The size of nodules is usually a few millimeters [8]. VMCs are usually asymptomatic and being accidentally discovered during laparotomy or autopsies. Multilocular occurrence is possible although they are rarely spread throughout the whole liver, as it was observed in the presented patient. According to data from the literature VMCs can be in association with Caroli's syndrome, congenital hepatic fibrosis, autosomal dominant polycystic renal disease [9], cholangiocarcinomas and cholangitis [10, 11].

VMCs can be misdiagnosed on ultrasonography with interpretation that is as metastatic hepatic disease [12]. The definitive diagnosis of this lesion was confirmed by biopsy of the liver. The ultrasonographic findings of the von Meyenburg complex are diversiform lesions which include hyperechogenic and hypoechogenic nodules with poorly or well limited margins [11, 13–15]. Nagano et al. [16] reported that magnetic resonance cholangiography is the best method for examination of hamartomas of the bile duct. This method makes possible to distinguish the different forms of bile duct abnormality, such as sacular dilatation of the biliary system (Caroli disease) and ductal cystic dilatation. This method also makes possible to detect the presence of cholangiocarcinoma or diffuse liver metastases.

Histologically, VMCs include dilated small bile ducts, surrounded by fibrous stroma. Microscopically, they are characterized by abundant fibrous stroma with cystic dilatations of the bile duct which can be of various sizes, and sometimes with associated periductal glands [17, 18].

VMCs are usually found along the portal tract. The bile ducts are covered by a single layer cuboidal or flattened cells, with regular oval nuclei. A cystic dilated bile duct have irregular shape and may contain eosinophilic debris in lumen [19].

In the literature we have not encountered studies in a larger number of cases describing the malignant potential of these lesions. Xu et al. [20] in two case reports described association of von Meyenburg complex with cholangiocarcinoma, for commented it as lesion which carries an increased risk for the development of cholangiocarcinoma [20].

Except for cholangiocarcinoma, VMCs can be associated with hepatic cysts, polycystic renal disease and cholangitis [21]. Owing to the association between these diseases, Vitule et al. [22] emphasized that in cases of adult polycystic disease, screening for VMCs is very important.

Conclusion

Von Meyenburg complexes are an important differential diagnosis of liver metastases which also includes other benign liver lesions, like hemangiomas, adenomas or infectious lesions. Due to the presence of liver metastases it is of great importance for therapeutic decision making in patients with malignant diseases, distinguishing metastatic and benign changes that mimic them. As VMCs are small, usually less than 5 mm in size, they can escape preoperative radiologic diagnostics. The macroscopic appearance of VMCs can mimic liver metastasis as demonstrated in our reported patient.

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CASE REPORT

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Transitional cell carcinoma in orthotopic ileal neobladder 12 years after radical cystectomy

Karcinom prelaznog epitela ortotopske ilealne neobešike 12 godina posle potpune cistektomije

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Abstract

Introduction. Some cases of secondary adenocarcinoma developing in the replaced bowel segment of urinary diversions have been reported so far. Secondary adenocarcinoma develops 20 years after surgery in about 0.5% of those in whom an ileal segment is used. There have been several reports in the literature describing extensions of transitional cell carcinoma (TCC) from the distal urether into an ileal conduit. Histology of loop tumor in 50% was TCC. The site of tumors in the majority of cases is the area at the uretheral orifices or the stoma. **Case report.** We presented a rare case of transitional cell carcinoma in an orthotopic ileal neobladder 12 years after radical cystoprostatectomy and ileal neobladder with the substitution by the procedure Camey II. A 65-year-old man with high-grade urothelial carcinoma of neobladder underwent partial resection of neobladder and right nephroureterectomy. Pathological analysis revealed high-grade urothelial carcinoma to the ileal neobladder (G II, Stage T2b). The patient died of laryngeal cancer a year after the surgery. **Conclusion.** Surgery of tumors in orthotopic neobladders is possible if diagnosed in time. In the presented case surgery resulted only in a decrease in the capacity of the neobladder without having an effect on the continence itself.

Key words:
urinary bladder, neoplasms; cystectomy; ileum; colonic pouches; recurrence.

