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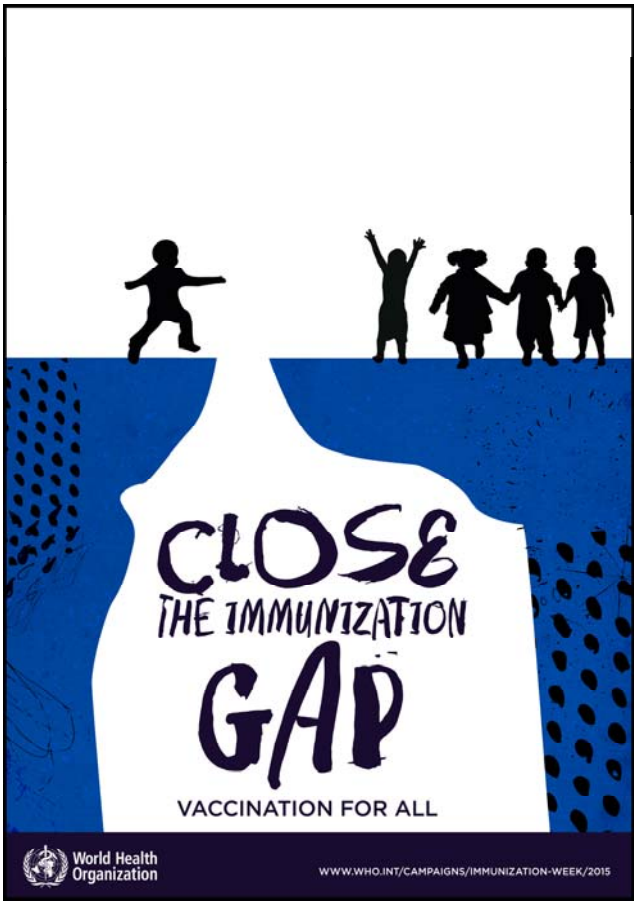

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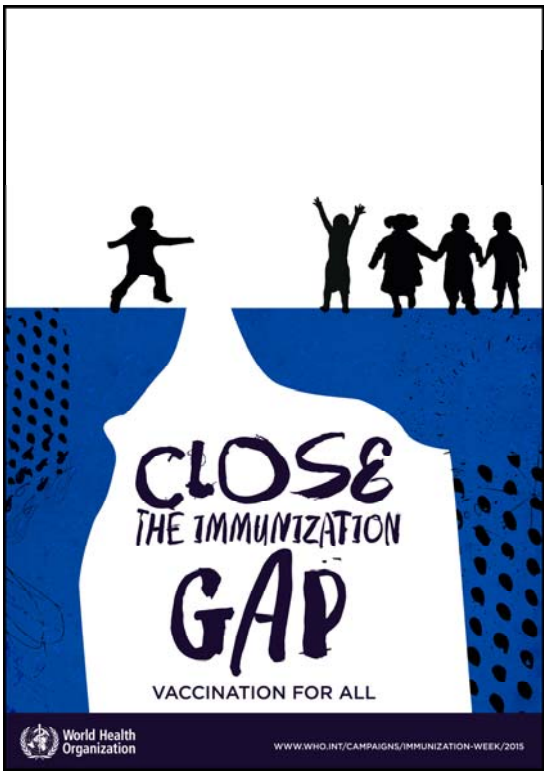

World Immunization Week is celebrated in the last week of April each year with the aim to promote the use of vaccines to protect people of all ages against communicable diseases. This year`s World Immunization Week campaign focuses on a renewed global, regional, and national effort to increase awareness of the whole society on need for immunization and improving regular vaccination delivery service (see Editorial, pp. 395-6).

Svetska nedelje imunizacije obeležava se svake godine u poslednjoj nedelji aprila sa ciljem da se promoviše upotreba vakcina u zaštiti ljudi svih starosnih grupa od zaraznih bolesti. Kampanja povodom ovogodišnje Svetske nedelje imunizacije stavlja u fokus obnovljene napore na globalnom, regionalnom i nacionalnom nivou čiji je cilj podizanje svesti celog društva o potrebi za vakcinacijom i poboljšanju rada službi za njeno redovno sprovođenje (vidi Uvodnik, str.395-6).

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

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Significance of immunization for public health

Značaj imunizacije za javno zdravlje

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Contagious diseases have been the major cause of death, especially in younger population, for thousands of years. People used various means to fight infection, unfortunately with poor results due to the fact that they knew nothing about the etiology and the mechanisms of disease transmission. Thus, big epidemics used to extinguish only by natural immunization or death. The discovery of vaccine and immunization saved more lives than any other intervention for health protection. Immunization is the fastest and cheapest way to control, eliminate, and finally eradicate many contagious diseases, being also the most powerful means that modern medicine could offer to humanity. Smallpox, a pernicious and severe viral disease, has been eradicated at a global level, so vaccination has been stopped. In 1972 was the last epidemics of smallpox in Serbia when 175 cases were registered out of who 35 died. That epidemics was stopped by urgent application of obligatory vaccination within the entire then Yugoslavia, and a wide action here and worldwide resulted in a complete eradication of this disease in 1979.

There is a long tradition of immunization in Serbia and many decades of efforts led to eradication of infantile paralysis (last cases registered back in 1996 in the area of Kosovo and Metohija, and in 1963 in the Central Serbia), elimination of diphtheria, while some diseases that could be prevented with immunization were reduced to only individual cases. Up to the 80s of the 20th century measles, rubella, and parotitis were common diseases at infant age, sometimes manifested by hard forms and complications, even death. Thanks to vaccination they are very rare today, so it is no wonder we almost forgot them. Before immunization measles caused death in thousands of children in Europe and the USA. More than 30,000 children used to get sick, and a few hundred die. In the 40s and 50s of the last century many tens of thousands of children got disability after catching infantile paralysis. The number of children with this disease in Serbia in 1956 was 854 having permanent consequences, while 44 of them died. These diseases have not changed, they still could cause paralysis, pneumonia, suffocation, brain damage, cardiac and many other problems in not vaccinated children. Children still die of these diseases in some parts of the world.

Immunization leads to a gradual reduction of the number of diseased due to a double effect: there appears individual immunity, similar to the natural one, on one side, and a vaccinated person is protected against infection for many years, sometimes even permanently. On the other side, there is the effect of making collective immunity. A cause present in the population could stay into it, could not spread since there are no sensitive individuals to grow and multiply in and to further transmit from. The cycle of natural spreading of infection ends, thus no epidemics could burst. To keep on the obtained results and achieve new aims (elimination of measles, rubella, and congenital rubella syndrome) it is necessary that each child get a dose of each vaccine, to the maintain immunization coverage of more than 95% that is one of the most important issues for public health.

A decrease in the vaccination coverage implies the appearance of epidemics. In the past years the countries of the European region were faced with epidemics of measles, rubella, and parotitis in sensitive, not vaccinated population. There are no borders for infections, so epidemic spreads from country to country. In 2009, for example, a total of 7,175 cases of measles were registered, out of whom 91% in 5 countries (Bulgaria, France, Switzerland, Great Britain, and Germany), while 10 individual died (3 months to 39 years old, 7 in Bulgaria, 2 in France, and 1 in the Netherlands). In 2010 a total of 30,367 cases of measles were registered, the majority in Bulgaria, France, Germany, and Italy. Out of that number 21,877 cases were treated in the hospital, 21 died (18 in Bulgaria, 2 in France, and 1 in Romania). A decrease in the vaccination coverage caused the reappearance of diphtheria in the countries of former SSSR, whooping cough in Britain, measles in Europe, infantile paralysis in numerous countries. Under such conditions it is

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more difficult or impossible to implement vaccination to the necessary coverage and rate.

Anti-vaccination movement in Serbia, culminating in the 2009/2010 swine flu pandemic, now shows its adverse effects on vaccination coverage as the increasing number of diseased children and the announced measles epidemic in a few parts of our country. Public confidence in vaccines and vaccination has been weakened, so in time it could compromise all the positive effects of immunization that were difficult to obtain over decades. Anti-vaccination movement worldwide tends to lessen the significance and points out, even fabricates, adverse effects of immunization, with no arguments and proofs, all the time doubtful about the ethics and humanity of the promoters of immunization, that is health workers. Every single negative information about vaccination spreads around quickly, also thanks to the Internet. Unfortunately, this is not the first time since that anti-vaccination movement appeared in history before. In the 70s in England spread the story about adverse effects of the whooping cough vaccine, about it as a conspiracy hatched by pharmacology, that resulted in the decrease of the vaccination coverage to 39%. Whooping cough, eradicated disease, came back and killed a great number of children, then people started to vaccinate children again. If anti-vaccination movement succeeded today, it would be easy to imagine the consequences. So, countries that take care of their nations work hard to promote immunization.

Studies performed worldwide indicate immunization as the most secure way to protect health of children. It is known for sure today that vaccine does not cause autism, diabetes, multiple sclerosis, asthma, nor attention disorder (hyperactivity) in children. Numerous studies confirmed no association between autism and MMR (measles/mumps/rubella) vaccine, such as the one performed in Denmark on half a million children born in 1991–1998. A total of 82% of those children were vaccinated with MMR, but the risk for autism was the same both in vaccinated and not vaccinated children: there is no association of MMR vaccine and autism.

Vaccines used in Serbia are safe. There are adverse effects of any drugs, while those of a vaccine are mild pain in a hand or leg to which it is administered or negligible higher temperature, very rarely they are severe. Eventual adverse vaccine effects are prevented by temporary or permanent contraindications prior to vaccination, safe vaccination, strictly recommended vaccines combination, time period between two succeeding vaccines, etc. Anyhow, the advantages of vaccination surpass the risks for eventual adverse effects.

Immunizations are realized as national programs that are based on a long-standing tradition, experience, and research. Disease prevention, including vaccination as the most effective and specific measure, is far more cost-effective than treatment itself. There is even no need to mention the significance of the reduction in death rate. So, there is no a single person, the doctor, nor health institution behind immunization, but the whole community and national interest, the whole state. Immunization with no support of the whole state is not possible. Thus, citizens themselves, as well as the parents alone, could not stand the burden of making decision on immunization.

It is a general interest of all of us, of our posterity, their health and well-being that the common sense prevails and immunization as the public health measure turns into more modern vaccines and discovering new using and appreciating the major principle of medical ethics – *Premium non nocere* (First, do no harm).

ORIGINAL ARTICLES

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Influence of suturing material on wound healing – An experimental study on dogs

Uticaj materijala za šivenje na zarastanje rana – eksperimentalna studija na psima

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Abstract

Background/Aim. The most common materials implanted in the human organism are suture materials that are classified on the basis of several criteria, usually the origin, structure, and properties. The properties of suture materials are related to its absorbability and non-absorbability. When using resorbable materials it is of great importance to determine whether its absorbability and tensile strength help wound healing in function of time. Sutures themselves can become a source of inflammation, that may reduce or compromise the potential of reparation and regeneration. The aim of this experimental study on dogs was to ascertain whether the absorption rate and the degree of local tissue reactions differ from information provided by the manufacturers, whether there are differences between the applied suture materials and which of the used suture materials have better effect on wound healing. **Methods.** Experimental testing of the selected suture materials basic characteristics was performed on 6 German Shepherd dogs, which, after induction of general anesthesia, were made 3 identical incisions each in all 4 quadrants (left and right side of the upper and lower jaws), so that 12 horizontal incisions were formed, 10 mm long, 20–25 mm distant from one another, on each animal. Randomly, incisions were stitched up in the following order, starting from back to front: catgut, Dexon®, Vicryl-Rapid®. The experiment was terminated by histopathological examination of tissue samples, taken on postoperative day 3, 7, 14 and 21 in order to identify the effect of healing and the degree of local reaction. **Results.** The obtained results suggest that catgut has the highest absorption rate, while Dexon® the lowest. Vicryl-Rapid® causes the lowest level of local reactions, while Dexon® the highest. **Conclusion.** There is no ideal suture material because various patient factors also influence the wound healing process.

Key words:
oral surgical procedures; suture, techniques; catgut; wound healing; dogs; histological techniques.

Apstrakt

Uvod/Cilj. Najčešći materijali koji se ugrađuju u organizam čoveka jesu materijali za šavove koji se klasifikuju prema više kriterijuma, najčešće prema poreklu, strukturi i osobinama. Osobine šavnog materijala odnose se na njegovu resorptivnost, odnosno neresorptivnost. Pri upotrebi resorptivnih materijala važno je utvrditi da li njegova resorptivnost i tenziona snaga pomažu zarastanju rane u funkciji vremena. Sami konci mogu postati izvor upale koji može sniziti ili ugrožititi potencijal reparacije i regeneracije. Cilj ove eksperimentalne studije bio je da se utvrdi da li se brzina resorpcije i stepen lokalne reakcije tkiva razlikuju od podataka koje daje proizvođač, da li postoje razlike između primenjenih materijala za šavove, kao i koji od njih bolje utiče na zarastanje rane. **Metode.** Eksperimentalna studija sprovedena je na šest nemačkih ovčara, kojima su načinjene po tri identične incizije u sva četiri kvadranta (sa leve i desne strane u gornjoj i donjoj vilici). Tako je kod svake životinje formirano 12 horizontalnih incizija, dužine 10 mm, međusobno udaljenih 20–25 mm, koje su ušivene trima različitim šavnim materijalima: ketgat, Dexon®, Vicryl-Rapide®, a eksperiment je okončan patohistološkim pregledom preparata, uzetih trećeg, sedmog, četrnaestog i dvadesetprvog postoperativnog dana, kako bi se video efekat zarastanja i stepen lokalne reakcije. **Rezultati.** Rezultati pokazuju da najbržu resorpciju ima ketgat, a najsporiju Dexon®. Najmanji stepen lokalne reakcije izaziva Vicryl-Rapide®, a najveći Dexon®. **Zaključak.** Idealan materijal za šavove ne postoji. Različiti faktori koji su povezani sa pacijentom takođe utiču na proces zarastanja rane.

Ključne reči:
hirurgija, oralna, procedure; šavovi, tehnike; šavovi, materijali; rana, zarastanje; psi; histološke tehnike.

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Introduction

Surgical suture materials, in the strict sense suture, denote material used for reconstruction of tissue, hemostasis (ligation of blood vessels), fixing of tissue, as well as various transplants.

The most common materials implanted in the human organism are suture materials. It is estimated that 3 million individual stitches are placed worldwide on daily basis [1]. Stitching up wounds is an ancient skill mentioned in Egyptian writings from 3,500 BC [2]. Ancient Egyptian mummies are found with body cavities stitched up with suture materials made of animal tissue (tendons), braided horsehair, leather strips and plant fibers [2].

Centuries later, in the writings De Medicina, Celsus wrote about stitching up soft tissue with human hair [3]. Galen, the physician of Roman gladiators, recommended using silk and hemp thread for hemostasis in the year 400 BC [4].

In Renaissance, Andreas Vesalius recommended stitching up fresh wounds, tendons and nerves [4]. Physick, professor of surgery at the University of Pennsylvania, made absorbable suture materials of goat and deer skin in 1806 [3]. Catgut sutures were coated with chromium in 1876, which resulted in stronger stitch. Wound healing was enhanced when Joseph Lister introduced carbolic acid as suture disinfectant [3].

The first absorbable synthetic suture material made of polyvinyl alcohol was manufactured in 1931. In the same year, BASF laboratories produced the first polyamide suture (supramid), which was introduced in clinical practice after WWII. In the second half of the sixties it was discovered that polyglycolic acid could provide a material of particularly favorable features, so the first derivative of this substance called Dexon® appeared in 1970 [5, 6].

Regardless of the fact that technological development of suture materials starts in the 30s of the 20th century, owing to development of new technologies and research, there is a great variety of these materials of different characteristics nowadays. However, the ideal suture material has not been found yet.

Suture materials are classified on the basis of several criteria, usually the origin, structure, and properties [7].

By origin, surgical sutures can be natural and synthetic; by structure they can be monofilament, multifilament (braided, twisted and spun) and pseudo-monofilament; by properties in tissue they can be absorbable and non-absorbable.

Healing of superficial wound tissue usually takes five to ten days, but some surgical procedures require sutures to persist 14 to 28 days [8].

Absorbable sutures are degraded and thus gradually lose strength in supporting tissues. As defined by the US Pharmacopoeia, most tensile strength of absorbable sutures is lost during the period of 60 days, as opposed to non-absorbable that retain it for longer than 60 days.

Synthetic absorbable sutures are dissolved by hydrolysis, which takes place in rather precise timeframe. Given that absorbable suture materials are dissolved under the influence of proteolytic enzymes or hydrolysis, it would be preferable not to remain in tissue longer than necessary.

Sutures themselves can become a source of inflammation, which may reduce or compromise the potential of reparation and regeneration. There are not many references comparing or discussing properties and quality of absorbable suture materials [9–12].

Oral surgery interventions are customarily finished by stitching surgical wound up [13], and choice of most appropriate suture material depends on the site and depth of tissue to stich. Given that this type of intervention includes stitching up overlying tissue, routinely used are non-absorbable materials (usually silk sutures), which are removed after 5–7 days.

In some situations, however, when it is not possible to remove the placed sutures, absorbable materials are used for oral surgery interventions in case of persons with disability who are not able to cooperate, when removing sutures would imply induction of general anesthesia, for children and for soldiers from remote units, without adequate conditions for revisit and removing sutures (this can be quite common in states of emergency and during wartime).

From the viewpoint of their usage in oral surgery, absorbable suture materials are required to preserve their tensile strength long enough, not to have absorption time shorter than necessary for wound to heal, to keep adherence of soft sediments on sutures as slight as possible and, despite enzyme absorption process, to keep inflammatory reaction least intensive possible [14–20].

Information concerning these characteristics of specific absorbable materials is included in the manufacturers instruction manuals and stipulated prior to their mass production. However, neither measuring methods nor types of tissues used for placing these materials during tests, are known.

The aim of this study was to ascertain whether the absorption rate and degree of local tissue reactions differ from information provided by the manufactures, whether there are differences between the applied suture materials and which of the used suture materials have better effect on wound healing.

Methods

Experimental testing of basic characteristics of the selected suture materials was performed on 6 German Shepherd dogs, which, after induction of general anesthesia, were made 3 identical incisions each in all 4 quadrants (left and right side of the upper and lower jaw), so that 12 horizontal incisions were formed, 10 mm long, 20–25 mm distant from one another, on each animal.

The experimental part of the study was fully implemented in accordance with the Act "The ethical principles of scientific experimental animals research", Military Medical Academy, Act No. 282-10, issued on November 20, 2002.

All experimental animals were 6 years old. Dogs were marked randomly with numbers 1 to 6. Dog number 1 male, body weight 25 kg; dog number 2, female, body weight 30 kg; dog number 3, male, body weight 24 kg; dog number 4,

male, body weight 27 kg; dog number 5, male, body weight 21 kg and dog number 6, female, body weight 19 kg.

The animals were administered atropine in the dose 0.02–0.04 mg *per* kg of body weight, subcutaneously (SC), 15 min prior to administering anesthetics. After 15 min, they were administered propionylpromazine (Combelen®), 0.03 mL *per* kg of body weight intravenously (IV) and Ralatek® (ketamin) 5%, 0.3 mL *per* kg of body weight intramuscularly (IM) [20]. Upon induction of anesthesia, 3 horizontal incisions, 1 cm long each were made in the vestibula of the animals, on the left and right side of upper and lower jaws, with equal spacing (Figure 1A).

Randomly, incisions were stitched up in the following order, starting from back to front: 1. catgut, 2. Dexon®, 3. Vicryl-Rapid®. Each incision was stitched up with individual stitch in the given order. Incisions were stitched up with catgut 3.0, Dexon® 3.0, Vicryl-Rapid® 3.0, with identical 3/8 circle needles (Figure 1B). Knots were tied using identical technique – the first knot was tied in clockwise direction, the second one counter-clockwise, and the third one in the same direction as the first one. So each animal had 4 incisions stitched up with each of the 3 types of material, or 12 incisions total. A total of 72 incisions were made and 72 individual stitches were tied with triple knots, or 24 *per* type of suture material on day zero when the experiment commenced. The appearance of the stitched up incisions on the postoperative day 7 is shown in Figure 1C.

Tissue samples for histological assessment of the speed of absorption and the degree of local reaction to implanted suture material were taken at predetermined time intervals. Each animal was taken biopsy on postoperative days 3, 7, 14 and 21 in the following way: biopsy from the upper right quadrant was taken on postoperative day 3, biopsy from the upper left quadrant was taken on postoperative day 7, biopsy from the lower left quadrant was taken on postoperative day 14 and biopsy from the lower right quadrant was taken on postoperative day 21 (Figure 1D) for all 3 types of suture material. Each section-taking on the preset days included 18 biopsies.

After reinduction of general anesthesia in the same way as on day zero, biopsy was taken from the upper jaw right on day 3, from the upper jaw left on day 7, from the lower jaw left on day 14 and from the lower jaw right on day 21, for all 3 types of suture materials by taking circular sections of gingiva and submucosal tissue, 2 cm diameter, including sutures.

Section wounds were stitched up (Figure 1 E) with catgut 4.0, which was not removed, and sections were fixed in 10% neutral buffered formalin solution, packed in sterile vials and marked with letters A (catgut), B (Dexon®) and C (Vicryl-Rapid®) and the numbers 3, 7, 14 or 21. Prepared specimens were sent to the Institute of Pathology, Military Medical Academy, where they were embedded in paraffin molds (Figure 1F), cut with microtom 5–7 microns thick, and stained using hematoxyllin and eosin, and the specific histochemical techniques: Masson trichrome, Paff Halmi periodic acid-schift (PAS) and PAS diastasis. Histopathological examination of specimens was then performed.