Apstrakt

Uvod. Opisani su pojedinačni slučajevi sekundarnih adenokarcinoma koji su se javili u crevnom segmentu kod urinarnih derivacija. Sekundarni adenokarcinom razvio se 20 godina posle kod oko 0.5% bolesnika kod kojih se koristi segment ileuma. Takođe, u literaturi su opisani pojedinačni slučajavi propagacije tumora iz distalnog dela uretera u ilealni konduit. Histološki, ovi tumori su kod 50% bolesnika karcinomi prelaznog epitela. Kod najvećeg broja bolesnika lokalizacija tumora je u blizini novoformiranog orificijuma. **Prikaz bolesnika.** Prikazali smo redak slučaj pojave karcinoma prelaznog epitela u ortotopskoj ilealnoj neobešici 12 godina nakon radikalne cistoprostatektomije. Formiranje ilealne neobešike je učinjeno primenom postupka Camey II. Bolesniku, starom 65 godina, sa karcinomom u ilealnoj neobešici, učinjena je desnostrana nefroureterektomija i parcijalna resekcija ortotopske ilealne neobešike. Patohistološkom analizom utvrđeno je da se radi o urotelnom karcinomu ilealne neobešike visokog gradusa (G II, stadijum T2b). Bolesnik je umro od laringealnog karcinoma godinu dana nakon operacije. **Zaključak.** Hirurške resekcije tumora ortotopske ilealne neobešike moguće su ukoliko se tumori dijagnostikuju na vreme. Kod prikazanog bolesnika resekcija je dovela samo do sniženja kapaciteta ortotopske ilealne neobešike, bez uticaja na kontinenciju.

Ključne reči:
mokraćna bešika, neoplazme; cistektomija; crevo, tanko; creva, rezervoari; recidiv.

Introduction

Continent urinary reservoirs represent the state of the art in urinary diversions. A low incidence of tumors in continent urinary diversions is very encouraging. The surgeons who perform continent urinary reservoirs are urged to institute provisions for careful long-term follow-up in these patients [1, 2].

Patients found to have muscle-invasive disease of the urinary bladder commonly undergo radical cystectomy and urinary diversion. The decision about which urinary diversion will be best suited to the individual patient is complex

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and depends of multiple factors relating to the patient and the tumor as well, as a clear understanding about the risks and benefits of each diversion [3, 4]. Orthotopic neobladders can be constructed in the anatomic position and anastomized with the native urethra. Volitional voiding is achieved by increasing the abdominal pressure and relaxing of the external sphincter. Neobladders can be fashioned from ileum, colonic tissue or sigmoid colon. During dissection special attention must be payed to protect the urethra, periuretheral muscles and the sphincter. Numerous variations of the orthotopic neobladder have been introduced, but the Studer's type pouch, because of its versatility, is currently most often used. Other neobladders include the Camey, a 60 cm segment of ileum fashionised into a U-shape; the Hauptmann, which is similar to other neobladders but W-shaped to increase capacity; and the Mainz, Le Bag, and UCLA pouches wich all use an ileocecal segment. Enterocystoplastic is the procedure which is reserved for patients with long life expectancy and good functional results. Those patients are under 70, not obese, not diabetic, have no cardiopulmonary or neurological diseases, and should be highly motivated, informed and cooperative. It is very important that muscle invasive tumor is away from the bladder neck and the urethra is with no strictures. The upper urinary tract should be evaluated and performing urethral biopsy is mandatory [5].

Neobladder forming using the procedure Camey II involves detubularising the distal ileum bowel segment and creating an orthotopic urinary reservoir of low intraluminal pressure. Ureters are implanted in the newly formed neobladder using the antireflux Camey-Le Duc technique creating a "le lit ureterale". Detubularisation cancels out the rise of intraluminal pressure in a reservoir created by bowel movement resulting in better continence in patients during the day and night time which is the main advantage of this technique over the procedure Camey I (were the bowel segment isn't detubularised, increasing the quality of life in these patients). Leandri et al. [5] compared the results of Camey I orthotopic neobladder replacement in 275 patients with Camey II procedure in 36 patients and concluded that the continence in detubularised orthotopic neobladder – Camey II was 100% during the day and 70% at night. These patients void every 3–4 hours and the voiding routine is stabilized after three months.