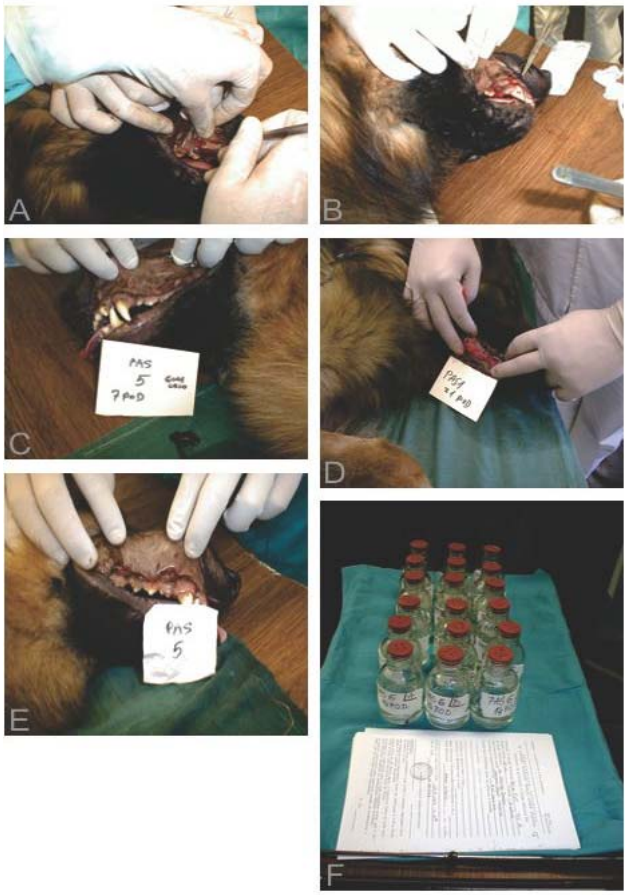


Fig. 1 – A) Incisions on experimental animals; B) Stitching up incisions on experimental animals; C) The appearance of the stitched up incisions on the postoperative day 7; D) The appearance after taken biopsies on the postoperative day 21; E) Stitched up biopsy sites; F) Vials with the taken sections soaked in formalin and in order for pathohistological analysis.

Results

Experimental part of the study was finalized with histopathological analysis of the specimens.

Histopathological findings on the postoperative day 3

On the postoperative day 3, on the sections taken from the postoperative wound sites stitched up with catgut, from all 6 dogs, a part of stratified squamous epithelium, and the connective tissue with blood vessels underneath were visible. Parts of suture were visible in one spot and gingival defects were also present in that area. In the connective tissue surrounding sutures, rare cells of chronic inflammation, predominantly lymphocytes were visible. On the postoperative day 3 intensive inflammation surrounding the implanted sutures was detectable, with inflammation cells including lymphocytes, leukocytes and plasma cells. There was no formation of new connective tissue nor the presence of new blood vessels (Figure 2a).

On the postoperative day 3, on the sections taken from all 6 dogs from the sites of postoperative wounds stitched up with Dexon®, preserved stratified squamous gingival epithe-

lium was visible, and connective tissue underneath with fresh bleeding spots. Gingival epithelium was missing in one spot and deep cavity protruding into the connective tissue was detectable, with remains of suture in it (Figure 2b).

In the wide area around the described cavity, rich nonspecific chronic inflammation cells infiltration, prevailingly lymphocytes were visible. There was sporadically a formation of foreign body type cells. On the postoperative day 3, intensive inflammation surrounding the implanted sutures was detectable, with inflammation cells that included lymphocytes, leukocytes and plasma cells. There was no formation of new connective tissue nor the presence of new blood vessels.

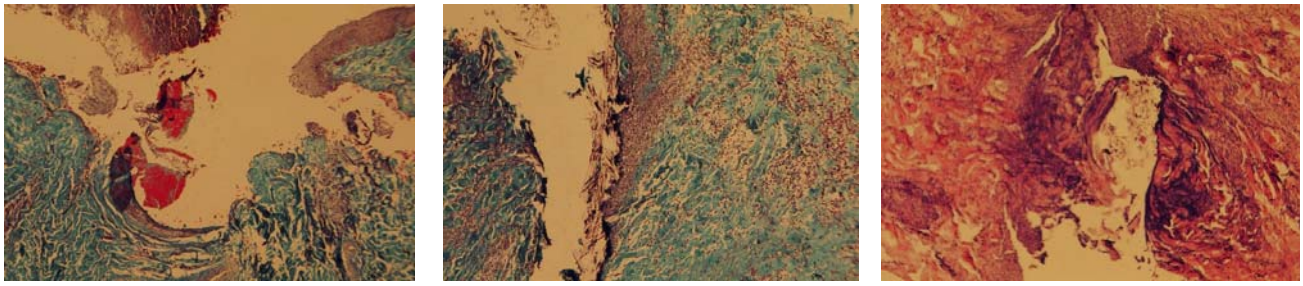
On the postoperative day 3, on the sections taken from all 6 dogs from the sites of postoperative wounds stitched up with Vicryl-Rapid®, partly preserved stratified squamous gingival epithelium was visible. Remains of surgical sutures were visible in one spot. Around this area, as well as in somewhat broader range, in the connective tissue, rich nonspecific chronic inflammation cells infiltration was present, mainly lymphocytes (Figure 2c).

sue revealed a point rich in lymphocytes and leukocytes, with a great number of new blood vessels (Figure 3b).

Slight residues of surgical sutures were sporadically visible. Lymphocytes and leukocytes infiltration were present also in other parts of connective and muscle gingival tissues.

On the sections taken from all 6 dogs at the sites of postoperative wounds stitched up with Dexon®. Preserved stratified squamous gingival epithelium was visible. Rare lymphocytes were found at the site of implanting surgical sutures in the connective tissue, while many fibroblasts and fibrocytes with initiated formation of granulation tissue were present (Figure 3c).

A large number of new blood vessels could be seen. Inflammatory changes were still present, as well as remnants of surgical sutures. One histological specimen (tissue section taken from dog number 2 showed a cavity in the connective gingival tissue with minor fragments of surgical sutures. This cavity was mainly surrounded by rich clusters of lymphocytes and leukocytes, with eosinophilic albuminous mass between them. The remaining connective and muscle tissue



a) **b)** **c)**

Fig. 2 – Histopathological finding on the postoperative day 3.
a) Catgut (Masson Trichrom staining, 40 ×); b) Dexon®: gingival defect and remains of suture (Masson trichrom, 10 ×); c) Vicryl-Rapid®: gingival defect and the remains of suture (hematoxylin and eosin, 10 ×).

On the postoperative day 3, intensive inflammation was detectable with inflammation cells that include lymphocytes, leukocytes and plasma cells. There was no formation of new connective tissue nor the presence of new blood vessels.

Besides taking sections for histopathological examination on the postoperative days 3, 7, 14 and 21 there were records about the presence of each single stich implanted on the day-zero of the experiment (for all 3 types of suture materials).

Histopathological findings on the postoperative day 7

On the sections taken from all 6 dogs on sites of postoperative wounds stitched up with catgut, a part of stratified squamous epithelium, and connective tissue with blood vessels underneath were visible. Parts of suture were visible at one spot and gingival defect was also present in that area. In the connective tissue surrounding sutures rare cells of chronic inflammation, predominantly lymphocytes were visible (Figure 3a).

Inflammatory changes were still evident, but to a somewhat lesser extent. Microscopically visible residues of sutures were present. Histopathological specimen (tissue section taken from dog number 3 of the gingival connective tissue was permeated by inflammatory cells, prevailingly lymphocytes and leukocytes.

On the sections taken from all 6 dogs at the sites of postoperative wounds stitched up with Vicryl-Rapid®, the connective gingival tissue still included a significant number of lymphocytes, but also increased number of fibroblasts and fibrocytes, with formation of new granulation tissue. There was a spot with residues of surgical sutures in its center, which was surrounded by strongly increased number of fibroblasts, fibrocytes and new blood vessels with formation of young granulation tissue (Figure 3d).

Rare lymphocytes and leukocytes were visible. The site of Vicryl-Rapid® placement showed still present cell inflammation, but significantly milder. Rare fragments of surgical sutures and increased number of new blood vessels were visible.

Histopathological findings on the postoperative day 14

On the sections taken from all 6 dogs at the sites of postoperative wounds stitched up with catgut, mainly preserved gingival epithelium was visible. In the gingival connective tissue surrounding the site of implanted surgical sutures, proliferation of new connective tissue with large number of new blood vessels, fibroblasts and fibrocytes were

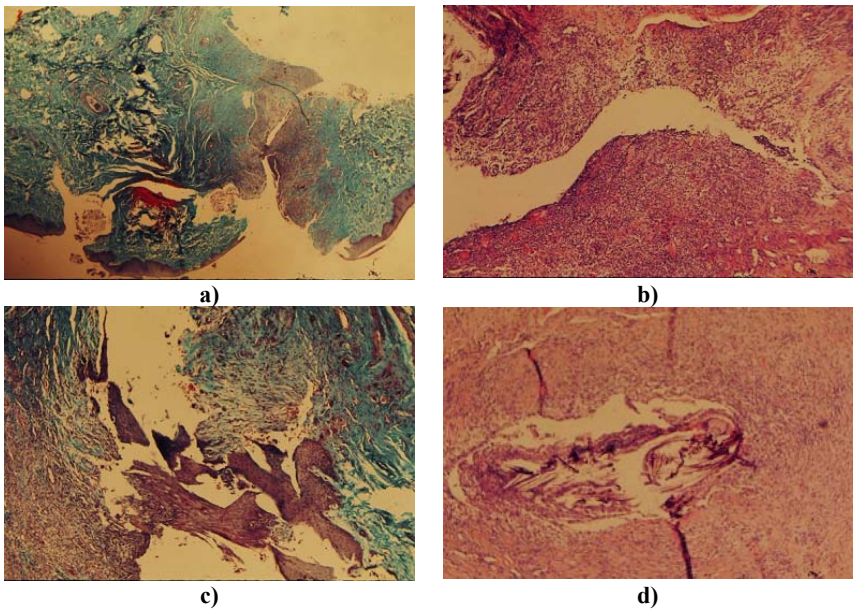


Fig. 3 – Histopathological finding on the postoperative day 7.
a) Catgut (Masson Trichrom, 10 ×); b) Catgut (hematoxylin and eosin, 10 ×); c) Dexon®: (Masson Trichrom, 10 ×); d) Vicryl-Rapid® (hematoxylin and eosin, 10 ×).

found whose lumen was filled with blood. The granulation tissue was surrounded by clusters of chronic inflammation cells, mainly lymphocytes (Figure 4a). Rare giant cells were also present. Traces of chronic inflammation were still visible in the connective tissue, as well as remnants of surgical sutures. The number of new blood vessels was somewhat increased.

On the sections taken from all 6 dogs at the sites of postoperative wounds stitched up with Dexon®, cavity with fragments of surgical sutures was evident. This cavity was surrounded by multiplied fibroblasts and fibrocytes, as well as new blood vessels. Rare lymphocytes and giant cells were present. Traces of chronic inflammation in the connective tissue and fragments of surgical sutures were still visible. A somewhat increased number of new blood vessels were present (Figure 4b).

cal sutures were visible only at the surface beneath epithelium. The surrounding tissue contained individual chronic inflammation cells, a large number of new blood vessels, increased number of fibroblasts and the newly formed granulation tissue (Figure 4c).

Histopathological findings on the postoperative day 21

On the sections taken from all 6 dogs at the sites of postoperative wounds stitched up with catgut, in the gingival connective tissue surrounding the site of implanted surgical sutures numerous macrophages, lymphocytes and leukocytes and slightly increased numbers of fibroblasts and fibrocytes were visible. Traces of chronic inflammation were still present. There were increasing numbers of new blood vessels and fibroblasts and the beginning of formation of new elastic tissue was evident (Figure 5a).

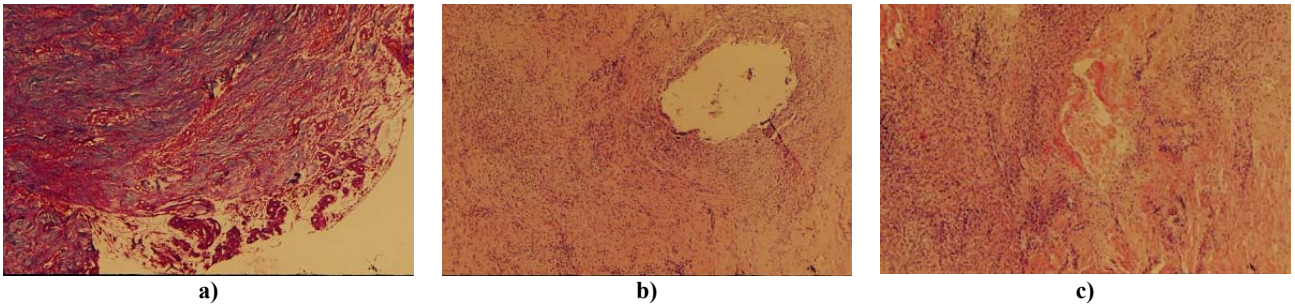


Fig. 4 – Histopathological finding on the postoperative day 14.
a) Catgut (Periodic acid-schiff, 10 ×); b) Dexon® (hematoxylin and eosin, 10 ×); c) Vicryl-Rapid® (hematoxylin and eosin, 10 ×)

On the sections taken from all 6 dogs at the sites of postoperative wounds stitched up with Vicryl-Rapid®, a focus of newly formed connective tissue with large number of fibroblasts and fibrocytes was visible in the gingival connective tissue on the site of implanting surgical sutures. No chronic inflammation cells were present. Fragments of surgi-

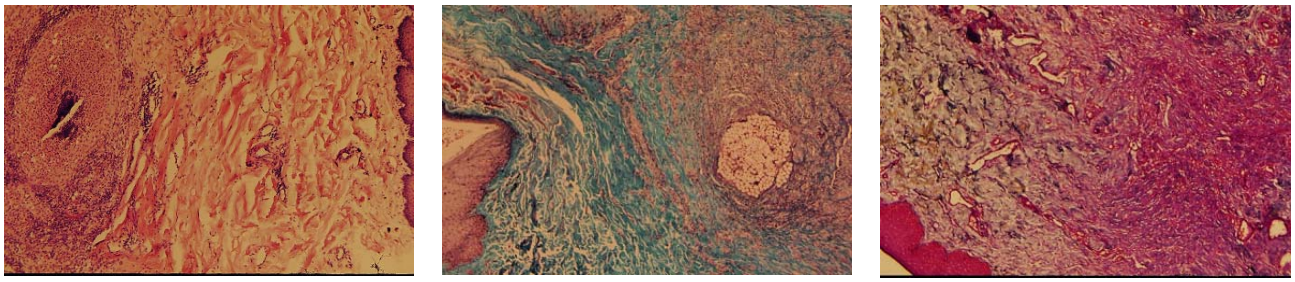
On the sections taken from all six dogs at the sites of postoperative wounds stitched up with Dexon®, the gingival connective tissue contained residues of surgical sutures, surrounded by proliferation of fibroblasts and fibrocytes, new blood vessels, phagocytes and still rich chronic inflammation cells infiltration (Figure 5b). Mature newly formed elastic

tissue with increased number of newly formed blood vessels in it was present. The connective tissue surrounding the sites of the newly formed elastic tissue was preserved, made of collagen fibers. Multiplication of new blood vessels and formation of elastic fibers with rich chronic inflammation cells infiltration was evident.

On the sections taken from all 6 dogs at the sites of postoperative wounds stitched up with Vicryl-Rapid®, the connective gingival tissue at the site of sutures contained new connective tissue, with spots towards mature connective tissue covered by a layer of epithelium and with rare lymphocytes (Figure 5c). Some connective tissue sections included spots of new granulation tissue with numerous fibroblasts, fibrocytes and new blood vessels. The number of chronic inflammation cells was still significant (Figure 5c). The presence of suture in the wound as a function of time is shown in Figure 6.

ences between implanting these materials in various human tissues [21–29].

Nary Filho et al. [21] conducted a comparative study on Wistar albino rats, comparing Poliglecaprone 25, Polyglactin 910 and Politetrafluoroethylene suture materials. They observed inflammation, the degree of fibroblastic and angioblastic proliferation and the presence of fibrous tissue around implanted sutures. Nary Filho et al. [21] say that Polyglactin 910 is one of the preferred in dentistry. It shows no intensive local reaction. Its implantation includes mediocre acute inflammatory response and early fibroblastic and angioblastic proliferation. It also allows subsequent organization of connective tissue around suture filaments. Our experiences with Vicryl® are positive and we partly agree with these authors. A shortcoming of Vicryl-Rapid® is the possibility of loosening of knots (because it is coated). This shortcoming may be overcome by tying 3–5 knots. As for



a) b) c)

Fig. 5 – Histopathological finding on the postoperative day 21.
a) Catgut (hematoxylin and eosin, 10 ×); b) Dexon® (hematoxylin and eosin, 10 ×); c) Vicryl-Rapid® (Paff-Halmi, 10 ×)

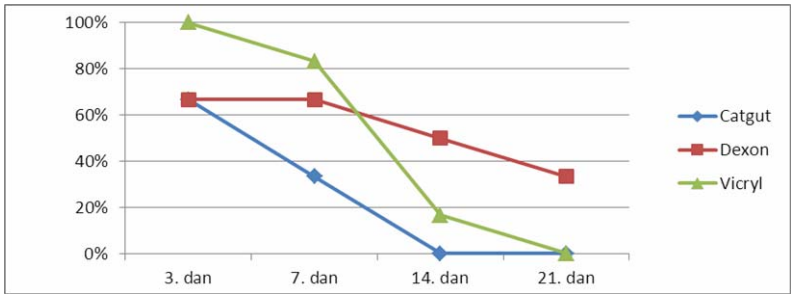


Fig. 6 – The presence of suture in the wound as a function of time.

Discussion

Information concerning characteristics of specific absorbable suture materials is included in the instruction manuals of manufacturers and stipulated prior to their mass production. However, neither measuring methods nor types of tissues used for placing these materials during tests, are known.

There are not many references comparing or discussing properties and quality of absorbable suture materials [9–12].

There is no ideal suture material, and it is therefore necessary to conduct a large number of studies that will help clinicians consider all properties of suture materials, with the emphasis on biological properties, since manufacturers indicate them very concisely and without specifying the differ-

local reaction, Nary Filho et al. [21, 25] find biological reaction to Vicryl® good, including mediocre acute inflammatory response in the early postoperative period and early fibroblastic and angioblastic proliferation. It also allowed organization of connective tissue fibers around it subsequently.

Duprez et al. [27] conducted an experimental study, which included stitching up human cadaver skin grafts on the dorsal side of experimental animals (mice) with Vicryl-Rapid®. The absorption mechanism included hydrolysis. Inflammatory reaction was present, with abundant macrophages containing suture material fragments. It was also possible that inflammation cells released lytic enzymes, which increased spontaneous lysis and lead to fragmentation of suture material. Suture material absorption time depends on the degree of local

reaction, the amount of present electrolytes and basal metabolism. Duprez et al. [27] find Vicryl-Rapid® perfect, tolerant and breaking after 12 to 16 days, with moderately present reaction of macrophages.

All data from the literature suggest that inflammatory tissue reaction is strongest with catgut. Our research shows the strongest inflammatory response with Dexon®, then with catgut, and the mildest with Vicryl-Rapid®.

Sutures used in oral surgery behave differently than when implanted in other body parts, due to tissue quality, the presence of saliva and specific microorganisms, strong vascularization, as well as the present functions of speaking, chewing and swallowing [22]. Mirković and Đurđević-Mirković [30] have studied the way suture material affects accumulation of soft deposits, soft tissue decubitus and wound dehiscence, comparing silk, Vicryl® and nylon. They did not confirm superiority of Vicryl® over the other two, suggesting that silk, followed by nylon, causes the least decubital lesions, while silk accumulates mostly soft deposits, followed by Vicryl® and nylon. Suture dehiscence is equally present with nylon and silk, while it is somewhat more extensive with Vicryl® [30]. Another study of Mirković et al. [31] examining influence of suture material on mechanical damage to the mouth, also suggest superiority of synthetic monofilaments (nylon).

Wallace et al. [32] compared polyglycolic acid, silk, chromic and flat catgut used on 52 respondents. Polyglycolic acid suture (Dexon®) caused mildest tissue reactions, less intensive than those caused by silk, flat and chromic catgut. Besides mentioning easier stitching up with Dexon® than with silk, Wallace et al. [32] suggest that Dexon® stays present in tissue 16–20 days after implanting. Catgut is absorbed after 3–5 days, and chromic after 7–10 days. According to these authors, Dexon® has properties closer to the ideal suture material than any other tested.

Besides being conditioned by the degree of local tissue reaction, suture material absorption period is also conditioned by present electrolyte concentrations and by basal metabolism. The only logical explanation of the difference in absorption period of Dexon® can be that Wallace et al. [32] perhaps equalize disappearing of knot from the mouth with complete absorption, although disappearing of knot does not necessarily imply complete absorption of suture material. Full absorption of suture material can be proven only by microscopic inspection of tissue sections taken from sites stitched up with suture material, which was not mentioned in this study as a research method.

In our experimental study, using microscopic inspection of tissue sections taken from sites stitched up with suture material, we find that catgut is absorbed most quickly, while Dexon® is absorbed most slowly.

The results of patohistological examination of specimens taken from experimental animals suggest that Dexon® causes most intensive local tissue reaction, while reaction is a lot milder with catgut. Vicryl-Rapid® caused the least intensive tissue reaction, but it was not tested by Wallace et al. [32].

In our study, histopathological examination on postoperative day 21 of specimens taken from wounds stitched up with Dexon® suggests the presence of suture fragments visible in mucosa and submucosal tissue. The hypothesis that catgut is absorbed most quickly, while Dexon® is absorbed most slowly was confirmed. It was also confirmed that Vicryl-Rapid® causes the slightest local reaction, while reaction is strongest not with catgut, but with Dexon®, which differs from information suggested in most studies and manufacturers' instructions. It is also confirmed that wounds heal more quickly with Vicryl-Rapid®, with lower dehiscence incidence and milder local reaction, than with catgut or Dexon®. Besides these properties, it is also important that local reaction is mildest possible (low antigenic potential), with the lowest possible wound dehiscence incidence. The displayed properties of Vicryl-Rapid® suggest its application whenever indication for usage of absorbable suture material is present.

Based on this study results, Vicryl-Rapid® ranks among the better suture materials.

Based on everything suggested here, we may conclude that the aim of this study was achieved, except in case of local reaction, which is not most intensive with catgut, but with Dexon®. This differs from the information suggested in most studies and manufacturers' instructions. According to histopathological analysis, Vicryl-Rapid® has properties most suitable for oral surgery of all absorbable suture materials tested. For this reason, we suggest and recommend its usage whenever there is a need for application of absorbable suture materials, until some better material is launched.

Conclusion

There is no ideal suture material for the simple fact that various patient factors also influence the wound healing process.

Based on the results obtained in the experimental part of the study, it can be concluded that catgut has the highest absorption rate, while Dexon® has the lowest one. Vicryl-Rapid® causes the lowest level of local reactions, while the highest level of local reactions is not caused by catgut, but by Dexon®. Application of Vicryl-Rapid® makes wounds heal more quickly, with a lower dehiscence rate and milder local reactions than with catgut or Dexon®.