Cases of urothelial carcinoma developing in urinary diversions using the ileum have been infrequently reported [2] and it underlines the importance of regular follow-up of patients with orthotopic neobladder replacement after radical cystectomy.

Some cases of secondary adenocarcinoma developing in a replaced bowel segment of urinary diversions have been reported. Secondary adenocarcinoma developed 20 years after surgery in about 0.5% of those in whom an ileal segment was used.

There have been several reports in the literature describing extensions of transitional cell carcinoma (TCC) from the distal urether into an ileal conduit. Histology of loop tumor in 50% was TCC [2]. The site of tumors in the majority of cases was the area at the urethral orifices or the stoma [1, 2].

Case report

We presented 65-year-old man, smoker, with urothelial carcinoma in orthotropic neobladder 12 years after radical cystoprostatectomy for bladder cancer and orthotopic ileal neobladder, the procedure Camey II.

Radical cystoprostatectomy with orthotopic neobladder, Camey II procedure, was performed in 1985 as a method of choice in treating muscle invasive bladder tumor. After initial surgery the patient was fully continent during the day and at night after a 3-moth period. Hystopathological examination after radical cystoprostatectomy revealed urothelial carcinoma, grade 2, Stage T2NoMo. The patient underwent regular follow-up, which included urethropouchoscopy every 6 months following the first two years after the surgery. Later on urethropouchoscopy and intravenous pyelogram examination were performed yearly. Blood and urin analyses were also performed, levels of electrolytes, urea and creatinin were monitored every 3 months and there were no clinical signs of recurrence of the primary disease.

The patient was admitted for diagnostic examinations 12 years after the surgery for complains of gross hematuria. The diagnosis of the tumor in the orhotopic neobladder was established by intravenous pyelography (Figure 1), showing

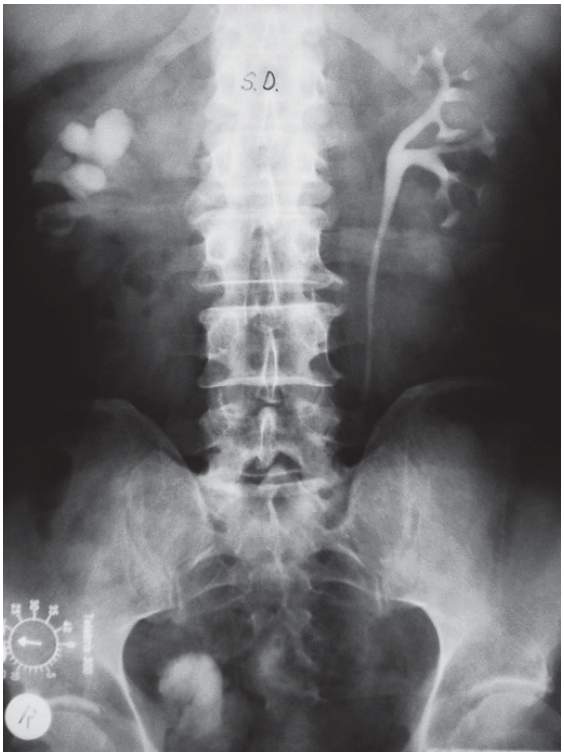

Fig. 1 – Intravenous pyelography of the right kidney.

ureterohydronephrosis on the right side and ehosonography confirming the ureterohydronephrosis on the right side and also revealing 20 × 10 mm size tumor on the right wall of neobladder. Computed tomography was not used as standard procedure in that period. Urethropouchography showed filling defect of the right wall of the neobladder (Figure 2),