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ORIGINAL ARTICLE

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Relationship of hepatotoxicity and the target tissue dose of decabrominated diphenyl ether in subacutely exposed Wistar rats

Odnos hepatotoksičnosti i doze dekabromovanog difeniletra u ciljnom tkivu kod subakutno izloženih Wistar pacova

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Abstract

Background/Aim. Based on numerous studies in animals, the most prominent toxic effects of decabrominated diphenyl ether (BDE-209) are observed in the liver, thyroid hormone homeostasis, reproductive and nervous systems. BDE-209 exhibits its toxic effects partly through the aryl hydrocarbon (Ah) receptor and consequent induction of hepatic microsomal enzymes. The aim of this study was to assess the hepatotoxic effect *vs* target tissue dose of BDE-209 in the subacutely orally exposed Wistar rats. **Methods.** Effects were examined on male Wistar rats, weighing 200–240 g, exposed to doses of 1,000, 2,000 or 4,000 mg BDE-209/kg body weight (bw)/day by gavage during 28 days. Animals were treated according to the decision of the Ethics Committee of the Military Medical Academy, No 9667-1/2011. Evaluation of the hepatotoxic effect was based on: relative liver weight water and food intake, biochemical parameters of liver function [aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gama glutamyl transferase (γ-GT)], and oxidative stress parameters in liver homogenates [malondialdehyde (MDA), superoxide dismutase (SOD), -SH] and morphological and pathohistological changes in the liver. For the assessment of internal dose – response relationship, lower confidence limit of Benchmark dose (BMDL) of 5% or 10% i.e. $BMDL_5$ or $BMDL_{10}$, were calculated using PROAST software. **Results.** After the application of 1,000, 2,000 or 4,000 mg BDE-209/kg bw/day, the concentrations of BDE-209 measured in liver were 0.269, 0.569 and 0.859 mg/kg of liver wet weight, (ww) respectively. Internal doses correlated with external ($r = 0.972$; $p < 0.05$) according to equation: internal dose (mg BDE-209/kg of liver ww) = 0.0002 × external dose (mg/kg bw/day) + 0.0622. Hepatotoxicity was demonstrated based on significant increase in AST and γ-GT activities and the degree of histopathological changes. The lowest $BMDL_5$ of 0.07228 mg BDE-209/kg of liver ww, correlating to external dose of 39 mg/kg/day, indicated the increase of AST activity as the most sensitive biomarker of BDE-209 hepatotoxicity in subacutely exposed rats. **Conclusion.** The results of the present work add up to the issue of BDE-209 toxicity profile with a focus on relationship between internal dose and hepatotoxicity. Critical internal dose for the effect on AST of 0.07 mg/kg of liver ww, corresponding to external dose of 39 mg/kg/day, is the lowest dose ever observed among the studies on BDE-209 hepatotoxicity. For the persistent substances with low absorption rate such as BDE-209, critical effect based on internal dose in majority of cases is considered as more precisely defined than the effect established based on external dose, particularly.

Key words:
halogenated diphenyl ethers; liver; toxicity test; rats.

Apstrakt

Uvod/Cilj. Prema podacima iz brojnih studija na životinjama, dekabromovani difeniletar (BDE-209) najznačajnije toksične efekte ispoljava na jetri, homeostazi hormona štitaste žlezde, reproduktivnom i nervnom sistemu. BDE-209 ispoljava toksične efekte delom preko receptora za aromatične ugljovodonike (Ah) i posledične indukcije mikrozomalnih enzima jetre. Cilj rada bio je procena hepatotoksičnog efekta u odnosu na dozu BDE-209 u ciljnom tkivu kod su-

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bakutno oralno eksponovanih Wistar pacova. **Metode.** Efekti su ispitivani na mužjacima Wistar pacova, mase 200–240 g, koji su putem oralne sonde primali doze od 1 000, 2 000 ili 4 000 mg BDE-209/kg telesne mase (tm) dan, tokom 28 dana. Životinje su tretirane u skladu sa odlukom Etičkog komiteta Vojnomedicinske akademije u Beogradu br. 9667-1/2011. Procena hepatotoksičnih efekata bazirana je na merenju relativne mase jetre, unosa vode i hrane, biohemijskih parametara funkcije jetre [aspartat aminotransferaza (AST), alanin aminotransferaza (ALT), alkalna fosfataza (ALP), gama glutamil transferaza (γ-GT)], parametara oksidativnog stresa u homogenatima jetre [malondialdehid (MDA), superoksid dizmutaza (SOD), -SH)] i morfoloških i histoloških promena na jetri. Za procenu odnosa interna doza – odgovor izračunavana je donja granica pouzdanosti granične Benchmark doze (BMDL) od 5% ($BMDL_5$) ili 10% ($BMDL_{10}$) primenom PROAST softvera. **Rezultati.** Koncentracije BDE-209 iznosile su 0,269, 0,569 i 0,859 mg/kg jetre nakon aplikacije 1 000, 2 000, odnosno 4 000 mg BDE-209/kg tm/dan. Interna doza u našoj studiji korelisala je sa eksternom dozom prema jednačini: interna doza (mg BDE-209/kg jetre) = 0,0002 × eksterna doza (mg/kg tm/dan) + 0,0622 (r = 0,972; p < 0,05). Hepatotoksičnost je potvrđena na osnovu nalaza o povećanju aktivnosti enzima AST i γ-GT, kao i stepena patohistološkog oštećenja jetre. Najniža $BMDL_5$ u eksperimentu od 0,07228 mg BDE-209/kg jetre, koja koreliše sa eksternom dozom od 39 mg/kg tm/dan izračunata je za aktivnost AST i ukazuje na to da je aktivnost AST ujedno i najosetljiviji biomarker hepatotoksičnosti BDE-209 kod subakutno eksponovanih pacova. **Zaključak.** Rezultati prezentovane studije daju doprinos pitanju toksikološkog profila BDE-209 sa fokusom na odnos između interne doze i hepatotoksičnih efekata. Kritična interna doza za efekat na AST od 0,07 mg/kg jetre, koja koreliše sa eksternom dozom od 39 mg/kg tm/dan, jeste ujedno i najniža kritična doza do sada definisana za hepatotoksične efekte BDE-209. Kritičan efekat koji se bazira na dozi u ciljnom tkivu u većini slučajeva može se smatrati preciznije definisanim od kritičnog efekta definisanog na bazi oralno primenjene doze, naročito za nedegradabilne supstance sa niskim stepenom apsorpcije, kao što je BDE-209.

Ključne reči:
difeniletri, halogenovani; jetra; toksičnost, testovi; pacovi.

Introduction

Polybrominated diphenyl ethers (PBDEs) are considered to be extremely efficient flame retardants [1, 2]. Decabrominated diphenyl ether (BDE-209) is a major component of deca-BDE commercial mixture [3–6] that can migrate from the product, get into the environment and pose a risk for the human health. The main source of exposure to BDE-209 is from the diet, however inhaled air could also contribute to its entire body burden [7, 8]. After entering the organism, BDE-209 exhibits its toxic effects partly through the aryl hydrocarbon (Ah) receptor and consequent induction of hepatic microsomal enzymes. It may also induce production of reactive oxygen species [7] found to be related to DNA damage. Based on numerous studies [8–17] on animals, the most prominent toxic effects of PBDEs are observed in the liver, thyroid hormone homeostasis, reproductive and nervous systems.

While the liver is one of the main target organs of its toxicity, the aim of this study was to assess the hepatotoxic effect of BDE-209 in the subacutely orally exposed Wistar rats. Considering that BDE-209 has poor solubility the level of its absorption and the amount reaching target tissues are difficult to predict. We assumed that the dose measured at target site is more appropriate than orally given dose, allowing us to more accurately perceive the liver changes from the relationship of internal dose and intensity of effects. Evaluation of hepatotoxic effects was based on serum liver enzymes activity: aspartat aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (γ-GT), alkaline phosphatase (ALP), degree of histopathological changes, as well as oxidative stress parameters in liver homogenates: malondialdehyde (MDA), activity of total superoxide dismutase (SOD) and content of sulfhydryl (-SH) groups.

Methods

Chemicals

BDE-209, 98% (Sigma-Aldrich, St. Louis, MA, SAD) and dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MA, SAD) were purchased from commercial sources.

Experimental animals

Male albino Wistar rats, weighing 200 g to 240 g were obtained from a disease-free stock bred at the Military Medical Academy in Belgrade, Serbia. The animals were housed in plastic cages with wire mesh top, in a climate-controlled facility with a constant 12-hour day and night cycle at 20°C to 24°C and relative humidity between 40% and 60%. The animals had free access to food and water throughout the study and were treated according to the guidelines for animal studies (No. 9667-1/2011), issued by the Academy's Ethical Committee.

Experimental protocol

After a quarantine period of 14 days, each group of animals (n = 8 *per* group) was receiving treatment solution by gavage in a volume of 0.5 mL/kg body weight (bw) day for 28 days. Control animals were receiving water (the control group), while rats in the vehicle control group were receiving DMSO alone the (DMSO group). Three groups were receiving BDE-209 as a suspension in DMSO in the doses of 1,000, 2,000, or 4,000 mg/kg bw/day (groups assigned as: $BDE\text{-}209_{1000}$, $BDE\text{-}209_{2000}$, and $BDE\text{-}209_{4000}$, respectively). The doses of BDE-209 were chosen based on literature data [18, 19]. Bws, water and food intake were recorded weekly. Clinical signs of poisoning were observed each day of the experiment.

After the period of exposure, animals were sacrificed, and samples of blood and liver were taken. Liver weight and observed morphological changes were recorded immediately. Liver enzyme activity was measured in serum samples. Samples of the liver were taken for histopathological analysis as well as for homogenisation, and these samples were stored at -80°C prior to the analysis of the oxidative stress parameters. The rest of the liver had been stored at -20°C before the analysis of BDE-209 concentration could be performed.

Determination of BDE-209 in liver

The method was based on multistep extraction from homogenised liver, extract cleaning and determination of BDE-209 by gas chromatography (GC) – electron capture detection (GC – ECD) and GC – mass spectrometry (MS). Homogenised sample of 0.5–2 g was dried and fragmented into fine powder and transferred in a glass centrifuge tube. A total of 8 mL of mixture n-hexane:dichlormethane [(8:2; (V/V)] was added to the powder, and the total amount was vortexed, sonificated and centrifuged. Supernatant was separated, dried in air flow and than resolved in 5 mL of acetonytrile. Further cleaning of sample was done using QuEChERSs (Quick Easy Cheap Effective Rugged Safe) (ENVIRO-CLEAN r EUMIV50CT, amchro GmbH, Hattersheim, Germany). After shaking, the content was centrifuged and aliquot was transfered into the tube for solvent evaporation. The dried residue was reconstituted in 1 mL of n-hexane and injected in GC. The retention time (Rt) and mass spectra (m/z values) were used for determination, and the pik area was used for quantification. Quality control of the analytical method was performed using certified reference material CIL-EDF-2524 Clean Fish (slurry) - Organic contaminants (LGC standards).

Determination of liver enzymes activities

Activity of AST, ALT, γ GT, and ALP in serum samples was determined on automatic analyser using commercial tests (Roche Elecsys 2010-cobas c 111 analyser, Roche Diagnostics, Mannheim, Germany).

Histopathological analysis of liver tissue

The liver sample was excised and fixed with 10% neutral formaldehyde. Dehydratation of tissue samples was done with graded aethanol and embedded in paraffin blocks. For semiquantitative tissue analysis, sections in 2 μm thick paraffin were stained by hematoxylin and eosin (HE) method and analyzed (Olympus-2 microscope).

Following criteria were used for semiquantitative evaluation of histopathological changes: 0 – unchanged liver`s parenchyma; 1 – single cells with intracellular oedema, vasodilatation of blood vessels and the appearance of inflammatory response cell; 2 – groups of cells with cytoplasmic vacuolation, distinct vasodilatation of numerous blood vessels (over 50%) and the accumulation of cellular infiltrate in the surrounding tissue; 3 – most cells with prominent vascular degeneration and karyopyknosis, focal accumulation of cellular infiltrate, 4 – vacuolar degeneration and karyolysis in all cells, single cell necrosis and diffuse accumulation of cell infiltrate, and 5 – massive and diffuse necrosis, foam cells and tissue degeneration. For determine these parameters, whole longitudinal and cross-sectional tissue was analysed and observed under high magnification (40×).

Determination of oxidative stress parameters

Oxidative stress parameters were measured after homogenisation of liver samples in saccharose medium. Following parametres were determined: concentration of MDA, activity of SOD and concentration of -SH groups. Proteins were measured in homogentes by Bradford [20] method.

The level of MDA in liver homogenates was based on reaction of MDA with thiobarbituric acid under acidic condition for 15 min at 95°C in termostatic water bath. In this reaction light yellow to pink colour complexes were formed depending on the concentration of MDA in tissue homogenates. Intensity of colour was measured at 523 nm and 600 nm.

Activity of SOD, EC 1.15.1.1. in homogenates was measured using the method of Misra and Fridovich [21], based on ability of SOD to inhibit spontaneous autooxidation of adrenaline under alkaline condition (pH 10.2).

The total content of -SH groups in liver homogenates was determined by the Ellman's [22] method which is based on the reaction of 2,2-dinitro-5,5-dithio-benzoic acid (DTNB) with alifatic thiol compounds in alkaline condition (pH 9.0).

Statistical analysis

A significance of the difference among the data in the groups for certain effect was determined by analysis of variance (ANOVA) and post-hoc Tukey test ($p < 0.05$) (Statistica 7.0).

PROAST software was used (RIVM, Bilthoven, Netherland) for the determination of dose-response relationship and calculation of benchmark dose lower confidence limit [(BMDL i.e. $BMDL_5$ if 5% of change in effect was considered as critical effect size – (CES)]. Moreover, associated benchmark dose to critical dose was assigned as critical effect dose (CED) [23–25].

Correlation analysis was used to estimate the relationship between external and internal dose ($p < 0.05$). The following equation was derived: internal dose [mg BDE-209/kg of liver wet weight (ww) = 0.0002 × external dose (mg/kg bw/day) + 0.0622

Results

In life toxicology

During the study period no clinical signs of poisoning were observed. Water consumption during the period of exposure decreased after the first week (Figure 1) and at

the end of study was significantly lower in rats treated with medium and the highest dose of BDE-209 (Table 1).

During the experiment there were no significant changes in food intake related to DMSO group (Figure 1).

Animals body weights were increasing during the period of exposure, but statistically significant differences in weight gain were not observed (Figure 2).

After the application of 1,000, 2,000 or 4,000 mg BDE-209/kg/day, the concentrations of BDE-209, measured in the liver, were 0.269, 0.569 and 0.859 mg/kg of the liver ww, respectively. In the control and the DMSO group concentrations of BDE-209 were below the quantification limit.

The lowest concentration of BDE-209 in the liver of Wistar rats, resulted in a significant increase in relative liver weight comparing to the control, while two highest concentrations, resulted in a significant decrease in relative liver weight compared to the results recorded in the DMSO group (Table 1). Relative liver weight was uniformly changing in the dose dependant manner, and calculated BMD_5 for internal dose of BDE-209 was 0.224 mg/kg of liver ww, corresponding to external dose of 971.4 mg BDE-209/kg/day (Table 1).

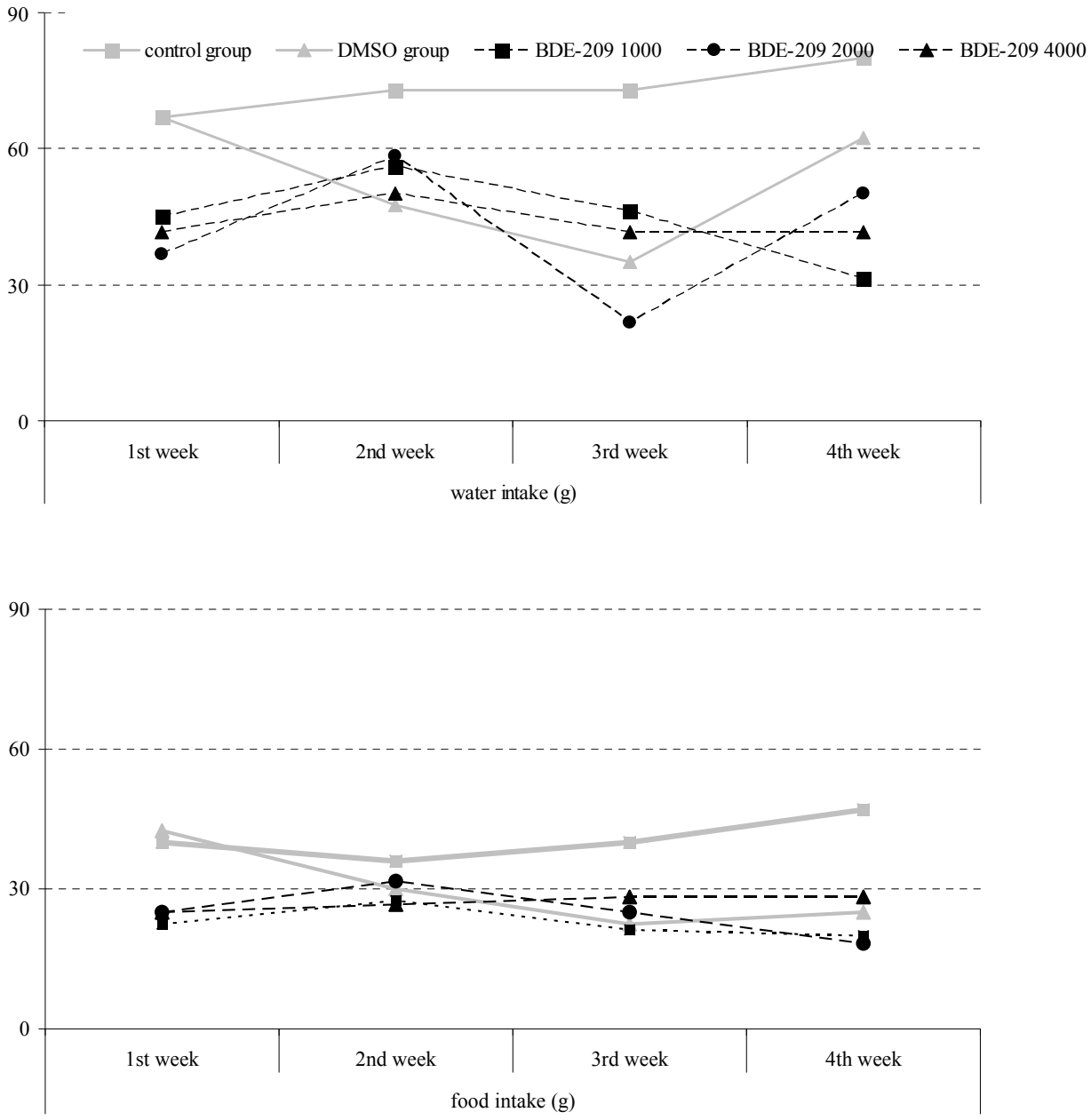


Fig. 1 – Weekly records on water and food intake in *Wistar* rats subacutely exposed to decabrominated diphenyl ether (BDE-209); DMSO – dimethyl sulfoxide.

Table 1

Influence of increasing doses of decabrominated diphenylether on liver weight, relative liver weight, and water and food intake in subacutely exposed Wistar rats

Internal dose (mg/kg)/ Dose (mg/kg bw/day)	Liver weight (g)	Relative liver weight (%)	Water intake[§] (g)	Food intake[§] (g)
0/0 $_{control}$	8.50 ± 0.38	2.72 ± 0.26	73.25 ± 5.32	40.75 ± 4.50
0/0 $_{DMSO}$	9.11 ± 0.77	3.63* ± 0.37	53.00 ± 14.61	30.00 ± 8.89
0.269/1000	10.93 ± 1.21	3.44* ± 0.29	44.69 ± 10.28	22.81* ± 3.29
0.569/2000	8.39 ± 0.83	2.75[#] ± 0.16	41.68* ± 16.02	25.00* ± 5.47
0.859/4000	6.90 ± 1.02	2.38[#] ± 0.24	43.78* ± 4.15	27.08* ± 1.58
		Results on benchmark dose and model internal dose / external dose		
BMD/BMDL	nt	<10 / <10	-	-
dose response	nt	(+) / (+)	(-)	(-)
Model	nt	E3 / E5	-	-
BMD_5	nt	0.2244 / 971.4	-	-

*** – A statistically significant difference from the control group; [#] – A statistically significant difference from the DMSO group (ANOVA, post-hoc Tukey test); [§] – Average obtained from 4 measurements, recorded weekly, during the 4-week of experiment; nt – dose response relationship was not tested; (+) or (-) – dose response relationship confirmed or not confirmed; E1-E5 – type of dose-response model given by PROAST software; BMD – benchmark dose; BMDL – 95% lower confidence limit of BMD; DMSO – dimethyl sulfoxide.**

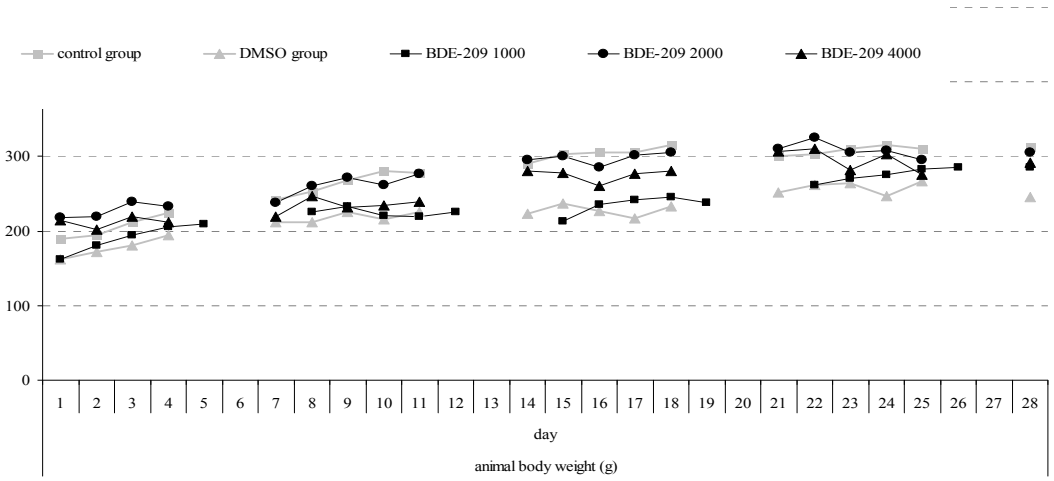


Fig. 2 – Daily records (Records were made five times a week) on body weight (g) of Wistar rats subacutely exposed to decabromineted diphenyl ether (BDE-209); DMSO – dimethyl sulfoxide.