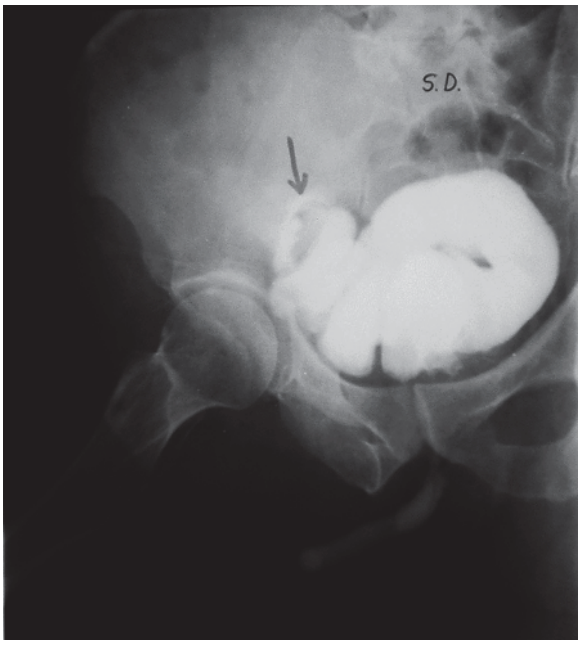

Fig. 2 – Urethropouchography shows filling deffect of the right wall of the neobladder.

where papillary tumor was disclosed by urethropouchoscopy examination and the biopsy of the tumor was performed. Hystopathological examination revealed urothelial carcinoma TCC, grade 2. Blood analysis was normal, urine analysis was positive for red blood cells.

Right nephroureterectomy with partial resection of the neobladder were performed by the midline transperitoneal approach. The tumor was loacalised on the newly formed uretheral orifice of the right kidney. Since ureterohydronephosis was early diagnosed with the decrease in the renal parenchyma it was decided that right nephroureterectomy to be performed. During the 3-hour surgery blood loss was minimal and the patient received one dose of transfusion and administration of antibiotics was intraoperatively performed according to the urinoculture. His immediate postoperative course was uneventful with 2 days of fever. Urethral catheter was removed 14th day after the surgery and the patient was fully continent. Surgery resulted in the decreased capacity of the orthotopic urinary reservoir, the patient urinated more frequently than before, but had no effect on day and night urinary continence. Hystopathological finding revealed urothelial carcinoma, grade 2, Stage T2b. Tumor probably developed in a bowel segment from the distal part of the right urether.

The patient died of laryngeal carcinoma a year after the described surgery.

Discussion

Principles of low pressure orthotopic ileal neobladder reconstruction are well-known and the urologists interested and experienced in reconstructive surgery should be encouraged to perform reconstructive orhotopic ileal neobladder surgery after radical cystectomy in younger and motivated patients [4].

Careful follow-up is important for patients with continent urinary reservoirs. It is important to regularly follow-up these patients due to possible complications when forming orthotopic neobladder which include strictures of uretherointestinal anastomosis, kidney damage and tumors in newly formed neobladder. Intravenous pyelography with descendent pouchography, ehotomography and urethropouchoscopy should be performed regularly. Urine cytology and endoscopy are being used in addition to standard radiographic procedures to follow-up those patients for potential tumor development [6–8]. Several institutions decrease their surveillance to once a year after the first 5 years of follow-up. Tumors in isolated bowel segments tend to be much less frequent in those bowel segments that have contact with both urine and feces. The site of tumor in the most number of cases was the area at the ureteral orifices or the stoma [3, 4]. Intraluminal tumor cell seeding appears to be an important mechanism of transitional cell carcinoma recurrence in the ileal mucosa of a neobladder [1, 8].

Conclusion

Surgery of tumors in orthotopic neobladders is possible if diagnosed in time. In the presented case surgery resulted only in a decrease in the capacity of the neobladder without having an effect on the continence itself.

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CASE REPORT

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Group art therapy as adjunct therapy for the treatment of schizophrenic patients in day hospital

Grupna art terapija kao pomoćna terapija za lečenje shizofrenih bolesnika u dnevnoj bolnici

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Abstract

Introduction. The schizophrenics are frequently disinterested and resistant to standard care. **Case report**. We presented clinical observations of group art therapy of two schizophrenic patients during integrative therapy in Day Hospital. We modified the original "Synallactic collective image technique" (Vassiliou G, Vassiliou V.). The group is open, heterogeneous, meets once a week and discusses on exhibited drawings, drawn by free associations. The patients' drawings and group protocols showed clinical improvement by lowering depressive themes, more human figures and self-confidence. The obvious severity of markedly impairment on Clinical Global Impression (CGI) and Global Assessment of Functioning (GAF) scales on admission with minimal improvement at discharge was rated. **Conclusion.** Group art therapy enables visual expression of emotions, perceptions and cognitions, develops creative potentials and support within the group, thus facilitating the integrative therapeutic process of schizophrenics. It may be useful adjunctive therapy for schizoprenic patients.