Liver enzymes

As for the results of liver enzyme activity, a significant increase of the AST was induced by the medium and the highest concentrations, while a significant increase of γ-GT was induces by the lowest and the highest liver BDE-209 concentrations (Table 2).

The dose-response relationship was confirmed only for these two effects on liver enzymes. For the effect on AST, calculated $BMDL_5$ was 0.07228 mg BDE-209/kg of liver ww and

Table 2

Influence of increasing doses of decabrominated diphenylether on serum liver enzyme activity of subacutely exposed Wistar rats

Internal dose (mg/kg)/ dose (mg/kg bw/day)	AST (U/L)	ALT (U/L)	ALP (U/L)	γ-GT (U/L)
0/0 $_{control}$	211.0 ± 47.9	76.3 ± 21.2	295.5 ± 27.3	2.2 ± 1.0
0/0 $_{DMSO}$	221.0 ± 15.6	61.3 ± 4.11	173.5* ± 54.8	1.8 ± 0.5
0.269/1000	218.1 ± 17.4	63.4 ± 5.7	250.0 ± 37.7	4.9*,[#] ± 1.1
0.569/2000	330.0* ± 73.0	85.2 ± 22.7	166.0 ± 48.9	2.0 ± 1.3
0.859/4000	295.2* ± 33.9	72.2 ± 10.8	184.8 ± 31.4	4.4*,[#] ± 2.3
	Results on benchmark dose and model internal dose / external dose			
BMD/BMDL	<10 / -	- / -	- / -	- / -
dose response	(+) / (-)	(-) / (-)	(-) / (-)	(+) / (+)
Model	E2 / -	- / -	- / -	E1 / E1
BMD_5	0.07228/ -	- / -	- / -	- / -

*** – A statistically significant difference from the control group; [#] – A statistically significant difference from the dimethyl sulfoxide (DMSO) group (ANOVA, post-hoc Tukey test); (+) or (-) – dose-response relationship confirmed or not confirmed; E1-E5 – type of dose-response model given by PROAST software; BMD – benchmark dose; BMDL – lower confidence limit of BMD; AST – aspartat aminotransferase; ALT –alanine aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; γ-GT – gamma glutamyl tranferase.**

the curve representing the target tissue dose-response relationship is shown in Figure 3. The dose of 0.07228 mg BDE-209/kg of liver ww, corresponding to external dose of 39 mg/kg/day, was the lowest $BMDL_5$ among all the calculated benchmark doses indicating the effect on AST as the most sensitive biomarker of BDE-209 hepatotoxicity in subacutely exposed rats.

Histopathology

Tissue sections of the rat liver from the control and the DMSO group did not show any deviation from the normal histological structure (Figure 4, A and B).

With the lowest concentration of 0.269 mg/kg, normal histology of the liver was preserved only in the central parts. Edema of hepatocytes, mild hyperemia and small focal hemorrhages in the sinusoids were observed. Sinusoids were slightly narrowed because the moderate edema of the most hepatocytes. Cytoplasm was very eosinophilic and filled with small vacuoles. Nucleus in these cells were rounded, irregular and hyperchromatic, and nucleoli were difficult to be observed (Figure 4C). Numerous pathological mitoses were present, in most hepatocytes, particularly in the group where internal dose of BDE-209 was 0.569 mg/kg of liver ww (Figure 4D). All blood vessels were dilated, with moderate to severe polymorphonuclear cell infiltration (Figure 4E). A response higher than 10% was considered as extra risk for the degree of histopathological changes and therefore $BMDL_{10}$ was calculated. The values of the degree of histological damage as ordinal type of the variable were converted in quantal type of the variable [23, 24]. Dose response was confir-

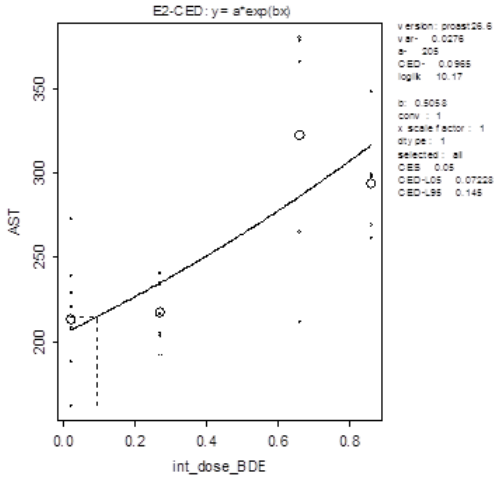
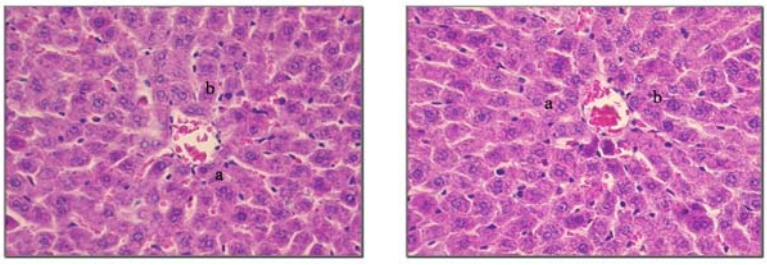
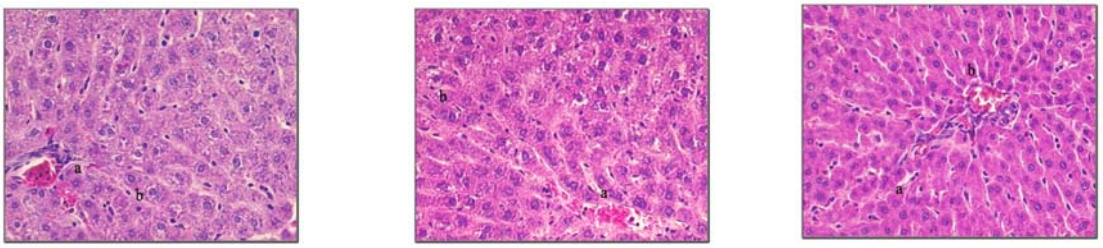


Fig. 3 – Dose-response curve for the effect on aspartat amino transferase (AST) activity against liver concentrations of decabromineted diphenyl ether (BDE-209); CED – critical effect dose.



A) Control group **B) Dimethyl sulfoxide group**



C) 0.269 mg BDE-209/kg liver **D) 0.569 mg BDE-209/kg liver** **E) 0.859 mg BDE-209/kg liver**

Fig. 4 – Histopathological changes in the liver induced by decabromineted diphenyl ether (BDE-209) after 28 days oral exposure; (HE, 40×): A) a – radially placed hepatocytes, b – unchanged hepatocyte; B) a – no histological lesions found, b – hepatocytes in different phases of mitosis; C) a – small number of polymorphonuclears around dilated blood vessel, b – local bleedings in sinusoids; D) a – moderate focal bleeding, b – pathological mitosis of hepatocytes; E) a – discontinuity of vascular wall, b – different phases of hepatocytes degenartion.

med for either internal or external dose of BDE-209 and corresponding $BMDL_{10}$ doses were 0.324 mg BDE-209/kg of liver ww and 812 mg/kg bw/day, respectively.

Oxidative stress parameters

A significant influence of BDE-209 on oxidative stress parameters, MDA, SOD, -SH, in liver homogenates was not confirmed, however internal dose-response relationship was confirmed as a linear model assigned as E1 according to PROAST software (Table 3).

Table 3

Influence of increasing doses of decabrominated diphenylether on oxidative stress parameters in liver homogenates of subacutely exposed Wistar rats

Internal dose (mg/kg)/ dose (mg/kg bw/day)	MDA (nmol/mg of proteins)	-SH groups (nmol/mg of proteins)	SOD (U/g of proteins)
$0/0_{control}$	108.71 ± 47.77	29.47 ± 8.92	103.03 ± 43.64
$0/0_{DMSO}$	127.44 ± 44.01	26.94 ± 14.23	102.75 ± 40.56
0.269/1000	90.91 ± 39.93	27.40 ± 12.94	100.88 ± 21.43
0.569/2000	114.84 ± 57.04	25.40 ± 9.99	100.50 ± 21.77
0.859/4000	161.99 ± 82.12	27.86 ± 3.70	97.69 ± 18.05
	Results on benchmark dose and model internal dose / external dose		
BMD/BMDL	- / -	- / -	- / -
dose response	(+) / (+)	(+) / (+)	(+) / (+)
Model	E1 / E1	E1 / E1	E1 / E1
BMD_5	- / -	- / -	- / -

ANOVA with post-hoc Tukey test was used for statistical analysis; (+) or (-) – dose-response relationship confirmed or not confirmed; E1-E5 – type of dose-response model given by PROAST software; MDA – malondialdehyde; SH – sulfhydryl; SOD – superoxide dismutase; BMD – benchmark dose; BMDL – lower confidence limit of BMD.

Discussion

Determining the relation between external and internal doses, and measurements of BDE-209 concentrations in the liver show that the ratio among the external doses (1 : 2 : 4) is different from the ratio among internal doses (1 : 2 : 3.5), implying the lower absorption when the highest dose is applied. Internal doses correlate with external ones ($r = 0.972$; $p < 0.05$) according to the equation: internal dose (mg BDE-209/kg of liver ww) = 0.0002 × external dose (mg/kg bw/day) + 0.0622. Hepatotoxicity is demonstrated based on significant increase in AST and γ-GT activities, while the lowest $BMDL_5$ is calculated for the serum AST activity. The value of 0.07228 mg BDE-209/kg of liver ww, corresponding to external dose of 39 mg/kg/day, indicates the increase of AST activity as the most sensitive biomarker of BDE-209 hepatotoxicity in subacutely exposed rats. The degree of histological damage increases in the dose-dependent manner, and the corresponding $BMDL_{10}$ dose is 0.324 mg BDE-209/kg of the liver.

For decaBDE the United States Environmental Protection Agency provides no observed adverse effect level (NOAEL) dose of 2.22 mg/kg bw/day as a reference point, however this dose is related with neurobehavioral effects [26–28]. As for the liver, in a 2-year long NTP study (1986), male and female B6C3F1 mice, administered decaBDE in the diet at the doses of 0,3200 or 6,650 mg/kg bw/day for males and 0,3760 or 7,780 mg/kg bw/day for females, the following effects were recorded: liver granulomas and liver hypertrophy in males. Based on these results no NOAEL for liver effects is derived. However, the study identifies a NOAEL for a portal-of-entry effect for females of 3760 mg/kg bw/day [29]. The data sets from another NTP (1986) 2-year rat and mouse studies are selected for BMD modeling: thrombosis in the liver, liver degeneration, in male rats and centrilobular hypertrophy in livers of male mice. The lowest calculated $BMDL_{10}$ was 406 mg/kg bw/day for liver degeneration effect in male rats [29]. Only in one study internal dose, in site, was used for the assessment of BDE-209 subacute oral toxicity in rats [30]. In this experiment internal doses arround 0.25 mg/kg of liver ww induced slight centrilobular hypertrophy in the liver together with increased expression of hepatic CYP1A and CYP2B ($BMDLs_{10}$ 0.5–0.7 mg/kg bw/day) and dose-dependent decrease in serum ALP ($BMDL_{10}$ 0.6 mg/kg bw/day, corresponding to 0.222 mg/kg of liver ww). Histopathological changes seen in our experiment started from the concentration of 0.269 mg/kg of liver ww, which is very close to the value published by Van der Ven et al. [30]. Hepatotoxicity of BDE-209 either *in vivo* or *in vitro* is usually connected with: a) the increased formation of reactive oxygen species, oxidative stress and decreased efficacy of antioxidative defence system [7, 31–34]; induction of apoptosis related with oxidative stress in human hepatoma cells HepG2 [33]; aryl hydrocarbon receptor (AhR) binding particularly expression of luciferase reporter gene mediated by AhR in H4II hepatoma cell line [5]; induction of CYP1A1 mRNA and CYP1A1 protein mediated by AhR [5, 30]. The lack of effect on oxidative stress parameters in our experiment can be understood from the differences, since the values were measured at the end of exposure in which activation of adaptive mechanisms of enzyme or non-enzyme antioxidative defense could take place [35].

Conclusion

The results of the present work add up to the issue of decabrominated diphenyl ether toxicity profile with a focus on the relationship between internal dose and hepatotoxicity. Critical internal dose for the effect on AST of 0.07 mg/kg of liver ww,

corresponding to external dose of 39 mg/kg/day in our study is the lowest dose ever observed in the studies on decabrominated diphenyl ether hepatotoxicity indicating the increase of AST activity as the most sensitive biomarker of decabrominated diphenyl ether hepatotoxicity in subacutely exposed rats. Critical effect based on internal dose in the majority of cases is considered as more precisely defined than the effect established based on external dose, particularly for the persistent substances with low absorption rate, such as decabrominated diphenyl ether.

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Assessment of depression and anxiety in patients with chronic liver disease

Procena depresije i anksioznosti kod bolesnika sa hroničnim bolestima jetre

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Abstract

Background/Aim. In recent years mental health of patients including those with chronic liver disease (CLD), has become interesting because its disturbance leads to reduced quality of life, that is associated with worsening of clinical outcome, reduced compliance and increased mortality. The aim of the study was to determinate the frequency and severity of depression and frequency of anxiety in patients with CLD and to assess the contribution of selected socio-demographic, clinical and laboratory risk factors for depression and anxiety. **Methods.** In this cross-sectional study, we used the Hamilton depression rating scale (HDRS) and Hamilton anxiety rating scale (HARS) in patients with CLD. **Results.** The study included 54 male and 43 female patients. Depression was present in 62.9%, and anxiety in 13.4% of the patients. A higher HDRS was noted in the patients older than 50 years ($p = 0.022$) and unemployed patients ($p = 0.043$). The patients with at least one episode of gastrointestinal bleeding had a significantly higher frequency of anxiety than those without bleeding ($p = 0.018$). A higher HARS score was present in the women ($p = 0.011$), unemployed patients ($p = 0.008$) and those with non-alcoholic liver disease ($p = 0.007$). There was a significant correlation between the mean corpuscular volume (MCV) and the value of the HDRS score, and between serum potassium and sodium levels and HDRS score. **Conclusion.** Age and the mean corpuscular volume have significant influence on the HDRS score while unemployment, gastrointestinal bleeding, serum potassium and serum sodium have predictive value for HARS score.

Ključne reči:
liver diseases; chronic disease; depression; anxiety; questionnaires.

Apstrakt

Uvod/Cilj. U poslednje vreme raste interesovanje za mentalno zdravlje bolesnika sa hroničnim bolestima jetre (HBJ), zbog toga što poremećaj mentalnog zdravlja redukuje kvalitet života i udružen je sa pogoršanjem kliničkog ishoda, smanjenjem komplijanse i povećanjem mortaliteta. Cilj ove studije bio je određivanje učestalosti i težine depresije i anksioznosti kod bolesnika sa HBJ, kao i procena uticaja različitih faktora na skorove depresije i anksioznosti. **Metode.** U studiji preseka, na uzorku bolesnika sa HBJ, korišćeni su Hamiltonova skala depresije (HDRS) i Hamiltonova skala anksioznosti (HARS). **Rezultati.** U studiju je bilo uključeno 54 muškarca i 43 žene obolelih od HBJ. Depresija je bila prisutna kod 62.9%, a anksioznost kod 13.4% bolesnika. Veći HDRS skor imali su bolesnici stariji od 50 godina ($p = 0.022$) i nezaposleni bolesnici ($p = 0.043$). Bolesnici sa barem jednom epizodom gastrointestinalnog krvarenja imali su značajno veću učestalost anksioznosti od bolesnika bez epizode krvarenja ($p = 0.018$). Viši HARS skor bio je prisutan kod žena ($p = 0.011$), nezaposlenih bolesnika ($p = 0.008$) i bolesnika sa nealkoholnom bolešću jetre ($p = 0.007$). HDRS skor značajno je korelirao sa prosečnom zapreminom eritrocita (MCV), a HARS skor sa koncentracijom serumskog kalijuma i natrijuma. **Zaključak.** Starost i prosečna zapremina eritrocita imaju prediktivni značaj za HDRS skor dok su nezaposlenost, gastrointestinalno krvarenje, vrednost serumskog kalijuma i natrijuma prediktori HARS skora.

Key words:
jetra, bolesti; hronična bolest; depresija; anksioznost; upitnici.

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Introduction

Chronic liver disease (CLD) encompasses a wide spectrum of diseases, ranging from liver steatosis (alcoholic and non-alcoholic), hepatitis B and C virus infection, cirrhosis and other less common conditions [1]. Due to the high frequency of occurrence and its consequences, this group of diseases is becoming an increasingly important public health issue worldwide. Depression is one of the leading causes of disability in the adult population and expected to become the second leading cause of disability in all age groups by 2020 [1]. Furthermore, it has been pointed out that this illness is as one of the most common clinical manifestations in a broad range of different diseases [2]. Additionally, results from numerous studies have shown that CLD are often associated with psychiatric comorbidity, particularly mood disorders (depression and anxiety), personality, sleep and other behavior and cognitive deficits [2–6]. The evidence about the presence of these symptoms in CLD patients is important because they have an adverse effect upon the course of illness in the form of amplification of physical symptoms, functional impairment, reduced treatment compliance, and decreased quality of life [2, 6–13].

Most of the previous studies have been focused on depression in CLD [4, 6, 7, 12, 14–17], while only a few have been devoted to anxiety in these patients [6, 14, 16, 17]. Published studies data were heterogenic, both in terms of study population, different diagnostic criteria and assessment instruments that were used for the diagnosis of depression and anxiety.

The aim of the study was to determinate the frequency and severity of depression and frequency of anxiety in patients with CLD and to assess the contribution of selected socio-demographic, clinical and laboratory risk factors for depression and anxiety.

Methods

Data were collected on a sample of patients in the Chronic Liver Disease Questionnaire (CLDQ) validation study, and detailed methodology was previously published [18]. The study was conducted at the Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, in the period of one year. Consecutive outpatients and inpatients with chronic liver disease were evaluated for inclusion and exclusion criteria. Inclusion criteria was CLD (chronic hepatitis and liver cirrhosis), while exclusion criteria were: age < 18 years, psychiatric disorders (psychosis or dementia), acute complications of CLD, hepatic encephalopathy (grade > 2), liver transplantation and patients undergoing antiretroviral therapy [18].

The severity of liver disease was quantified using modified Child-Pugh classification into three groups – A, B and C class – to determinate the stage of liver insufficiency [19]. The patients were stratified into the two groups: no cirrhosis/early cirrhosis (Child A) and advanced cirrhosis (Child B/Child C).

According to the etiology, the patients were stratified on alcoholic and non-alcoholic liver disease. The group of patients with non-alcoholic etiology included: viral (hepatitis B and C), autoimmune (primary biliary cirrhosis, primary sclerosing cholangitis and autoimmune hepatitis), non-alcoholic steatohepatitis, Morbus Wilson and cryptogenic CLD.

Assessment of the presence and the level of depression was based on the Hamilton depression rating scale (HDRS) [20]. It is clinician-administrated depression estimation scale conducted using semi-structured interview in which 21 signs and symptoms of depression were evaluated. Most of the items were rated on the 0 to 4 scale, reflecting the severity of symptom manifestation, while others were rated on the 0 to 2 scale reflecting the absence, probable, or the definite existence of the symptom. HDRS can have values from 0 to 64, with the values higher than 8 labeled as abnormal (clinically meaningful depression). In relation to the value of the HDRS score, severity of depression was classified to: mild depression, 9–17; moderate depression, 18–24; severe depression, higher than 24 [20].

For estimation of anxiety, we used the Hamilton anxiety rating scale (HARS), which includes evaluation of 14 symptoms and signs. The presence of these symptoms is graded from 0 (not present) to 4 (severe). HARS score can range from 0 to 56, with the values higher than 17 as pathological (clinically significant anxiety) [21].

Testing with the scales HDRS and HARS was conducted by clinicians.

In addition, the socio-demographic, clinical and psychometric data were collected, as well as the following laboratory parameters: hematological, biochemical and viral hepatitis profiles. Data were collected using a questionnaire, based on medical records and anamnesis. Data were collected by clinicians.

This study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade (Decision No. 29/I-2). All the subjects gave written consent to participate in the study.

Statistical analysis

The methods of descriptive and analytical statistics were applied. The continuous variables were presented as mean value ± standard deviation, while the categorical ones as proportions (percentages). For statistical analysis, we used parametric and non-parametric tests and correlation tests. A p value < 0.05 was marked statistically significant.

Hierarchical multiple regression analysis was conducted to identify predictors of depression and anxiety. The analysis was conducted separately for HDRS and HARS as outcome variables. The predictor variables were separated into three blocks (models). Selected socio-demographic characteristics were entered in the first block, clinical characteristics comprised the second block followed by laboratory parameters in the third block. After adjustment for potential confounding factor, we determined final models for HDRS and HARS scores.

Results

The study included 103 patients with CLD. The depression and anxiety scale were completed by 97 (94.1%) of the patients, and only they were analysed. Men were 54 (55.7%). The average age was 53.5 ± 12.8 years. Clinically meaningful depression was present in 61 (62.9%) of the patients, with the average HDRS score 13.4 ± 9.4. Mild depression was present in 33 (34.0%) of the patients, moderate in 16 (16.5%), and severe in 12 (12.4%) of the patients. Clinically significant anxiety was present in 13 (13.4%) of the patients. The average value of the HARS score was 10.0 ± 6.4.

The distribution of the selected socio-demographic and clinical characteristics according to the presence and category of depression measured by the HDRS score was presented in Table 1 and Table 2. There were no statistically significant differences in the frequency of varying severity of depression between the patients with different socio-demographic and clinical characteristics.

Analysis of differences in the average HDRS score, indicated that the patients older than 50 years had a significantly higher HDRS score than the patients ≤ 50 years (15.2 ± 9.7 *vs* 10.6 ± 8.3), (z = 2.290, $p = 0.022$). Additionally, the unemployed patients had a significantly higher HDRS score than the employed patients (14.6 ± 9.4 *vs* 10.5 ± 8.9), (z = 2.024, $p = 0.043$). For the other investigated socio-demographic and clinical characteristics, statistically significant difference was not obtained (data not shown). Furthermore, correlation analysis between the level of investigated laboratory parameters and depression status showed that only the mean corpuscular volume (MCV) significantly correlated with HDRS scores (r = 0.215, $p = 0.034$).

Hierarchical regression analysis showed that socio-demographic variables (age, employment) explained 6.9% of the variance ($p = 0.036$) of the HDRS as outcome measure. Addition of the variables "ascites" and "gastrointestinal

Table 1

The distribution of selected socio-demographic characteristics according to the Hamilton depression rating scale (HDRS) degree of depression severity

Socio-demographic characteristics	The HDRS degree of depression severity				*p*-value
	no depression	mild	moderate	severe	
Gender, n (%)					
male	25 (46.3)	14 (25.9)	10 (18.5)	5 (9.3)	0.095
female	11 (25.6)	19 (44.2)	6 (14.0)	7 (16.3)	
Age, n (%)					
≤ 50 years	18 (47.4)	12 (31.6)	6 (15.8)	2 (5.3)	0.217
> 50 years	18 (30.5)	21 (35.6)	10 (16.9)	10 (16.9)	
Education, n (%)					
≤ 12 years	24 (38.1)	19 (30.2)	11 (17.5)	9 (14.3)	0.609
>12 years	12 (36.4)	14 (42.4)	4 (12.1)	3 (9.1)	
Employment, n (%)					
employed	14 (48.3)	8 (27.6)	5 (17.2)	2 (6.9)	0.406
unemployed	22 (32.4)	25 (36.8)	11 (16.2)	10 (14.7)	
Marrital staus, n (%)					
single	8 (28.6)	12 (42.9)	2 (7.1)	6 (21.4)	0.103
married	26 (39.4)	20 (30.3)	14 (21.2)	6 (9.1)	
Children, n (%)					
yes	29 (36.7)	25 (31.6)	14 (17.7)	11 (13.9)	0.584
no	7 (38.9)	8 (44.4)	2 (11.1)	1 (5.6)	
Smoking, n (%)					
yes	8 (25.8)	14 (45.2)	5 (16.1)	4 (12.9)	0.355
no	28 (42.4)	19 (28.8)	11 (16.7)	8 (12.1)	

Table 2

The distribution of the selected clinical characteristics according to the Hamilton depression rating scale (HDRS) degree of depression severity

Clinical characteristics	The HDRS degree of depression severity				*p*
	no depression	mild	moderate	severe	
Disease severity, n (%)					
no cirrhosis	12 (27.3)	17 (38.6)	9 (20.5)	6 (13.6)	0.324
Child's B/ C	24 (45.3)	16 (30.2)	7 (13.2)	6 (11.3)	
Etiology, n (%)					
alcoholic	17 (47.2)	9 (25.0)	6 (16.7)	4 (11.1)	0.389
non-alcoholic	19 (31.1)	24 (39.3)	10 (16.4)	8 (13.1)	
Ascites, n (%)					
yes	16 (47.1)	10 (29.4)	5 (14.7)	3 (8.8)	0.502
no	20 (31.7)	23 (36.5)	11 (17.5)	9 (14.3)	
Gastrointestinal bleeding, n (%)					
yes	6 (27.3)	7 (31.8)	5 (22.7)	4 (18.2)	0.457
no	30 (40.5)	26 (35.1)	11 (14.9)	7 (9.5)	

bleeding", in the second model caused an increase by 5.4% in variance ($p = 0.067$). Furthermore, after adding the MCV in the third block an additional 5.4% of the variance was explained in HDRS ($p = 0.018$). The final model showed that age, employment, ascites, gastrointestinal bleeding and MCV accounted for 17.6% of the variance in HDRS (Table 3).

The patients with at least one episode of gastrointestinal bleeding had a significantly higher frequency of clinically meaningful anxiety than the patients without bleeding ($\chi^2 = 5.561$, $p = 0.018$). For the other socio-demographic and clinical characteristics, a difference in clinically significant anxiety was not found. Analysis of distribution of anxiety according to the selected demographic variables indicated that women had a significantly higher average HARS score compared to the males (11.6 ± 6.6 *vs* 8.8 ± 6.0), ($z = 2.546$, $p = 0.011$), as well as the unemployed patients had a significantly higher HARS score than the employed patients (11.0 ± 6.7 *vs* 7.5 ± 5.0), ($z = 2.659$, $p = 0.008$). Moreover, the patients with non-alcoholic liver disease had a significantly higher HARS score than the patients with alcoholic liver disease (11.2 ± 6.6 *vs* 8.0 ± 5.5), ($z = 2.688$, $p = 0.007$). For the other investigated socio-demographic and clinical characteristics, a statistically significant difference was not obtained (data not shown). Furthermore, investigation of the association between anxiety and the level of laboratory parameters showed that HARS scores significantly correlated with the value of serum potassium ($r = 0.443$, $p < 0.001$) and serum sodium ($r = -0.363$, $p < 0.001$).

With HARS as dependent variable, the first model in hierarchical regression analysis (consisting of the selected socio-demographic variables), accounted for 9.3% of the variance in the outcome variable. Moreover, "gastrointestinal bleeding", "ascites" and "etiology of CLD" explained additional 8.7% in the total change in HARS, in this analysis. Concentration of serum sodium and potassium, in the third model, accounted an additional 23.5% of the variance in HARS ($p < 0.01$). Therefore, the final model (employment, gastrointestinal bleeding, serum potassium and serum sodium) explained 41.5% of the variance in HARS ($p < 0.01$) (Table 4).

Table 3

The results of hierarchical multiple regression analysis for the Hamilton depression rating scale (HDRS) as the dependent variable

Variable	Model I			Model II			Model III		
	B	SE(B)	β	B	SE(B)	β	B	SE(B)	β
Age	3.63	2.10	0.18	3.80	2.07	0.19	4.09	2.02	0.21*
Employment	-2.53	2.24	-0.12	-2.36	2.20	-0.11	-1.83	2.15	-0.09
Ascites				-3.27	1.94	-0.16	-3.67	1.90	-0.18
GIT bleeding				3.68	2.20	0.16	3.91	2.14	0.17
MCV							0.18	0.07	0.23*
R^2		0.069*			0.123			0.176*	
F for change in R^2		3.448*			3.185*			3.856**	

GIT– gastrointestinal; MCV – mean corpuscular volume of erythrocytes.
*** $p < 0.05$; ** $p < 0.01$.**

Table 4

The results of hierarchical multiple regression analysis for the Anxe depression rating scale (HARS) as the dependent variable

Variable	Model I			Model II			Model III		
	B	SE (B)	β	B	SE (B)	β	B	SE (B)	β
Gender	-2.30	1.31	-0.18	-1.71	1.51	-0.13	-1.6	1.30	-0.13
Employment	-3.06	1.42	-0.21*	-2.95	1.38	-0.21*	-2.68	1.18	0.19*
Etiology				-1.29	1.47	-0.10	-0.71	1.27	-0.05
Ascites				-1.45	1.51	-0.10	-0.15	1.34	-0.01
GIT bleeding				3.83	1.51	0.25*	6.64	1.53	0.43**
S-Potassium							0.51	0.12	1.16**
S-Sodium							0.42	0.14	0.73**
R^2		0.093*			0.181*			0.415**	
F for change in R^2		4.639*			3.838**			8.629**	

GIT – gastrointestinal; S – serum; * $p < 0.05$; ** $p < 0.01$.

Discussion

The correlation between depression and anxiety with CLD has been known for a long time [6], as well as the fact that patients with CLD had a significantly higher incidence of depression compared to healthy population [5, 12, 22–24]. Pathogenesis of depression in patients with CLD is insufficiently clarified. Neuropsychological deficits in this group of patients usually include cognitive impairment and depression [5]. These disorders occur as a consequence of accumulation neuropathogenic molecules and toxins in blood due to the inadequate clearance in a damaged liver [25]. Also, in patients with end stage liver disease, immunological mechanisms can lead to the development of depression [26]. However, it remains unclear why up to 50% of patients with noncirrhotic CLD have these disorders long before the occurrence of cirrhosis [25].

Although this psychiatric comorbidity is common in all CLD, most of the published studies are based on an estimate of depression and anxiety in liver transplant candidate or patients after liver transplatation [7, 29, 14, 15, 27], and patients with chronic viral hepatitis [28–30]. As reported in the literature, there is no difference in the level of depression and anxiety among candidates for liver transplantation and patients with liver disease who are not on the list for transplantation [24], and therefore we estimate that the scores for depression and anxiety between these two groups of patients are comparable [15].

In our study 62.9% of patients with CLD had depression, while 13.4% of patients had anxiety which was in accordance with previous findings [4, 6, 15]. Namely, in the patient with CLD, incidence of depression ranged from 20% to 70.6% [4, 6, 7, 12, 14–17], and anxiety from 13% to 71.6% [6, 14, 17]. Similar data were obtained in the group of patients with chronic hepatitis C [27, 31–34] and in patients with alcoholic cirrhosis [18]. The analysis of the severity of depression, in our patients, have revealed that 34% of patients had mild, 16.5% moderate, and 12.4% severe stage of this disorders. Similar data were obtained by Bianchi et al. [4]. Besides neuropathogenic impact of CLD [1, 5, 25], such a high frequency of this mental disturbances might be explained by the fact that a majority of these patients belonging to a high risk population for developing psychiatric disorders (alcoholism, drug addiction, etc.) [17].

The results of our study show that women and man do not differ in severity of depression, which is consistent with previous communications [14, 17]. However, although the frequency of anxiety did not differ between the genders, we confirmed a higher average HARS score in women, which is also described in other studies [35–37]. The reason for this difference may be in different biological and social factors between men and women [35, 38], respectively in greater responsibility to the health of women and the importance of their health on families and children.

The results from numerous studies demonstrated that age had no influence to the severity of depression, which is consistent with our result [14, 15, 17, 22]. However, in our sample of CLD patient, it was found that patients > 50 years old have a significantly higher mean HDRS score, than younger patients. The effect of age on depression in CLD is described by Kraus et al. [22], and Theofilou [36], which suggest that older patients have higher levels of depression.

Our study showed that employed and unemployed patients did not differ in the frequency and severity of depression and frequency of anxiety, as described by other authors [7, 22]. We also obtained the results in favor of it, that unemployed patients have a significantly higher HDRS and HARS score than employed patients. The reason for these results might be the fact that unemployment can be stressful factor because patients are exposed to existential problems, and lack of professional activity, which could at least partially occupy the patient, and turn his attention to the somatic state.

Our results indicate that patients with different disease severity have no differences in depression and anxiety. There are divided opinions about the way of liver disease severity affects the levels of depression and anxiety. Some authors state that the Child-Pugh and Model for end-stage liver disease (MELD) score does not affect depression [3, 4, 7, 39]. However, another studies suggest that the severity of liver assessed by Child-Pugh score directly affects depression and anxiety [14, 40].

Patients with at least one episode of gastrointestinal hemorrhage had significantly higher rates of anxiety. Dramatic clinical presentation of bleeding and awareness of the possibility of recurrence, mortality and treatment methods have a negative impact on the patient's mental status and favor the development of anxiety. Certainly it should not be forgotten that bleeding usually occurs in more advanced CLD. As obtained in our research, and based on the literature data [7], it is not confirmed that gastrointestinal bleeding has influence on the level of depression.

In our study, patients with alcoholic and non-alcoholic etiology of CLD did not differ in depression and anxiety. Our results confirm previously published data, indicating that if we exclude overall psychiatric comorbidity (neuroses, affective disorders, and anti-social personality), which is more common in patients with alcoholic liver disease than in patients with non-alcoholic liver disease [41, 42], these two groups will not differ in depression and anxiety [15, 39, 43]. However, in patients with non-alcoholic etiology of the disease significantly higher HARS score was confirmed than in patients with alcoholic CLD. Besides the anxiolytic effects of alcohol, based on the modulation of neurotransmission predominantly via gamma-amynobuteric acid (GABA-A) [44, 45], the reason for the lower HARS score in patients with alcoholic liver disease may be unrealistic in their relation to health, causing CLD experience less severely than patients with non-alcoholic liver disease. In determining the differences between patients of different gender, age, employment status, and etiology of the disease, it was found that they do not differ in depression and anxiety. However, if we analyze the average HDRS or/and HARS scores a statistically significant difference in these scores is registered for some categories. The reason for this phenomenon may be that the cut-off values for determining the presence of depression and anxiety, arbitrary, cover a relatively wide range of values of these psychometric tests, and that significant changes in the absolute values of scores are not sufficient to lead to a change in category (presence/absence of disorder).

Nonmegaloblasic (normoblastic), macrocytic anemia and macrocytosis without anemia is common in chronic liver diseases [46]. The pathogenesis is not fully known. It is believed that in patients with alcoholic cirrhosis, it is a result of direct toxic effects of alcohol on red blood cells, which affects the modification of lipid components of the erythrocyte membrane [46, 47]. Also, macrocytic anemia occurs as the result of vitamin B12 and/or folic acid deficit. In the recent literature, it is described that these micronutrients deficit is associated with depression [48–50]. Also, the use of vitamin B12 with a selective serotonin reuptake inhibitor, significantly improved the symptoms of depression [51]. Our study established a positive correlation between depression score and MCV. In previous study no correlation between HDRS score and MCV was found [52]. However, Alves de Rezende et al. [53], de-

scribe a positive correlation between depression score and MCV in women. In order to investigate the association between MCV and HDRS score among CLD patients included in our study, we performed the hierarchical regression analysis.

In our study, it was found that serum potassium correlated positively with HARS score, and serum sodium negatively with the score of anxiety. More complex pictures of these correlations are obtained by hierarchical regression analysis. Namely, after controlling for potential confounding factors (gender, employment, etiology, ascites and gastrointestinal bleeding), final model showed that serum potassium and serum sodium have significant influence on the development of anxiety. For potassium, a possible explanation is based on the GABA effect. In fact, a study conducted on the experimental animals in 2012, confirmed that the use of loop diuretics, which reduce the concentration of potassium, lead to anxiolytic effect based on the modulation of GABA-A receptors, through antagonism of cation-chloride cotransporters [54]. Also, sodium imbalance is important in patients with advanced cirrhosis, which is associated with developing of numerous complications [55, 56] and even better predictor of mortality than MELD score [57]. The study of Solà et al. [58], from 2012 concluded that the concentration of serum sodium is a predictor of mental components of quality of life in CLD patients, as a result of the effect of hyponatremia on central nervous system. Based on our results, measuring MCV, serum potassium and serum sodium, could have a significance in detection of patients with CLD, which should be further examined to diagnose depression and/or anxiety.

Conclusion

In this study 62.9% of the patients with chronic liver disease had depression, while 13.4% of the patients had anxiety. The women and the patients ≤ 50 years old, had a significantly lower the HDRS score. The unemployed patients had a significantly higher HDRS and HARS score than the employed patients. The patients with different disease severity and different etiology of chronic liver disease did not differ in depression and anxiety. Of all the studied characteristics, it was concluded that only age and mean corpuscular volume had a predictive value in the development of depression, while employment, gastrointestinal bleeding, serum potassium and sodium levels had a significant influence on anxiety score.

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Matrix metalloproteinase-9 index as a possible parameter for predicting acute coronary syndrome in diabetics

Indeks matriks metaloproteinaze-9 kao mogući parametar predviđanja akutnog koronarnog sindroma kod dijabetičara

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Abstract

Background/Aim. Matrix metalloproteinase-9 (MMP-9) index is the ratio of active MMP-9 and total MMP-9 levels. It reflects the importance of MMP-9 in acute coronary syndrome (ACS). **Methods.** The study included 3 groups of patients (n = 87): the group 1 – non-diabetic subjects without ACS (control); the group 2 – diabetic patients with ACS [subgroups with unstable angina pectoris (UAP), myocardial infarction (MI) or reinfarction]; and the group 3 non-diabetics patients with ACS. Total and active MMP-9 were measured and used to create MMP-9 index. **Results**. MMP-9 index, as a marker showed good sensitivity and specificity, of ACS in diabetics, with a cut-off value over 58.2. MMP-9 was higher in the study groups than in the control one. MMP-9 correlated with ACS occurrence and type of cardiovascular event. A statistically significant difference was found among the groups according to active MMP-9 ($p < 0.001$). The same was found with active MMP-9 between the control and the group with MI ($p < 0.001$). The control was highly statistically significantly different from the group of patients with UAP ($p < 0.01$). Statically significant differences in MMP-9 index was found between the control and the diabetics with ACS ($p < 0.001$). Statistically significant difference of MMP-9 index was also found in the controls compared to the value in non-diabetic patients with ACS ($p < 0.01$). **Conclusion.** MMP-9 index may be a possible marker of atheromatous plaque rupture in diabetics.

Key words:
diabetes mellitus; acute coronary syndrome; matrix metalloproteinase 9; prognosis.

Apstrakt

Uvod/Cilj. Indeks matriks metaloproteinaza-9 (MMP-9) predstavlja odnos nivoa aktivne i ukupne MMP-9. On odražava značaj MMP-9 u akutnom koronarnom sindromu (ACS). **Metode.** Ova studija obuhvatila je tri grupe bolesnika (n = 87): grupa 1 – nedijabetičari bez ACS (kontrola); grupa 2 – dijabetičari sa ACS [podgrupa sa nestabilnom anginom pektoris (UAP), miokardijalnim infarktom (MI) ili reinfarktom]; grupa 3 – nedijabetičari sa ACS. Određivan je nivo ukupne i aktivne MMP-9 da bi se dobio MMP-9 indeks. **Rezultati.** Index MMP-9, kao marker za ACS, pokazao je dobru senzitivnost i specifičnost za *cut off* (granične) vrednosti od preko 58.2. Kod dijabetičara MMP-9 bio je viši u ispitivanim grupama nego u kontroli. Index MMP-9 korelisao je sa pojavom ACS i tipom koronarnog događaja. Statistički značajna razlika dobijena je između grupa prema aktivnom MMP-9 ($p < 0,001$). Isto je nađeno sa aktivnim MMP-9 između kontrolne grupe i grupe sa MI ($p < 0,001$). Kontrola se visoko statistički značajno razlikovala od grupe bolesnika sa UAP ($p < 0,01$). Statistički značajne razlike u indeksu MMP-9 nađene su između kontrole i grupe dijabetičara sa ACS ($p < 0,001$). Statistički značajna razlika nađena je kod MMP-9 indeksa između kontrole u poređenju sa vrednostima kod nedijabetičara sa ACS ($p < 0,01$). **Zaključak.** Indeks MMP-9 može biti potencijalni marker za rupturu ateromatoznog plaka kod dijabetičara.

Ključne reči:
dijabetes melitus; akutni koronarni sindrom; matriks metaloproteinaza 9; prognoza.

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Introduction

Unstable atherosclerotic plaque is the pathophysiological substrate of acute coronary syndrom (ACS) [1]. Enzymes matrix metalloproteinases (MMPs), especially MMP-9 secreted by inflammatory cells of atheromatous plaque (macrophages), smooth muscle cells and endothelial cells belong to a large family of zinc-binding, calcium-dependent endopeptidases that are involved in the degradation and remodeling of extracellular matrix [2, 3]. They are a growing group of proteolytic enzymes involved in numerous processes including embryogenesis, interstitial metabolism, angiogenesis, etc. [4]. Matrix activity was observed in carcinogenesis, in some degenerative processes, and inflammatory conditions including atherogenesis. MMPs primarily MMP-9 has clinical significance in the serum of diabetic patients in inflammatory processes involving in plaque rupture and leading to acute coronary event with elevated levels [5–13]. MMP-9 is localized on the shoulders of plaque, a thin area which is suspected for rupture. The potential role of MMP-9 as a marker for risk stratification in patients with ACS was examined in a study on a larger number of patients showing that the values of MMP-9 were associated with future lethal cardiovascular events [6]. In patients with unstable angina pectoris (UAP) MMP-9 showed the increase of 70%, indicating active synthesis, compared with patients with stable angina. Although there is no conclusive data to show correlation of MMP with atheromatous plaques and localization of MMPs in the shoulder region of vulnerable lesions, a direct connection to the real rupture of unstable plaque has been described.

The aim of the study was to find out if the enzyme MMP-9 in the serum of diabetics and MMP-9 index could be early and safe markers of atheromatous plaque rupture and ACS.

Methods

We investigated patients admitted due to ACS to the Coronary Care Unit, Clinical Center of Serbia, Belgrade, during the period February, 2012 to February, 2013. Ethical principles were respected and all patients gave their consent to participate. A total of 87 patients of both sexes (57 male, 30 female) were examined. The patients were 40–80 years old (61.1 ± 10.3 year). The main criterion for inclusion was the presence of ACS (acute myocardial infarction or unstable angina pectoris). The two groups were formed depending on the diagnosis of diabetes mellitus (DM): diabetics with ACS (DM + ACS) and the group of non-diabetic patients with ACS. The diagnosis of ACS was based on clinical, electrocardiographic findings, biochemical analysis and diagnosis of DM based on the current American Diabetes Association criteria [14]. The patients with damaged hepatic function, severe anemia, neoplastic illness, infectious or autoimmune disease were excluded. DM + ACS was divided into two subgroups: UAP and the group with myocardial infarction or reinfarction (MI). The control consisted of healthy subjects (age 55.3 ± 8.9 years). MMP-9 activity was measured using detection enzymes in their pro-forms activated by binding to the active MMP-9 in the single-level enzymatic process by the method of Verheijen et al. [15]. The active form of MMP-9 linked to the detection enzyme was determined by binding to specific chromogenic peptide substrate complex which absorbs light of the wavelength of 405 nm. The concentration of active MMP-9 in the sample was determined by interpolation from the standard curve. Biotrak of MMP-9 activity assay system is equivalent to ELISA determination. The measuring range of the method is: the total MMP-9 1.0–32 ng/mL, and active MMP-9 0.5–16 ng/mL. MMP-9 index is defined as the relative ratio of the active and total form of MMP-9 multiplied by 100 [(MMP-9 active/MMP-9 total) ×100].

The results were reported as the mean value ± standard deviation and percentage. The differences between the groups were assessed by two-way analysis of variance (ANOVA-with Bonferroni post hoc analysis) for continuous variables and one-way non-parametric analysis of variance (Kruskal-Wallis) for category variables. Potential cutoffs for MMP-9 index were evaluated using receiver operating characteristic (ROC) curve analysis. The correlations between parameters were analyzed with Pearson's and Spearman's test. The differences were considered statistically significant at $p < 0.05$. SPSS 12.0 software was used for statistical analysis.