Key words:
schizophrenia; art therapy; psychotherapy, group; integrative medicine; treatment outcome.

Apstrakt

Uvod. Shizofreni bolesnici su često nezainteresovani i rezistentni na standardno lečenje. **Prikaz bolesnika**. Prikazane su kliničke opservacije grupne art terapije dva shizofrena bolesnika u toku integrativnog lečenja u Dnevnoj bolnici. Modifikovali smo originalnu "*Synallactic collective image technique*" (Vassilliou G, Vassiliu V). Grupa je otvorena, heterogena, sastaje se jedanput nedeljno, diskutuju se izloženi crteži, koji su nastali po slobodnim asocijacijama. Crteži i grupni protokoli prikazanih bolesnika pokazali su kliničko poboljšanje uz smanjenje depresivnih tema, više ljudskih figura i povećanje samopouzdanja. Registrovano je značajno bolesno stanje na *Clinical Global Impression* (CGI) i *Global Assessment of Functioning* (GAF) skalama na prijemu, sa minimalnim poboljšanjem na otpustu. **Zaključak**. Grupna art terapija omogućava vizuelnu ekspresiju emocija, percepcija i kognicija, podstiče razvoj kreativnih potencijala i grupnu podršku, olakšavajući integrativnu terapiju shizofrenih bolesnika u Dnevnoj bolnici. Ona može biti korisna pomoćna terapija kod shizofrenije.

Ključne reči:
shizofrenija; lečenje umetnošću; psihoterapija, grupna; medicina, integrativna; lečenje, ishod.

Introduction

Schizophrenia is a severe, chronic condition affecting person's cognition, emotions, perception and complete behavior. It most commonly occurs in adolescence with prevalence rates reaching 1% of the total population [1]. Treatment and rehabilitation of schizophrenia patients have become a big socio-medical concern [2]. In spite of the advances in pharmacotherapeutic treatment, symptoms of schizophrenia accompanied by the reduction in the normal flow of communication could be frequently seen in those patients. Therefore, art therapy coupled with other creative therapies used as an adjunctive method in combination with drugs, might be helpful in obtaining an insight into the patients' inner world, without making them feel threatened [3]. Having been developed some 60 years ago, art therapy was focused on developing good communication skills through arts [4].

After the acute treatment phase and hospitalization, or due to worsening of their condition during maintenance therapy, such patients are referred to day hospitals for further treatment, where, in addition to their pharmacotherapy, they attend various psychotherapeutic and sociotherapeutic sessions [5].

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Since the development of art therapy and its introduction into psychiatry clinical practice, the clinicians' attention has mostly been focused on the drawing's content as a diagnostic tool. Investigation of art therapy effectiveness in the schizophrenia treatment conducted over the recent decades, led to its inclusion into some national treatment guidelines for schizophrenia as a treatment option recommended for the management of all types of this disorder [6]. However, there is little or insufficient knowledge of the availability, organization and the way of conducting art therapy programs, not only when our community is concerned, but other national healthcare facilities as well [7].

More than 20 years ago, the author of this paper from the Day Hospital of the Military Medical Academy's (MMA's) Psychiatry Clinic modified, to some extent, the technique called by its creators, George and Vasso Vassiliou, the "Synallactic collective image technique" [8]. The original method was based on the systematic understanding of group therapy processes, and was focused on only one drawing voted in the course of session by the members in a homogeny group [9]. But in our case, we modified the said technique in a way to adjust it to the heterogenic composition of the group, as well as to the integrative therapy, which is used in our Day Hospital [10]. Thus, an open, heterogenic group of patients meets once a week. During the group therapy process, a therapist and a co-therapist lead the group in a structured way: the drawings that the patients have made by free associations and brought to group therapy are exhibited, then voted, and discussed for the ninety minutes. On completion of the session, the therapists conduct a qualitative analysis of the drawing form and content, and the group therapy protocols as well using the experience of co-observers.