Results

Table 1 shows the values of total MMP-9, active MMP-9 and MMP-9 index in the examined groups and the groups formed on coronary events.

Total MMP-9 in the examined groups

The values of total MMP-9 show a statistically significant difference among the three examined groups ($p < 0.01$). There was a statistically significant difference of total MMP-9 between the control and DM + ACS group ($p < 0.001$). Also, there is a statistically significant difference of total MMP-9 between the groups of diabetics and non-diabetics with ACS ($p < 0.05$). There was no statistically significant difference of total MMP-9 between the control and the group of non-diabetics with ACS.

Active MMP-9 in the examined groups

The values of active MMP-9 showed a statistically significant difference among the three examined groups ($p < 0.001$). The value of active MMP-9 was significantly different between the control and both groups with ACS, diabetics ($p < 0.001$) and non-diabetics ($p < 0.001$). The groups of diabetics and non-diabetics with ACS were statistically significantly different ($p < 0.001$).

MMP-9 index in the examined groups

A significant difference in the values of MMP-9 index among the three examined groups was found ($p < 0.001$), as well as between the control and DM + ACS group ($p < 0.001$). There was a significant difference in the value of MMP-9 index in the control compared to the value in the non-diabetics ($p < 0.0$).

Table 1

The values of matrix metallproteinaze-9 (MMP-9)

Marker	Examined groups ($\bar{x} \pm SD$)			*p*
	DM + ACS	ACS	Control	
Total MMP-9 level (ng/mL)	51.7 ± 13.4	42.2 ± 11.1	37.1 ± 12.4	< 0.01
Active MMP-9 level (ng/mL)	36.8 ± 11.2	28.4 ± 7.3	16.2 ± 7.9	< 0.001
MMP-9 index	70.6 ± 16.8	68.8 ± 15.6	42.9 ± 10.1	< 0.001
	Coronary events groups ($\bar{x} \pm SD$)			
	UAP	MI	Control	
Total MMP-9 level (ng/mL)	44.4 ± 15.5	46.7 ± 12.3	37.1 ± 12.4	< 0.05
Active MMP-9 level (ng/mL)	28.9 ± 10.4	32.7 ± 9.9	16.2 ± 7.9	< 0.001
MMP-9 index	66.7 ± 16.5	71.4 ± 16.0	42.9 ± 10.1	< 0.01

x – mean value; SD – standard deviation; DM – patients with diabetes mellitus; ACS – patients with acute coronary syndrome; UAP – patients with unstable angina pectoris; MI – patients with myocardial infarction or reinfarction.

Total MMP-9 in the coronary events groups

The values of total MMP-9 showed a statistically significant difference among the three groups formed on coronary events ($p < 0.05$). There was a statistically significant difference in total MMP-9 between the control group and the MI group ($p < 0.01$).

Active MMP-9 in the coronary events groups

The values of active MMP-9 show a statistically significant difference among the three groups formed on coronary events ($p < 0.001$). The value of active MMP-9 was significantly different between the control and both groups with ACS, UAP ($p < 0.001$) and MI ($p < 0.001$). The groups of UAP and MI were not significantly different in values of active MMP-9 ($p > 0.05$).

MMP-9 index in the examined groups

The values of MMP-9 index showed statistically significant difference among the three groups formed on coronary events ($p < 0.01$). The value of active MMP-9 was significantly different between the control and both coronary events groups, UAP ($p < 0.001$) and MI ($p < 0.001$). The value of MMP-9 index in the UAP group was not significantly different than the value in the MI group ($p < 0.05$).

Statistical analysis of the data presented in Figure 1 showed a significant difference in the percentage of patients with elevated total MMP-9 between the control group and the DM + ACS group ($p < 0.01$).

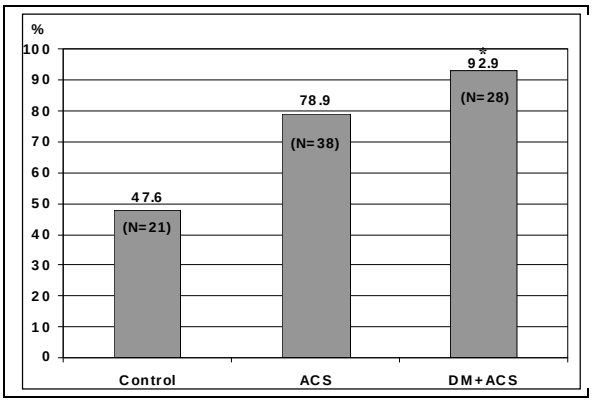

Fig. 1 – Distribution of elevated total matrix metalloproteinase-9 (MMP-9) levels.

*** *p* < 0.01 compared to control; N – number of patients; ACS – patient with acute coronary syndrome; DM – patient with diabetes mellitus.**

Statistical analysis of data presented in Figure 2 showed a significant difference in the percentage of patients with elevated total MMP-9 between the control group and the group of patients with myocardial infarction or reinfarction ($p < 0.01$).

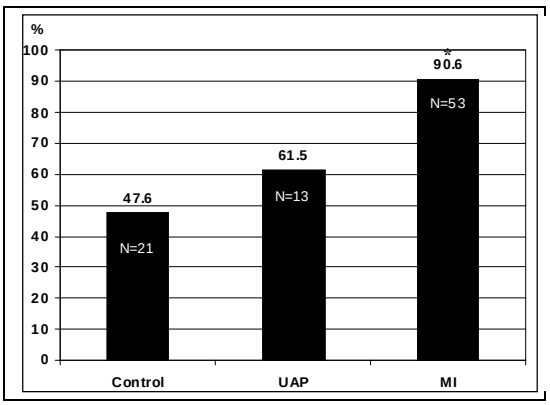

Fig. 2 – Distribution of elevated total matrix metalloproteinase-9 (MMP-9) levels in the coronary event groups.

****p* < 0.01 compared to control ; N – number of patients; UAP – patients with unstable angine pectoris; MI – patients with myocardial infarction or reinfarction.**

Data presented in Figure 3 show a significant difference between the control group and both groups with ACS (diabetics and non-diabetics) ($p < 0.001$). The groups of diabetics and non-diabetics with ACS did not differ significantly ($p > 0.05$).

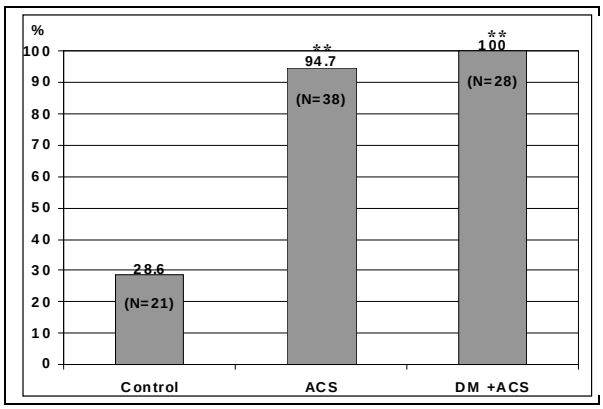

Fig. 3 – Distribution of elevated active matrix metalloproteinase-9 (MMP-9) levels

**** p < 0.001 compared to control; N – number of patients; ACS – patient with acute coronary syndrome; DM – patient with diabetes mellitus.**

Data presented in Figure 4 show a significant difference between the control and MI group ($p < 0.001$). The control group was significantly different from the UAP group ($p < 0.01$), too.

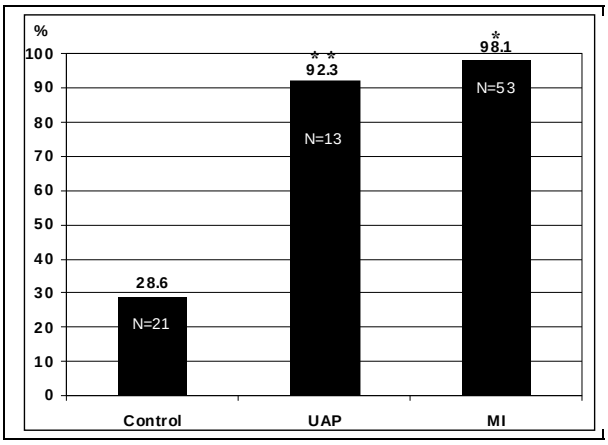

Fig. 4 – Distribution of elevated active matrix metalloproteinase-9 (MMP-9) levels in coronary event groups

***p < 0.01 compared to control; **p < 0.001 compared to control; N – number of patients; UAP – patients with unstable angine pectoris; MI – patients with myocardial infarction or reinfarction.**

Table 2 shows the significant correlations of total MMP-9 levels and other ACS parameters. In the DM + ACS group, elevated total MMP-9 level showed the strongest correlation with the elevated Homeostatic Model Assessment of Insulin Resistance (HOMA IR) score. Elevated cholesterol levels significantly correlated with elevated total MMP-9 level in the DM + ACS group. The presence of hypertension and smoking showed a statistically significant correlation between DM + ACS with total MMP-9.

The presence of hypertension and smoking in the DM + ACS group significantly correlated with serum levels of active MMP-9.

There was a good correlation between MMP-9 index and hyperinsulinemia and pain duration in the group of diabetics with ACS.

The largest area under the ROC curve (AUC) was of active MMP-9 level, the lowest AUC was of total MMP-9 level, and AUC of MMP-9 index was between active and total MMP-9 level. The AUC of total MMP-9 level showed a significant difference ($p = 0.0041$) compared to the area of 0.5. The AUC of active MMP-9 level and MMP-9 index showed a significant difference ($p = 0.0001$) compared to the area of 0.5. AUC of total MMP-9 is significantly different from the AUC of active MMP-9 ($p < 0.05$). The AUC for MMP-9 index was not significantly different compared to the AUC of total or active MMP-9 level. The AUC of MMP-9 index had the lowest standard error (SE). The resulting cut-off value for MMP-9 index was over 58.2.

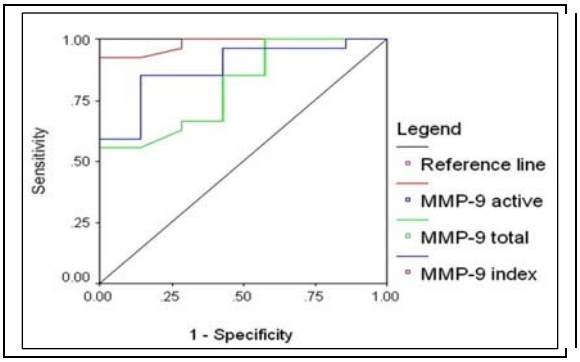

Fig. 5 – Receiver operating characteristic (ROC) curves of total matrix metalloproteinase-9 (MMP-9) level, active MMP-9 level and MMP-9 index in diabetics with acute coronary syndrom (ACS)

Table 3 shows the values of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of MMP-9 level as predictor of ACS in diabetics. Total MMP-9 level had good sensitivity, but poor specificity with a high PPV and NPV. Active MMP-9 had good sensitivity and specificity, with a high PPV and NPV. MMP-9 index showed good sensitivity, excellent specificity and PPV, and acceptable NPV.

Table 2

Correlations of matrix metalloproteinase-9 (MMP-9) with other important parameters of acute coronary syndrome (ACS)

Parameter	MMP-9	Group	*r*	*p*
Age	Total	All	0.353	< 0.01
Smoking	Total	DM + ACS	0.387	< 0.05
Hypertension	Total	DM + ACS	0.469	< 0.05
HOMAIR (high values)	Total	DM + ACS	0.557	< 0.05
Cholesterol (elevated levels)	Total	DM + ACS	0.422	< 0.05
Smoking	Active	DM +ACS	0.426	< 0.05
Hypertension	Active	DM + ACS	0.395	< 0.05
Insulinemia (high values)	Index	DM + ACS	0.542	< 0.05
Pain duration	Index	DM + ACS	0.382	< 0.05

***r* – coefficient of correlation; DM – diabetes mellitus.**

Table 3

Matrix metalloproteinase-9 (MMP-9) parameters as predictors of acute coronary syndrome (ACS) in diabetics

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV(%)
MMP-9 total level	100	44.4	84.4	100
MMP-9 active level	92.6	88.9	96.2	80
MMP-9 index	77.8	100	100	60

PPV – positive predictive value of the test; NPV – negative predictive value of the test.

Discussion

The levels of MMP-9 and tissue inhibitor metalloproteinases-1 (TIMP-1) are significantly increased in coronary arteries with unstable plaques [16]. As TIMP-1 is a potential inhibitor of MMP-9, its increase during the acute phase of MI may indicate production of MMP-9 [17–19]. Diabetes and/or hyperglycemia have a big influence on the structure and function of the heart and vascular tissue [20], inducing proinflammatory changes, including increased MMP and other factors potentially relevant to plaque rupture and thrombosis [21]. There are studies showing no changes of MMP-9 in diabetics [22]. Diabetes is also associated with plaque instability and carries a high risk for an acute coronary event. Sampling of MMP-9 values is related to the occurrence of acute coronary event and the diagnosis of ACS. Our results showed that the values of total MMP-9 level were higher in diabetics with ACS, than in the non-diabetics and the control. Elevated total MMP-9 level was highest in the patients with MI, then in the UAP and the control group. The mean value of total MMP-9 level in the control group was slightly above the upper limit of the reference values (37.1 ng/mL) and there was a statistically significant difference compared to the mean value obtained in the group of diabetics with ACS. The total MMP-9 level, active form, reflects the presence of ACS more precisely. The control group had statistically significantly lower values of active MMP-9 level compared to the groups of diabetics and non-diabetics with ACS. Elevated active MMP-9 level was highest in the patients with MI, then in the UAP group and the control group. The number of patients with highly active MMP-9 level in the control group was highly statistically significantly different compared to the groups with MI and UAP. The mean values of active MMP-9 level between the groups of diabetics and non-diabetics with ACS were highly significantly different. In the group of diabetics with ACS, a total MMP-9 level showed the strongest correlation with the values of HOMA IR index. There was a correlation of elevated cholesterol with the elevated total MMP-9 level in diabetics with ACS. Hypertension and smoking showed a correlation with the values of total MMP-9 level in the group of diabetics. Insulin resistance is closely related to the progression of atherosclerosis and total MMP-9 level may reflect the degree of existing atherosclerosis. In the group of diabetics there was correlation between active MMP-9 level with hypertension and smoking and without HOMA IR. IR is a chronic condition, and active MMP-9 level is a parameter of acute events. A correlation was found with hypertension, also a chronic condition, but may be a sudden increase of pressure in the form of hypertensive crisis, which can trigger an acute coronary event. A correlation with smoking can be the result of the influence of carbon monoxide on the development of coronary vasoconstriction of blood vessels, which in the ground of unstable plaque and hypertension increases the turbulent flow of blood which in turn contributes to plaque rupture. This study examined the active MMP-9 level in diabetics with ACS who are particularly exposed to numerous risk factors including IR [23]. Dysfunction in autonomic nervous system may result in pain absence during coronary event. It is necessary to predict coronary events in diabetics. The level of active MMP-9 level is associated with both plaque rupture and the massiveness of the rupture. There is no adequate information about genetic polymorphisms of MMPs. They can be partially explained by changes in the distribution of MMP-9 at the individual level. If the levels of total and active MMP-9 are directly related to the genetic polymorphism that may result in higher levels of both forms of MMP-9 without ACS. MMP-9 index reflects the impact of both forms of MMP-9. Therefore, we examined the relative ratio of active and total MMP-9, which we define as MMP-9 index. The mean value of MMP-9 index in the group of diabetics was highest, slightly lower in the non-diabetics with ACS, and lowest in the control group. MMP-9 index was very highly statistically significantly different among the groups. Our results showed that the mean MMP-9 index was slightly higher in the patients with MI than in the group with UAP but with no statistically significant differences. These results are expected because level of active MMP-9 is a marker of plaque rupture, which occurs in UAP and MI. MMP-9 index showed a statistically significant correlation with hyperinsulinemia and duration of pain in the group of diabetics with ACS. In order to prove the validity of MMP-9 index we determined sensitivity and specificity for each parameter individually (total, active MMP-9 and MMP-9 level index). Analyzing the ability of total MMP-9 level as a marker of ACS in diabetics we found an important AUC (amounted to 0.774) and the cut-off value for acute coronary events was over 27.6 ng/mL, while level of active MMP-9 in the same group the AUC was 0.936 and the cut-off value over 23.8 ng/mL. The AUC of active and total MMP-9 level differed significantly compared to the ACU of MMP-9 index. In diabetics with ACS, the AUC of MMP-9 index was 0.914 and highly significantly different compared to the AUC of 0.5. MMP-9 index cut-off value was 58.2. The AUC of MMP-9 index had the lowest values of SE.

The sensitivity of total MMP-9 level as a test for ACS in diabetics was 100%, but its specificity was low (only 44.4%). Active MMP-9 level had a good sensitivity and specificity. MMP-9 index showed a very good sensitivity and excellent specificity satisfying the requirements for screening test of ACS in diabetics. MMP-9 index is a relative number which eliminate several possible influences of the variability of active and total MMP-9 level values.

Analysing the available literature does not reveal similar results in terms of active MMP-9 level. MMP-9 index has been first postulated in this study. Total MMP-9 and active MMP-9 levels can be determined relatively quickly and easily. Their commercialization further reduces the costs and allows determination of the proposed MMP-9 index. This index could be a good marker for triage in population of diabetics and selection of candidates for elective coronary angiography. We believe that the use of this index in the secondary prevention of acute coronary events in diabetics may significantly improve cardiovascular outcomes. Without any doubt, it

is necessary to set a prospective randomized study on a larger number of patients, which may eventually correct the cut-off value of MMP-9 index determined by our study.

Conclusion

MMP-9 index could be a good marker for prediction of acute coronary syndrome in diabetic patients and for triage for elective coronary angiography.

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Disclosures

None of the authors have any competing interests.

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Bacterial infections associated with allogenic bone transplantation

Bakterijske infekcije povezane sa transplantacijom koštanog alografta

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Abstract

Background/Aim. Bone allografts are frequently used in orthopedic reconstructive procedures carrying a high risk for recipients. To assess the nature and frequency of allograft contamination and associated surgical infection the case records from our institutional bone bank were reviewed. **Methods.** We retrospectively analyzed the microbiology of discarded bone allografts and the surgical site of the recipients. A case series of patients who acquired surgical site infection after allogenic bone transplantation was presented. Swab culturing was conducted on 309 femoral heads from living donors who underwent partial and total hip arthroplasty between January 2007 and December 2013. To prevent potential bone allograft contamination we used saline solution of 2.0 mg/ml of amikacin during thawing. The overall infection rate was analyzed in 197 recipients. **Results.** Of the 309 donated femoral heads, 37 were discarded due to bacterial contamination, giving the overall contamination rate of 11.97%. The postoperative survey of 213 bone allotransplantations among 197 recipients showed the infection rate of 2.03%. The coagulase-negative *Staphylococcus* was the most commonly identified contaminant of bone allografts and recipient surgical sites. **Conclusion.** The allograft contamination rate and the infection rate among recipients in our institution are in accordance with the international standards. The coagulase-negative *Staphylococcus* was the most commonly identified contaminant of bone allografts and recipient surgical sites. There is no strong evidence that surgical site infections were associated with bone allograft utilization. We plan further improvements in allograft handling and decontamination with highly concentrated antibiotic solutions in order to reduce infection risk for recipients.

Key words:
bacterial infections; bone transplantation; postoperative complications; transplantation, homologous.

Apstrakt

Uvod/Cilj. Koštani alograftovi često se koriste u rekonstruktivnim ortopedskim procedurama. U cilju procene prirode i učestalosti kontaminacije koštanih alograftova i pratećih hirurških infekcija, analizirani su podaci koštane banke u našoj instituciji. **Metode.** Retrospektivno je analiziran mikrobiološki nalaz odbačenih koštanih alograftova i hirurškog mesta primalaca. Prikazana je serija bolesnika sa ispoljenom infekcijom operativnog mesta nakon transplantacije koštanog alografta. Zasejavanje briseva 309 glava butnih kostiju živih donora posle preloma vrata butne kosti ili primarne totalne artroplastike kuka obavljeno je od januara 2007. do decembra 2013. godine. U prevenciji kontaminacije koštanih alograftova korišćeno je 2 mg/mL amikacina prilikom njihovog topljenja. Stopa infekcije analizirana je kod 197 primalaca. **Rezultati.** Od 309 doniranih femoralnih glava, zbog bakterijske kontaminacije odbačeno je 37, dajući stopu kontaminacije od 11,97%. Postoperativnim praćenjem 213 koštanih alotransplantacija kod 197 primalaca ustanovljena je stopa infekcije od 2,03%. Koagulaza negativni *Staphylococcus* bio je najčešće identifikovani uzročnik kontaminacije koštanih alograftova i hirurškog mesta primalaca. **Zaključak.** Stope bakterijske kontaminacije koštanih alograftova i infekcije hirurškog mesta primalaca u našoj ustanovi u skladu su sa međunarodnim standardima. Koagulaza negativni *Staphylococcus* bio je najčešće identifikovani uzročnik kontaminacije koštanih alograftova i hirurškog mesta primalaca. Nema čvrstih dokaza da su infekcije operativnog mesta bile u vezi sa upotrebom koštanog alografta. Planiramo dalja unapređenja u rukovanju i dekontaminaciji koštanog alografta visokokoncentrovanim rastvorima antibiotika u cilju sniženja rizika od infekcije kod primalaca.

Ključne reči:
infekcija, bakterijska; transplantacija kosti; postoperativne komplikacije; transplantacija, homologna.

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Introduction

Bone allografts are frequently used in orthopedic reconstructive procedures carrying a high risk for recipients. An allograft-host non-unions and re-fractures may occur and are amenable to surgery, contrary to allograft associated infections which represent the most terrifying complication [1, 2]. Bone allograft-associated infections are largely dependent on its avascularity and porosity leading to biofilm formation by the contaminants [3, 4]. *Staphylococcus aureus* and coagulase-negative *Staphylococci* (mostly *Staphylococcus epidermidis*) are responsible for 36% to 38% of all allograft infections [1, 4]. Reported infection rate after bone allograft transplantation ranges from 1.6% to 12% [5–10]. An infection management after bone allograft transplantation is extremely challenging and may increase the treatment costs and have medico-legal implications.

To assess the allogenic bone related infection rate, the case records of 309 living donors and 197 recipients were reviewed. We report a case series of four surgical site infections following bone allograft transplantation in tertiary care academic medical center.

Methods

We retrospectively analyzed the microbiology of discarded bone allografts and the surgical site of the recipients. A case series of patients who acquired a surgical site infection after allogenic bone transplantation was presented. Swab culturing was conducted on 309 femoral heads from patients who underwent primary total hip arthroplasty (THA) or sustained a fresh femoral neck fracture between January 2007 and December 2013. Informed consent was obtained, and a detailed history was taken to exclude malignancy, systemic and infectious diseases before retrieval. Potential donors with severe degenerative changes or osteoporosis of the femoral head were excluded from bone harvesting. Patients that failed the selection criteria were excluded as potential donors. A prophylactic antibiotic (cefuroxime, 1.5 g or cefazolin 1.0 g) was given 30 minutes before surgery. We took swab samples from the surgical site and from the femoral head. The container was sealed tightly, immediately isolated with three sterile separate bags, labeled and stored in a freezer at -70°C within 30 minutes. Swab samples were sent to hospital laboratory for microbiological evaluation. Two cultures of aerobic and anaerobic microorganisms in blood agar, MacConkey agar, and chocolate blood agar were analyzed. The donors were tested for hepatitis B and C, HIV and syphilis at donation and at six months after surgery, according to the hospital bone bank protocol. All the blood test results at retrieval were documented in the donor's bone bank records. All donors and recipients were followed up periodically to detect any clinical surgical site infection (SSI). Postoperative SSI was defined as persistent wound discharge and erythema with positive isolation of organisms from wound swabs. According to the Center for disease control and prevention (CDC), a superficial incisional SSI occurs within 30 days after operation when only skin and subcutaneous tissue of the incision were involved. Deep incisional SSI occurs within 30 days after the operation if no implant is left in place, or within one year if implant is in place and infection appears to be related to operation, where infection involves deep soft tissues, such as fascia and muscles [11]. Acceptable bone allografts are stored for a maximum of 5 years. Before application, 213 allografts were thawed 30 minutes in 500 mL of 0.9% sterile saline at 37°C with 1g of amikacin (2 mg/mL). A prophylactic antibiotic (cefuroxime, 1.5 g, cefazolin 1.0 g or vancomycin 1.0 g) was given 30 minutes before surgery to all recipients. Intraoperative allograft culturing was not performed because there is no clinically relevant association between such positive cultures and postoperative wound infections [6, 9].

Results

Of the retrieved 309 femoral heads, 228 (73.78%) were harvested after primary total hip arthroplasty and 81 (26.21%) after fresh femoral neck fracture. Swab cultures were positive for at least one microorganism in 37 allografts giving an overall contamination rate of 11.97%. The coagulase-negative *Staphylococcus* was the most commonly identified contaminant of the bone allografts and the recipient surgical site (Table 1). Swab cultures of the surgical site were negative in all 37 donors. Surgical site infection was not recorded in those patients during the follow-up period of at least 12 months, according to the CDC [11]. A total of 213 (68.93.%) allografts were implanted to 197 recipients. Deep incisional SSI was identified in 4 out of 197 (2.03%) recipients (Table 2).

Table 1
Organisms cultured from allograft bone retrieved and surgical site of the recipient

Microorganism	Number of cultures	
	Bone allograft n (%)	Surgical site n (%)
Coagulase-negative Staphylococcus	9 (24.32)	2 (33.33)
Staphylococcus aureus	5 (13.51)	1 (16.67)
Staphylococcus epidermidis	6 (16.22)	
Streptococcus viridans	2 (5.40)	
Enterococcus faecalis	3 (8.11)	1 (16.67)
Gram-positive anaerobic cocci (GPAC)	5 (13.51)	
Proteus mirabilis	2 (5.40)	2 (33.33)
Acinetobacter species	2 (5.40)	
Bacillus subtilis	1 (2.70)	
Pseudomonas aeruginosa	1 (2.70)	
Providencia species	1 (2.70)	
Total	37 (100)	6 (100)

Discussion

We performed a retrospective case series study reporting the main causes of bone allograft contamination and associated surgical infection in tertiary care academic medical center. Deep wound infection after bone allograft transplantation may ha-

ve dreadful outcome. Literature confirmed that allograft- associated infection was not the same as allograft-transmitted infection. The most of the recipients who received contaminated allografts were clinically with no signs of infection [6, 8]. Three-quarters of these allograft-associated infections occured within 4 months of allogenic bone transplantation and led to osteomyelitis and osteolysis, which posed a huge challenge to both physicians and patients, as well [1, 4]. Sommerville et al. [12] performed a 4-specimen-culture study of 232 femoral heads, and found an overall 22% contamination rate. The majority of organisms cultured were *Staphylococcus epidermidis*. The overall infection rate was 2.4%. In our institution, the surgical site infection rate among recipients compared favorably with other reports as well as the allograft contamination rate. All 4 allograft related infections occurred within four months after transplantation. Three of 4 cases were high energy trauma patients with severe soft tissue injuries. The coagulase-negative *Staphylococcus* was the most commonly identified contaminant of the bone allografts and the recipient surgical site. Two or more pathogens were isolated in 2 of 4 patients with SSI. Thorough analysis of the patients' records revealed that none of these infections were obviously connected with bone allograft utilization. In case of distal femoral non-union, an infection occurred following revision surgery. In case 2, a 30-year-old patient suffered a comminuted Sandres IV left calcaneal fracture and right Schatzker II tibial plateau fracture after fall from height of 6 meters [13, 14]. He suffered an urinary tract infection immediately after surgery and positive urin cultures were found. No signs of surgical site infections were recorded postoperatively. The same bone allograft was used for two surgical sites. Three weeks after surgery, *Enterococcus faecalis* was isolated after debridement of necrotic skin on the medial side of the foot. A tibial plateau fracture healed uneventfully 12 weeks after surgery, with no signs of infection. Our assumptions are directed toward urinary tract infection following surgery as the primary source for hematogenous dissemination of bacteria into the severely injured hindfoot. In 3 of 4 cases, SSI successfully healed with full allograft incorporation. Two low virulent coagulase-negative *Staphylococcus* species and one highly virulent *Staphylococcus* aureus indicate the importance of strict monitoring system, aseptic handling technique and clean environment in the operating theatre. It is likely that graft surface is ideal for bacterial adherence and leads to selection of contaminants that exhibit marked adhesive properties, biofilm as well as increased resistance towards antibiotics. It seems that allograft associated infection may be prevented the same way as the implant associated infection. There are attempts to bond antibiotics to amine groups of allograft bone collagens and provide long-term bactericidal concentrations to prevent allograft associated infections [4]. The swab cultures had been proven insufficient to detect bacterial contamination of musculoskeletal allografts due to low sensitivity. Recent reports indicate that swab cultures after thawing were different from the wound cultures in most of the infected patients [15]. Our previous results based on

Table 2

Characteristics of infection cases after allogenic bone transplantation

No.	Gender/ Age (years)	Surgery	Bacteria	Clinical findings	Antimicrobial therapy
1	Woman/ 76	Fracture non-union Revision surgery	Methicilin-resistant *Staphylococcus aureus*.	Severe osteoporosis; Early low grade infection 3 months after revision surgery, wound debridement, implant removal	Vancomycin (1g/12 h) Rifampicin (600 mg/24 h) Trimethoprim/sulfamethoxazole (960 mg/12 h)
2	Male/ 30	Sanders IV calcaneal fracture, Schatzker II tibial plateau fracture	*Enterococcus faecalis, Proteus mirabilis Pseudomonas aeruginosa*	Positive urin cultures after surgery. Early high grade infection one month after surgery, multiple soft tissue revisions, implant removal, lower leg amputation	Ciprofloxacin (100 mg/12 h) Ofloxacin (200 mg/12 h) Amikacin (1.0/24 h) Amoxicillin/clavulanic acid (1.2g/8 h) Ceftazidime (2 g/8 h) Metronidazole (400/8 h)
3	Woman/ 39	Sanders III-calcaneal fracture	Coagulase-negative *Staphylococcus*	Early deep wound infection one month after surgery, multiple soft tissue revisions, implant removal	Vancomycin (1 g/12 h)
4	Male/ 55	Schatzker V tibial plateau fracture	Coagulase-negative *Staphylococcus, Proteus mirabilis*	Early deep wound infection two months after surgery, wound debridement, implant removal	Ceftazidime (2 g/8 h) Ceftriaxone (2 g/24 h) Gentamicin (240 mg/24 h)

the overall audit of bone bank performance, indicate that the highest risk of bone allograft contamination exists during its harvesting and thawing. We concluded that microbial contamination and allograft associated infection rate were predominantly influenced by the surgical team and its immediate environment [16]. Antibiotic rinsing of the allograft has been proposed by some authors, but it does not affect the risk of contamination in large studies with postmortem donors [17–21]. Bone allograft immersion in saline solution with high concentration of bactericidal antibiotics such as aminoglycosides may promote infection control and act as simple as effective secondary sterilization [16]. An antibiotic selection for such prophylactic decontamination should be variable and may be determined by the specific susceptibility of strains (if any) isolated in the operating theatre, or by the strains mostly isolated from the surgical site and coordinated with epidemiology department.

Conclusion

The allograft contamination rate and the infection rate among recipients in our institution are in accordance with the international standards. The organism most commonly identified as contaminant of bone allografts and surgical sites was coagulase-negative *Staphylococcus*. There is no strong evidence that surgical site infections are associated with bone allograft utilization. We plan further improvements in allograft handling and decontamination with highly concentrated antibiotic solutions in order to reduce infection risk for recipients.

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Treatment of low-grade gastric MALT lymphoma using *Helicobacter pylori* eradication

Lečenje MALT limfoma želuca niskog stepena maligniteta eradikacijom *Helicobacter pylori* infekcije

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Abstract

Background/Aim. Lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) of the stomach usually occurs as a consequence of *Helicobacter pylori* (*H. pylori*) infection. The aim of this study was to investigate the long-term effect of treatment of low-grade gastric MALT lymphoma with the *H. pylori* eradication method. **Methods.** In the period 2002–2012 in 20 patients with dyspepsia, mean age 55.1 years, the endoscopic and histologic diagnosis of gastric MALT lymphoma in the early stages were made. Histological preparations of endoscopic biopsy specimens were stained with hematoxyllin-eosin (HE), histochemical and immunohistochemical methods. **Results.** Endoscopic findings of gastritis were documented in 25% of the patients, and 75% of the patients had hypertrophic folds, severe mucosal hyperemia, fragility, nodularity, exulcerations and rigidity. Histopathologically, pathognomonic diagnostic criterion were infiltration and destruction of glandular epithelium with neoplastic lymphoid cells, the so-called lymphoepithelial lesions. In all 20 patients *H. pylori* was verified by rapid urease test and Giemsa stain. After the triple eradication therapy complete remission of MALT lymphoma was achieved in 85% of the patients, with no recurrence of lymphoma and *H. pylori* infection in the average follow-up period of 48 months. In 3 (15%) of the patients, there was no remission of MALT lymphoma 12 months after the eradication therapy. Of these 3 patients 2 had progression of MALT lymphoma to diffuse large-cell lymphoma. **Conclusion.** Durable complete remission of low-grade gastric MALT lymphoma is achieved in a high percentage after eradication of *H. pylori* infection, thus preventing the formation of diffuse large-cell lymphoma and gastric adenocarcinoma.

Key words:
lymphoma, b-cell, marginal zone; stomach neoplasms; helicobacter pylori; drug therapy; remission induction; prognosis; histology.

Apstrakt

Uvod/Cilj. Limfom limfnog tkiva mukoze (MALT limfom) želuca najčešće nastaje kao posledica *Helicobacter pylori* (*H. pylori*) infekcije. Cilj studije bio je da se ispita dugotrajni efekat lečenja MALT limfoma želuca niskog stepena maligniteta eradikacijom *H. pylori* infekcije. **Metode.** U periodu od 2002. do 2012. godine kod 20 pacijenata sa simptomima dispepsije, prosečne starosti 55,1 godinu, endoskopski i histološki je dijagnostikovan MALT limfom želuca u ranoj fazi. Histološki preparati endoskopskih biopsijskih uzoraka bojeni su klasičnom hematoksilin-eozin (HE) metodom, histohemijskim i imunohistohemijskim metodama. **Rezultati.** Endoskopski nalaz gastritisa imalo je 25% bolesnika, dok je 75% bolesnika imalo hipetrofiju nabora, jaku hiperemiju mukoze, fragilnost, nodularnost, egzulceracije i rigiditet. Patohistološki, patognomoničan dijagnostički kriterijum bila je infiltracija i razaranje žlezdanog epitela neoplastičnim limfoidnim ćelijama, odnosno limfoepitelijalna lezija. Kod svih 20 bolesnika bio je verifikovan *H. pylori* brzim ureaza testom i modifikovanom Giemsa metodom. Nakon trojne eradikacione terapije potpuna remisija MALT limfoma postignuta je kod 85% bolesnika, bez recidiva limfoma i *H. pylori* infekcije u prosečnom periodu praćenja od 48 meseci. Kod 3 (15%) bolesnika nije došlo do remisije MALT limfoma 12 meseci od eradikacione terapije. Kod dva od ova tri bolesnika došlo je do progresije MALT limfoma u difuzni krupno ćelijski limfom. **Zaključak.** Potpuna dugotrajna remisija MALT limfoma želuca niskog stepena maligniteta postiže se u visokom procentu posle eradikacije *H. pylori* infekcije. Na taj način sprečava se nastanak difuznog krupnoćelijskog limfoma, kao i adenokarcinoma želuca.

Ključne reči:
limfom, malt, želudac, neoplazme; helicobacter pylori; lečenje lekovima; remisija, indukcija; prognoza; histologija.

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Introduction

Primary lymphomas of stomach are rare tumors, which make less than 5% of primary stomach neoplasms. However, they are the most common extranodal lymphomas, representing 4–20% of all extranodal lymphomas [1, 2]. Lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is an extranodal lymphoma of the marginal zone, which contains morphologically heterogenic small B-cells with marginal zone (cells similar to centrocytes), cells that are similar to monocytes, small lymphocytes and singular immunoblasts, as well as cells similar to centroblasts [3, 4]. Infiltration of epithelium with neoplastic cells and forming of lymphoepithelial lesion is typical in epithelial tissues. Lymphoepithelial lesion appears as an indicator of MALT lymphoma at biopsy samples, which is the result of atypical lymphocytes invasion and reactive lymphoid follicles of tissue [5].

Most of MALT lymphoma are low-grade malignancy, small number are initially manifested as mid-grade malignancy no Hodgkin's lymphoma (NHL), which can develop as the evolution of low-grade malignancy lymphoma. Under normal circumstances, there is no organized lymphoid tissue in musoca of stomach, except poorly present lymphocytes. Besides that, most of MALT lymphomas develop in the stomach. This paradox can be explained by the fact that MALT and its product MALT lymphoma develop after colonization of the stomach with *H. pylori*, because in 70% of cases MALT lymphoma develops as the result of *H. pylori* infection. Several cytogenetic alterations are identified, most commonly trisomy 3 or t (11;18). Mutations are usually identified in NHL and are mostly not present in MALT lymphoma, although there are reports on the presence of BCL2 and TP53. However, specific genetic abnormalities which would be responsible for pathogenesis of MALT lymphoma are still not identified [6, 7].

In 1991, Wooterspoon et al. [8] announced for the first time that the patients with gastritic MALT lymphoma were ordinarily infected with *H. pylori*. After histomorphological examinations, recent epidemiological, molecular biological and experimental examinations showed the key role of *H. pylori* in the development and progression of gastric MALT lymphoma. These examinations led to the revolutionary shift in treatment of these patients. The tumor was cured with antibiotic therapy for the first time in the history of medical oncology [9].

The aim of this prospective non-randomized study was to investigate the long-term effect of low-grade gastric MALT lymphoma treatment applying *H. pylori* eradication.

Methods

In a period 2002–2012 in 20 patients (11 or 55% male and 9 or 45% female), average age 55.1 + 10.76 (32–73) years MALT lymphoma of the stomach was diagnosed in early stage, with localization in mucosa and/or submucosa.

Upper gastrointestinal endoscopy was done using video gastroscopes Olympus GIF-Q165 to all of the patients with symptoms of dyspepsia. At least 10 biopsies were taken from mucosa of the stomach for the histological examination, 3 of which from the antrum (the front, the back and incisura angulatu), 3 from the corpus (the front wall of the corpus, the back wall of the corpus and fundus) and at least 4 biopsies from suspicious lesion. Two additional biopsies were taken (one from the antrum, 20 mm from the pylorus towards big curve, and from the fundus) for *H. pylori* quick urease test.

Endoscopic bioptic material was fixated in 10% formaldehyde solution for 24 h. Then, laboratory testing of tissue in autotechnicon, with molding in paraffin and cutting of paraffin molds that were 3 μm thick was performed. Deparaffinated, enlightened through xylenes and rehydrated histological preparations were then colored using the following methods: classical hematoxylin-eozin (HE) method, histochemical methods like acian blue–periodic acid Schiff (AB-PAS, pH 2.5), van Gieson and modified Giemsa, immunohistochemical method Avidine-Biotine-Complex (ABC) by using antibodies on pancytoceratine, as a common marker for all tumors of epitelial origin, anibodies on CD20, as a marker for B-cell origin of lymphoma, antibodies on CD3, as the marker of T-cell origin and Ki-67, as the marker for mitotic activity of tumor cells.

Routine screening was done in all the patients in order to evaluate the eventual propagations of lymphoma. Routine screening included biohumoral analysis, ultrasound examination of upper abdomen, and radiografic examination of lungs and heart. Computed tomography (CT) and endoscopic ultrasound were also done in several patients.

Results

In 12 of the 20 (60%) patients diagnosed with MALT lymphoma of the stomach, the symptoms of dyspepsia were present, in the form of epigastric pain, nausea and intumescence of the abdomen, while 8 (40%) patients had alarming symptoms (poor apetite, weight loss, hematemesis and/or melena).

Endoscopic finding of gastritis, often present with nodular aspect, had 5 (25%) out of 25 patients, and the other 15 (75%) patients had hypertrophy of crease, a strong hyperemia of mucosa, fragility, nodularity, ulcerations and rigidity (Figure 1).

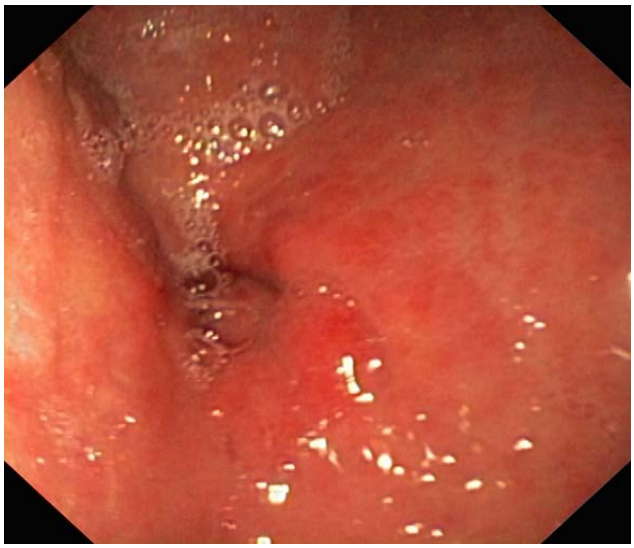

Fig. 1 – Endoscopic appearance of mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach antrum: edema, hyperemia, mucosal fragility and nodularity.

Most of the patients had the described changes that were located in the lower part of the corpus and the antrum of the stomach in 7 of 15 (46.7%) patients, only in the antrum in 4 (26.7%) patients, or only in the corpus of the stomach in 4 (26.7%) patients.

From patohistological perspective, in the early phase of MALT lymphoma, lymphoid follicles look like normal lymphoid follicles, which are hyperplastic with reactive germinative centers and with multiplied small B-lymphocytes of marginal zone, often densely distributed in *lamina propria* of mucosa. Because of the similarity with centrocites, monocites and plasmocites they are classified in centrocitoid, monocitoid and plasmacitoid types (Figure 2).

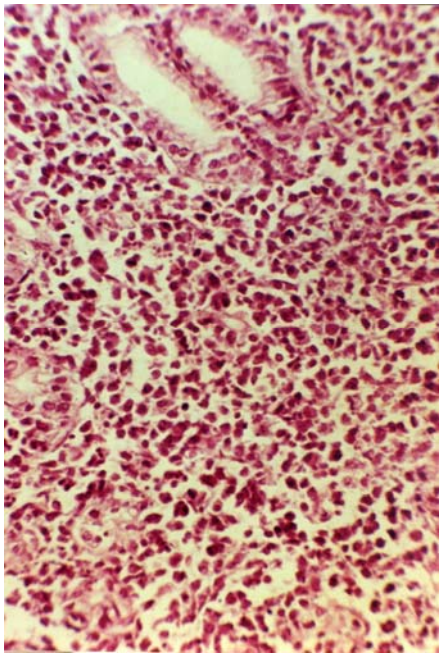

Fig. 2 – Mucosa-associated lymphoid tissue (MALT) lymphoma – centrocytoide type (HE × 150).

Atypical lymphoid cells type, centroblast and immunoblast, were rare and mixed with the dominant cells of marginal zone. Malignant cells did infiltration, destruction, and replacement of glandular parenhyma. Therefore, pathognomonic diagnostical criteria were infiltration and destruction of glandular epithelium by the nearby neoplastic lymphoid cells, apropos "lymphoepithelial lesion", verified by imuunohistochemical epithelial marker pancytokeratin (Figure 3). Antibodies on CD20, as the mutual marker for B-cell origin of lymphoma were positive (Figure 4). Antibodies on CD3 were negative, which excluded T-cell origin of MALT lymphoma. Mitotic activity verified by marker Ki-67 was negligible in MALT lymphoma, while in case of its progression to lymphoma of higher grade of malignity a significant increase of this marker was detected.

In all the 20 (100%) patients *H. pylori* was verified by bioptic urease test and the histologically modified Giemsa method. The triple eradication therapy was administered for 10 days, with protonic pump inhibitor in double dose, with clarithromycin 2 × 500 mg and with amoxicilin 2 × 1000 mg or metronidazole 3 × 500 mg. Two months after eradication therapy, control examinations of upper endoscopy and histological examination of endoscopic biopsies were done. Then, endoscopic-histological control examinations were done once in every 6 months. In case of histological verification of full remision of MALT lymphoma in the two consecutive results done once in every 6 months, the examination was done once a year. The average period of monitoring all of the 20 patients was 48 ± 19.8 months (from 2 to 98 months).