This paper is set of clinical observations and therapeutic possibilities of the art therapy through its application in the Day Hospital treatment of two schizophrenic patients, describing their general health condition and functioning at the time of their admission and discharge, two months later.

On both admission and discharge, an overall clinical assessment of the patients' condition and functioning was rated on a seven-point Clinical Global Impression Severity (CGI-S) Scale [11], and the Global Assessment of Functioning (GAF) Scale [12] by an independent psychiatrist not involved in the group art therapy, whilst the patients' improvement at the time of their discharge was rated on the Clinical Global Improvement (CGI-I) Scale [11].

The patients expressed their written consent for the participation in group therapy and the use of their drawings for such purposes.

Case report

Our first case was a 31-year-old female patient, graduated from the School of Arts, who was unemployed, of the average material status, single, lived with her mother who gave her considerable support during the treatment. For almost ten years, she was treated for paranoid schizophrenia associated with a considerable social withdrawal and other residual symptoms dominating her clinical picture over the last three years. However, there were no psychotic disorders observed in her family anamnesis. She was on the maintenance therapy with the long-acting depot preparation of classical antipsychotics, anxiolytics and antidepressants. As her condition accompanied by social isolation, distrust of people and mild paranoid delusions, the feeling of abandonment and depression worsened, she was referred to Day Hospital. Her admission GAF score was 51, while the CGI-S score was 5 (markedly ill). The theme of her first drawing (Figure 1), was

Fig. 1 – A drawing of flowers that looks depressed (the first patient, at the beginning of the treatment).

a depressive one depicting flowers bending over the sides of a vase dominantly standing out against a pale and empty background. Prior to her discharge, she drew a dead nature motive similar to that drawn upon her admission (Figure 2), but this time, with the addition of blooming flowers standing

Fig. 2 – Dead nature (drawing by the same patient prior to discharge).

straight up in a more colorful vase and background filled with new elements. However, the patient was fond of drawing, for it was her chosen career she couldn't ever pursue due to her illness. Her drawings were most often voted by the other patients during the group analysis sessions. That was practically the only activity through which she could demonstrate her genuine potentials and gain gratification. She became more open with the other members in the group, and her paranoid delusions were considerably minimally expressed. At discharge, her GAF score was 56, CGI-S was 5, while CGI-I was minimally improved.

The second case was a 27-year-old male patient, a second school graduate, a single young man living with his primary family, fled from the war-affected area, exposed to prolonged psychosocial stresses. He has a heterozygous twin brother and an elder one, both of whom are healthy. He felt secluded from his family, not being supportive enough. For the last nine years, he was treated for schizophrenia simplex with atypical antipsychotics and anxiolytics on the outpatient basis. However, as soon as his condition deteriorated along with the progression of negative symptoms accompanied by highly expressed social and emotional withdrawal and reduced efficacy, he was referred to the Day Hospital.

On his admission, the GAF score was 50, whilst the CGI score was 5 (markedly ill). During his first weeks, after admission, he kept drawing almost stereotypically the same house with evident signs of emptiness (Figure 3). At the group discussion sessions, his poor emotional experiences, difficulties with thinking and oral expression of poor meaningful contents, and very rare associations were evident. The other members in the group were very caring towards him, and recommended him, with a dose of humor, that he could add something else, at least a human figure, to the composition in his drawings. After several weeks he drew several houses. Then he continued to draw houses with windows wide open and those stick figures of men looking through it (Figure 4). Prior to his discharge, he drew a great many those figures out of the houses, thus, symbolically showing that he managed to overcome his feelings of social isolation (Figure 5). Each of his improvements in the content of his drawings was gratified by the members in group therapy, thus provoking a modest smile on his face. At the time of discharge, his GAF score was 55, CGI-S score was 5, and CGI-I was minimally improved.

Fig. 3 – A drawing of an empty house (the second patient, at the beginning of the treatment).

Fig. 4 – A drawing of houses with windows wide open and stick figures of men looking through them.

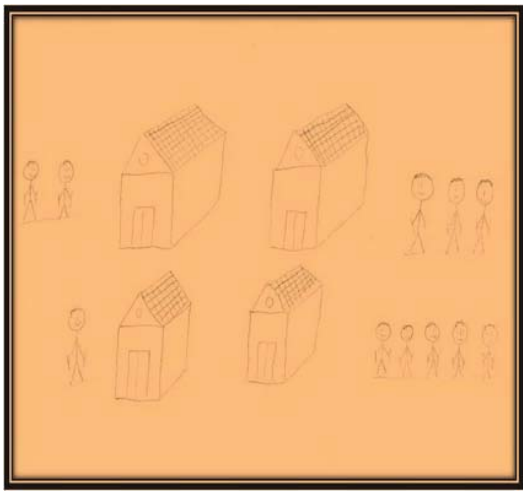

Fig. 5 – A drawing of many figures of men showing that the patient overcame his feelings of social isolation (prior to discharge).

Discussion

A personal feeling of alienation, helplessness, emotional deprivation, avoidable behavior and social isolation which are commonly seen in patients with schizophrenia [1], were also reported in the presented cases. However, there are reports on clinical trials, the results of which showe that group art therapy did not help improve the global functioning of patients suffering from schizophrenia, and the outcome of their treatment as well [13]. The applied scale scores confirmed only a minimal improvement of the general health condition and functioning achieved in those two patients upon the completion of the Day Hospital integrative treatment. It could be explained by the fact that it was about markedly ill, chronic patients with persistent and expressed negative symptoms of schizophrenia, due to what an intensive recovery could not be expected during a 2-month treatment period.

Clinical assessment scales are a universal tool useful in the clinical practice, and required in clinical trials. However, mental health status of a patient is colorful and illustrated through drawings in a unique way and specific individual style. So, in the first case, a vase of flowers was a theme the patient kept drawing, but her improvement in the course of the treatment in the day hospital setting was evident. Her first drawing was expressively depressive and poor in color and forms symbolically reflecting her isolated, lonely and depressive dyad surrounded by emptiness. In spite of the same motive she had repeatedly drawn as the central focus of the whole composition, her drawings at the end of her treatment were filled with expressive, warm colors, more richer background, without previously emphasized sharp lines, clean borders and high contrast between the vase and its background. In addition to such progress in her artistic expression, she managed as well to intensify her interaction with the other group members in the course of this well-structured therapy. The votes and clear gratification she got from the others helped her feel protected and accepted by the others, and encouraged her, at the same time, to build confidence in the group.

On the other side, poor performance and associations were evident in the second case. However, thanks to a warm support, and at the initiative of the other group members, he managed to enrich his stereotype expression and, to symbolically and clearly, demonstrate his reduced social withdrawal. It is an example of how the art of drawing is not considered to be either a dominant goal or a requirement for the art therapy, but an expressive tool that might help stimulate creative insights even in chronic patients. Thus, a relatively simple type of drawing made of lines has even the ability to make an impact on the patient to get an idea of what his/her own creative potentials are, and to facilitate communication within an intensive, interactive group.

What we could most clearly see from the drawings of presented patients was the reduction of both social and emotional withdrawal. The group played a significant role as a source of social support. It also encouraged the patient to regain and raise his/her self-esteem. Concurrently with the changes made to the composition of the drawings, the patients started to attend the other therapeutic activities in our Day Hospital. Similar clinical observations on raising the self-esteem in schizophrenic patients attending the group art therapy were reported by other authors [14]. The randomized clinical trial confirmed a statistically significant reduction of negative symptoms of schizophrenia along with minor changes of other symptoms [15], what was also observed in our cases. The same trial revealed as well a lower level of patients' attendance at the art group therapy, whilst our patients, along with the majority in the group, regularly participated in those therapy sessions. Another effects of integrative therapeutic process observed in schizophrenic patients, were a gradual shift of their focus from psychotic topics to an actual social context and the reality, and an approach and confrontation with the problems on the 'here and now' basis, what, thus, strengthened their sense of belonging to the group.

The basic limitations in assessing therapeutic possibilities of group art therapy presented here refers to the fact that it is only an adjunctive therapy that can be used as a part of the day hospital therapeutic program, what was confirmed by other authors [3, 16]. However, we have noticed that once the group process was initiated at the art therapy sessions, it intensified the therapeutic process itself, and the change of topics in the group psychotherapy, in which smaller, homogeny groups of psychotic patients meet three times a week. The gap between schizophrenic and other patients visiting the Day Hospital for occupational, working and creative therapies has also been reduced.

The Day Hospital concept has more intensive therapeutic effects as compared with the out-patient treatment [16]. Such a complex integrative therapy imposed the need to set the framework and define the role of group art therapy in our Day Hospital Program. Group art therapy should comprise a clearly structured group process with limited objectives accommodated to the entire therapeutic process. It particularly refers to psychotic patients since the projective potentials of their drawings may additionally provoke the feeling of being threatened and the opposite reaction in them. Therefore, there is a need for a constant supervision of group therapists as members of the Day Hospital therapeutic team dealing with comprehensive diagnostic explorations of each patient, and monitoring of the whole pharmacotherapy, psychotherapy and sociotherapy.

In order to assess the effectiveness of group art therapy in schizophrenic patients, a clinical investigation of the differences in functioning and the severity of the condition between the group undergoing conventional therapy, and the other one in which group art therapy is used as an adjunct method would be necessary.

Conclusion

Group art therapy is considered a useful adjunctive method applied within the framework of the integrative approach to diagnosis and treatment of schizophrenia. It stimulates the development of creative potentials, building of self-esteem and self-confidence, along with destigmatization of schizophrenic patients, facilitating, thus, the integrative

therapeutic processes in a day hospital setting, and patient's social reintegration within the community. On the other side, its application enriches the whole diagnostic and therapeutic processes conducted by the psychiatrists and psychologists in a day hospital team, and helps understand the inner world of patients. Ostensibly simple, it is a complex adjunctive therapy, which illustrates the course of therapy and the patient's rehabilitation. Close observation of series of drawings might be useful for getting an insight into the effects and the course of integrative therapy for each patient.

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Na drugoj stranici nalazi se strukturisani apstrakt sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **uvod i cilj** rada, osnovne procedure - **metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi - **rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt (**250** reči) ima podnaslove: *uvod/cilj, metode, rezultati* i *zaključak*. Za apstrakte na engleskom dozvoljeno je i do **450** reči. Strukturisani apstrakt je obavezan za metaanalize (istog obima kao i za originalne članke) i kazuistiku (do 150 reči, sa podnaslovima *uvod, prikaz slučaja i zaključak*). Ispod apstrakta, pod podnaslovom „Ključne reči“ predložiti 3–10 ključnih reči ili kratkih izraza koji oslikavaju sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Zaključak** može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije. U **uvodu** ponovo napisati naslov rada, bez navođenja autora. Navesti hipotezu (ukoliko je ima) i ciljeve rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo strogo relevantne podatke iz literature i ne iznositi opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

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Literatura se u radu citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i dodaje et al. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma.

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.

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

Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fusnoti, ne u zaglavlju. Za fus-notu koristiti sledeće simbole ovim redosledom: ***, †, ‡, §, ||, ¶, **, ††, ...** Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

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The second page should carry a structured abstract with the title for original articles, metanalyses and case reports. The abstract should state the purposes of the study or investigation, basic procedures (selection of study subjects or laboratory animals; observational and analytical methods), main findings (giving specific data and their statistical significance, if possible), and the principal conclusions. It should emphasize new and important aspects of the study or observations. S t r u c t u r e d abstract should contain typical subtitles: *background/aim, methods, results* and *conclusion*. The abstract for metaanalyses and obrginal papers should have up to 450 words, and up to 150 words for case reports (with subtitles *background, case report, conclusion*). Below the abstract authors should provide, and identify as such, 3–10 key words or short phrases that will assist indexers in cross-indexing the article and will be published with the abstract.

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

Discussion is to emphasize the new and important aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

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Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

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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