Eradication of *H. pylori* infection was considered succes-

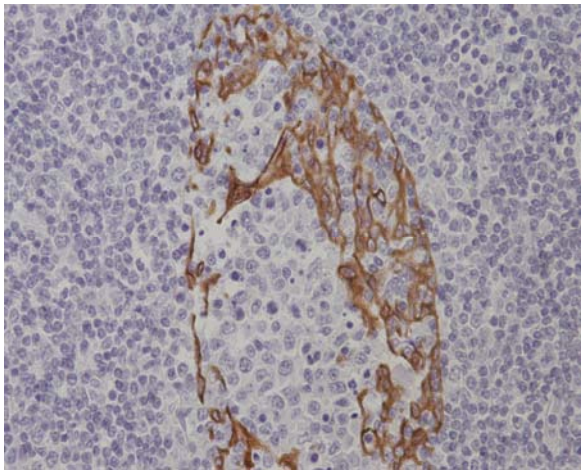

Fig. 3 – Lymphoepithelial lesion of MALT lymphoma (Pancytokeratin, × 400).

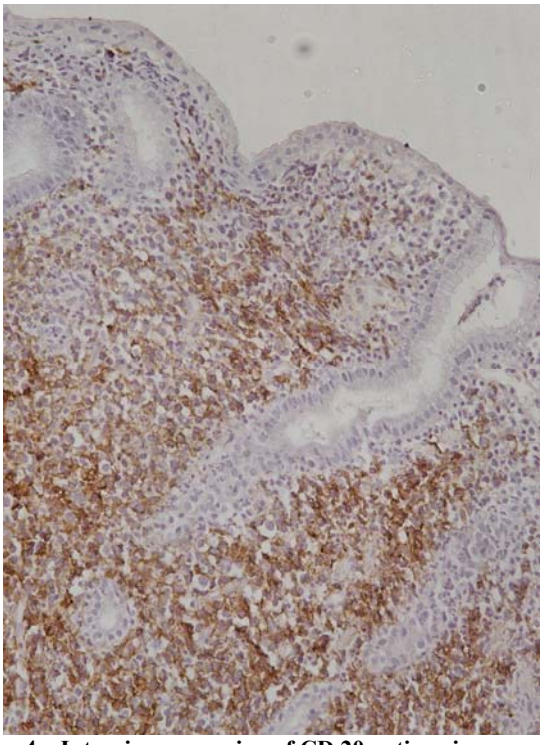

Fig. 4 – Intensive expression of CD 20 antigen in mucosa-assasiated hymphoid tissue (MALT) lymphoma (ABC, × 200).

sful in case of negative results of both tests (bioptic urease test and modified Giemsa) on *H. pylory* 2 months after the therapy. That was accomplished in all the 20 (100%) patients. Complete histological remision of MALT lymphoma was accompished in 17 of the 20 (85%) patients. In 5 out of 17 (29.4%) patients complete remision was accomplished 2 months after the

eradication therapy, in 8 out of 17 (47%) 6 months after, and in 4 out of 17 (23.5) 12 months after the therapy (Table 1).

Table 1
Period of complete remission of MALT lymphoma achieved after the *H. pylori* eradication therapy

Period after eradication therapy (months)	Number of patients	
	n	%
2	5	29.4
6	8	47
12	4	23.5
Total	17	100

MALT – mucosa associated lymphoid tissue

In the average perod of follow-up (48 ± 19.8 months) there were no relapses of MALT lymphoma, non relapses of *H. pylori* infection in the patients with complete remission that was accomplished. In 3 (15%) of the patients with localisation of MALT lyphoma in mucosa and submucosa there was not remission during the 12 month follow-up. Chemotherapy was administered due to worsening of sympthoms (poor apetite, weight loss), also due to persistention of endoscopic and histological signs of MALT lymphoma. In 2 of these 3 patients there was the progression of MALT lymphoma in diffuse large-cell lymphomas, after 2 and 5 years of the MALT lymphoma diagnose. Both patients died during the repeated chemotherapy.

In the patients with full remission of MALT lymphoma accomplished, there was a regression of endoscopic changes. Histologically, in the patients with complete remission of lymphoma *lamina propria* looked "empty", with the loss of glands. Rare lymphocites and monocites were spilled in the fur, and there were some focal accumulations of small lymphocites. There were no lymphoepithelial lesions. Epithelium was normal with the adequate secretion of mucine. Sometimes the empty spaces were filled with the typical foveolar hyperplasia (Figure 5).

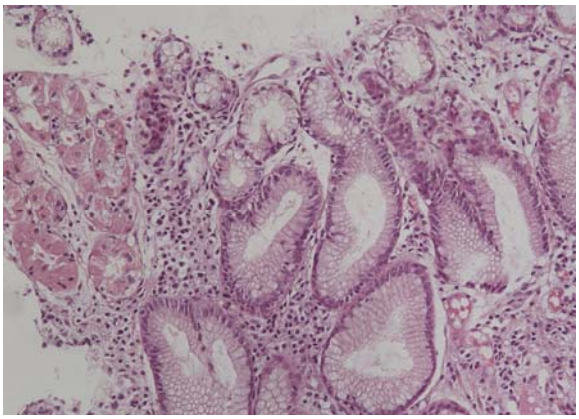

Fig. 5 – Effect of *H. pylori* eradication therapy: a rare lymphocytic infiltrate with typical foveolar hyperplasia of the covering epithelium (HE, × 250).

Discussion

MALT lymphoma is slightly more common in females, the average age of 65 years, and with the highest prevalence in seventh and eight life decade, although it can occur in children, adolescents, and younger people [10]. In our patients, MALT lymphoma of the stomach was similarly present in both males and females (males 55% and females 45%), and with the average age of 55 years.

In most of cases with MALT lymphoma it is diagnosed during upper gastrointestinal endoscopy, which is done due to usual dyspeptic symptoms. Alarming symptoms, as vomiting, weight loss, hematemesis and melena are rarely present [11]. In our study alarming symptoms were often present in 40% of patients.

Histological diagnosis of MALT lymphoma is often unexpected for an endoscopist because of the fact that only endoscopic signs of gastritis are found in more than 50% of cases, while in 41% of the cases there are singular or multiple active or rehabilitated ulcerations, while in 5% of cases there is erosion. Irregular and serpentine hypertrophic folds, which are described as a typical endoscpopic finding are present in 3% of patients and 1% of tumor mass. All toghether, endoscopic finding which would be characteristic for lymphomas is present in only the third of the number of patients [11]. In our study, a fewer patients (25%) with MALT lymphoma had only endoscopic signs of gastritis, which could be explained by the fact that within the previous years we did not routinely take biopsies of all patients with gastritis. A small number of bioptic samples makes the initial diagnosis of lymphoma more difficult. Since gastric MALT lymphoma can be multifocal, and diffuse large-cell lymphoma can exist at the same time, it is recommended to take more biopsy samples. There is no standardized protocol of taking biopsies, but multiple biopsies of every endoscopic lesion are recommended, as well as biopsies of macroscopically unchanged mucosa of all the main gastric regions (antrum, angulus, corpus and fundus), from the front and the back wall, and from the small and large curve. The identical protocol of taking biopsies should be applied on control endoscopic examinations, for the sake of adequate histologic comparation [11, 12], which guided us in our study.

Since there is no specific marker for immunohistochemical typisation of MALT lymphoma, histological HE method is "the gold standard" of lymphoma diagnosis. The pathohistological diagnosis is often difficult in the early phase of MALT lymphoma. The most common diagnostical dilemma is the differentiation between chronic atrophic gastritis and MALT lymphoma. That is why it is recommended to take multiple biopsies and to discover lymphoepithelial lesions with lymphocites that have polymorphic and atypical nucleus and increased mitotic activity [10]. The main diagnostic criterion that favors the diagnosis of MALT lymphoma is quantitative. The lymphoid infiltrate has to be thick and occupies most of the *lamina propria*, leading to the destruction and replacement of glandular structure, which is characteristic lymphoepithelial lesion. Among those present lymphoid follicles interfollicular space should be completely filled with lymphocytes [11]. The main cellular components of MALT lymphoma are cells similar to centrocytes, monocitoid cells, small circular lymphocytes, plasma cells, and scattered, individual, large centroblasts or immunoblasts similar cells. In gastritis dominate plasma cells and inflammatory activity manifested by granulocyte-leukocyte infiltration [12–14].

Histological parameters are usually sufficient for the diagnosis of gastric MALT lymphoma and rare lymphoma of the stomach, other similar histological structure, and follicular and "mantle cell" type. Nevertheless, the analysis of the panel of immunohistochemical markers types CD20, CD79a, CD3, CD5, CD10 and Cyclin D1 confirm the diagnosis of gastric MALT lymphoma. The neoplastic cells of MALT lymphoma showed expression of B-cell markers CD20 and CD79a. Dense CD20+ B cell infiltrate that invades and replaces the glandular structure is a common finding in MALT lymphoma. Markers CD5, CD10, BCl6 , and cyclin D1 were always negative in MALT lymphoma and allow exclusion of other small B cell lymphoma. There are also a number of "scattered" CD3+ non-neoplastic T-cells. Staining of pancytokeratin provides better visibility lymphoepithelial lesions of MALT lymphoma [11]. In our patients histological parameters were crucial for the diagnosis of gastric MALT lymphoma, also. For diagnostic confirmation of MALT lymphoma, we used immunohistochemical markers, such as antibodies to pancytokeratin, a common marker for the tumors of epithelial origin and antibody to CD20, a marker for B-cell lymphoma origin, who were positive and antibodies to CD3, which was negative, excluding the T-cell origin of lymphoma.

The exact prevalence of *H. pylori* infection in MALT lymphoma is unknown and varies depending on the study from 50% to almost 100%. This variability could be explained by the number of tests used for detection of *H. pylori* and their kind. If you use only one test more likely are false negative results. As for the types of tests, the prevalence of *H. pylori* infection is higher when using serological and urea based test compared to biopsy. This is explained by extensive mucosal lesions in MALT lymphoma that may lead to the reduction in *H. pylori* colonization, to undetectable levels. Also, the prevalence of *H. pylori* infection depends on the depth of invasion of lymphoma, so the lymphoma limited to the mucosa and submucosa prevalence of infection is higher than in lymphomas with a deeper propagation. These results support the hypothesis that *H. pylori* is present in the early stage of MALT lymphoma, but later, with the progression of lymphoma may result in the loss of *H. pylori* [15–17]. In all of our patients with MALT lymphoma *H. pylori* infection was proven. Such a high percentage of *H. pylori* infection in our patients could be explained by the fact of a large number of biopsy samples, and the two tests used for the detection of *H. pylori* (biopsy urease test and Giemsa stain) and that MALT lymphomas were at an early stage, limited to the mucosa and/or submucosa.

According to Maastricht IV Consensus, eradication of *H. pylori* is the first line therapy of MALT lymphoma of low-grade malignancy [18]. In 60–100% cases of MALT lymphoma remission is achieved with eradication of *H. pylori* infection [17]. Detection of genetic alterations, such as translocations t (11,18) (q21; q22) lead to poor therapeutic response to antibiotics and recommended for consideration other therapeutic modalities for MALT lymphoma, such as radio- or chemotherapy. Molecular biology techniques, such as polymerase chain reaction (PCR) methods contribute to the diagnosis of B cell clonality detection of MALT lymphoma, although a negative PCR result does not rule out MALT lymphoma [18, 19].

Similar to data in the literature, in 85% of our patients there was a complete remission of MALT lymphoma after eradication of *H. pylori* infection. Complete remission of lymphoma involves the absence of endoscopic and histological signs of lymphoma in two consecutive follow-ups. Histologically, neoplastic lymphoid infiltrate and lymphoepithelial lesions are completely withdrawn. Residual scattered lymphocytes and plasma cells are present in the *lamina propria*, and regressive changes such as stromal fibrosis and empty *lamina propria* devoided of glands [10, 11].

In our study, complete remission of MALT lymphoma was usually achieved 6 months after the eradication therapy (47%), after 2 months (29.4%) and after 12 months (23.5%). In a prospective study, Hong et al. [20] achieved complete remission in 78% of patients after 6 months, while in 93% it was achieved after 12 months of eradication therapy. In a systematic review by Zullo et al. [21] complete remission of lymphoma occurs in approximately 5 months, but in a small number of patients after a much longer period (3–4 years). Therefore, remission of lymphoma is a continuous process that can be fast (2–3 months), but in many patients longer (4–12 months or over 24 months) [19].

According to data in the literature, the majority of patients with MALT lymphoma after successful eradication of *H. pylori* take years to maintain stable remission [22]. In some studies with long-term monitoring the average of 5% or 10 % of relapses of lymphoma were shown [17, 23]. A higher percentage of relapse is shown in MALT lymphoma with previously undiagnosed foci of high grade malignancy. Overall, relapse of MALT lymphoma is mainly related to the recolonisation of *H. pylori* [19]. In the absence of these reasons, the transient relapse of MALT lymphoma is described, which is self-limiting with repeated spontaneous remission [21]. In our study, there was no recurrence of MALT lymphoma during the average follow-up of 48 months, which may be explained by the absence of reinfection, as well as the lack of focus of lymphoma with a high degree malignancy. Currently, it indefinite endoscopic-histologic follow-up of patients *per* year who are in stable remission is recommended, in order to detect possible reinfection and relapse of lymphoma [11]. In patients with histological remission achieved, the presence of persistent monoclonal B-cell population, detected by PCR, is the risk factor for relapse of lymphoma. Therefore, these patients are recommended for more intensive monitoring, and in the case of at least three negative findings by PCR analysis monitoring may be in longer intervals [24].

Remission was not achieved in 25% of our patients, possible because there was a translocation t (11, 18), which we could not, for technical reasons, found out. In cases with no complete remission of early stage MALT lymphoma after eradication of *H. pylori* infection achieved strategy of "watch-and-wait" for a period of 24 months prior to the consideration of alternative treatments are generally considered adequate. The interval could be shorter than 24 months in case of perigastric lymph node development and widespreaded disease [11]. However, a large number of protocols recommend that after 12 months of successful eradication therapy it can be considered that there is no response

to antibiotic therapy, so another form of treatment should be considered [19]. In our study, the patients with no remission of MALT lymphoma after *H. pylori* eradication after 12 months were sent to chemotherapy due to the worsening of clinical symptoms and persistence of endoscopic and histological signs of MALT lymphoma. Besides the possibility of the absence of MALT lymphoma remission, recurrence of lymphoma and MALT lymphoma transformation to diffuse large cell lymphoma, there is a 6 times higher risk of developing adenocarcinoma of the stomach, compared to the general population [21, 25].

The shortcomings of our study are a relatively small series of patients, and no molecular techniques such as PCR used for the detection of B-cell clonality and genetic abnormalities.

Conclusion

The results of our study show that complete remission of MALT lymphoma of low-grade malignancy is achieved in a high percentage (85%) by the eradication of *H. pylori* infection, generally up to 12 months after the eradication therapy. Remission of MALT lymphoma is held steady for years, if there is no *H. pylori* reinfection and focus lymphoma of high-grade malignancy in the initial MALT lymphoma. Treatment for MALT lymphoma of low-grade malignancy with eradication of *H. pylori* infection prevents the occurrence of diffuse large-cell lymphoma, and gastric adenocarcinoma.

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Correlation and characteristics of self-rating and clinically rating depression among alcoholics in the course of early abstinence

Povezanost i karakteristike samoprocene i kliničke procene depresije kod alkoholičara u toku rane apstinencije

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Abstract

Background/Aim. Depression is an alcoholism relapse risk factor, but frequently stays underdiagnosed among treated alcoholics. The correlation and characteristics of self-reported and clinically assessed depression in the course of early alcohol abstinence were explored. **Methods.** A total of 100 inpatient, primary male alcoholics (20–60 years) diagnosed according to Classificaton of Mental and Behavioural Disorders (ICD-10) and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) were recruited consecutively. The Hamilton Depression Rating Scale (HDRS) and Beck Depression Inventory (BDI) were scored on admission (T1), after 4 weeks (T2) and after 8 weeks (T3). Student's *t*-test, repeated measures ANOVA and Pearson's correlation between the scores were done ($p < 0.05$). Factor analyses of symptoms were performed. **Results**: On HDRS T1, T2, T3 90,7%, 39.5%, 17.4% alcoholics were depressive, respectively. The mean HDRS *vs* BDI scores on T1, T2 and T3 were 15.16 ± 6.34**,** 7.35 ± 4.18**,** 4.23 ± 2.93 *vs* 14.20 ± 9.56, 8.14 ± 7.35, 5.30 ± 4.94, respectively. Depression severity significantly lowered in the course of abstinence (ANOVA). The HRDS and BDI correlations on T1, T2 and T3 were significant **(**r1 = 0.763, r2 = 0.684, r3 = 0.613 respectively). Dysphoric mood, anxious, vegetative and cognitive HDRS subscales on T1, T2 and T3 were detected, but not BDI factors, thus BDI symptoms were analysed. **Conclusions.** The majority of alcoholics had depression on admission. A predominant mild-degree with a significant lowering of depression severity and positive significant correlations between HRDS and BDI scores in the course of abstinence were detected. The dysphoric mood on the HDRS subscale, and self-blame, anhedonia and guilt BDI symtoms were most prominent and persisted. The BDI could be a useful tool not only for routine screening and reassessment of depression, but also for exploring emotional content during early abstinence and planning tailored integrative therapy and relapse prevention for alcoholics.

Key words:
alcoholism; depression; comorbidity; psychiatric status rating scales; self-evaluation programs.

Apstrakt

Uvod/Cilj. Depresija predstavlja faktor rizika za relaps alkoholizma, ali je često nedijagnostikovana kod lečenih alkoholičara. Ispitani su povezanost i karakteristike samoprocene i procene depresije kod alkoholičara u toku rane apstinencije. **Metode.** U ispitivanje je bilo uključeno 100 primarnih alkoholičara muškog pola, starosti 20–60 godina (dijagnostikovani prema Međunarodnoj klasifikaciji bolesti – MKB-10 i Dijagnostičko-statističkom priručniku – DSM-IV). Depresija je bila procenjena Hamiltonovom skalom (HDRS) i Bekovim upitnikom (BDI) na prijemu (T1), posle četiri (T2) i osam nedelja (T3). Razlike skorova bile su testirane Studentovim *t*-testom i ponovljenim merenjima ANOVA. Primenjena je i Pearsonova korelacija ($p < 0.05$), kao i faktorska analiza simptoma. **Rezultati.** Na HDRS skali u vremenima T1, T2, T3 bilo je depresivno 90,7%, 39,5%, odnosno 17,4% alkoholičara. Prosečni skorovi HAMD *vs* BDI u vremenima T1, T2 i T3 bili su 15,16 ± 6,34**,** 7,35 ± 4,18**,** 4,23 ± 2,93 *vs* 14,20 ± 9,56, 8,14 ± 7,35, 5,30 ± 4,94. Opadanje težine depresije na ANOVA i korelacije između HRDS i BDI u vremenima T1, T2 i T3 (r1 = 0.763, r2 = 0,684, r3 = 0,613) bile su značajne. Na sva tri merenja bile su prisutne sve četiri HDRS supskale (disforično raspoloženje, anksiozna, vegetativna i kognitivna), ali BDI faktori nisu nađeni, pa su analizirani BDI simptomi. **Zaključak.** Na prijemu, većina alkoholičara imala je prosečno blagu depresiju. U toku osam nedelja, značajno je opao intenzitet depresije sa pozitivnom korelacijom između HDRS i BDI. Posle osam nedelja perzistirala je kao najprominentnija Hamiltonova supskala disforičnog raspoloženja, a na BDI simptomi samooptuživanja, osećaji gubika zadovoljstva i krivice. BDI bi mogao biti koristan ne samo za rutinsko isitivanje i praćenje depresije, već i za procenu unutrašnjeg sadržaja depresije u ranoj apstinenciji radi planiranja intergrativnog lečenja i prevencije relapsa kod alkoholičara.

Ključne reči:
alkoholizam; depresija; komorbiditet; psihijatrijski status, testovi; samoprocena, programi.

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Introduction

Alcoholism and depression often co-occur as confirmed in numerous both clinical and population investigations [1–3]. Several decades ago depression was not treated in dependent alcoholics because clinicians experience was that depressive symptoms were transitory during alcohol withdrawal [4, 5]. Alcohol-induced depression withdraws after the four-week abstinence and explains almost one half of depression episodes prevalence in alcoholics lives and it does not require treatment [6]. A prospective study showed that after a year of abstinence, male alcoholics were at more than twofold increased risk of severe depression comparing to general population [7]. A study on relapse prevention has shown that the most frequent determinant for relapse in the treatment of alcoholics was depressive mood in the early phase of abstinence [8]. Follow-up of the treated alcoholics has shown that relapse after 5 months more often occurs in those who had more expressed depression at the beginning of abstinence [9]. Depression disorder found out after 3 weeks from the beginning of abstinence represents a greater risk of relapse after 6 months of maintained abstinence [8]. Depression persistence after alcohol withdrawal makes treatment of alcoholics more complicated [10]. It is still insufficiently clear how comorbid depression changes during alcoholism therapy as well as which one is the most effective for both disorders [11]. Small differences observed in clinical features among alcoholics with and without depression are a particular problem because those with depression are more similar to the ones without depression than to depressive patients [12]. On the other hand, alcoholics have less clear insight about their own health and emotional condition at the beginning of the abstinence and it is also associated with the previous long period of masking emotions and self-medication with alcohol. There is a challenge for clinicians to recognize depression symptoms even on admission and then to anticipate whether these symptoms will be temporary or persistent [13, 6]. For this reason it is important not only to assess depression, but also to follow-up it in the course of early abstinence with the aim to apply timely therapy [14]. Interaction between depressive symptoms and therapist focus on the emotional status of the alcoholics is an important predictor of their successful treatment [15, 16]. In order to explore emotional status and plan the treatment, it would be useful to determine depression quality because alcoholics experience abstinence as the lost pleasure and they are reluctant to necessary treatment. It would be interesting to compare objective assessment and subjective depression rating, because usage of the scale by clinicians needs time and additional education, while self-rating for depression screening is simple and fast. Also, self-rating can be a simple method to obtain important information on emotional content of alcoholics who rarely spontaneously explain their depressive symptoms. For this reason it could be useful to do not only detection, but also to follow-up depression in the course of early abstinence phase and to analyze its content with the purpose to improve integrative therapies for alcoholics.

The aim of this study was to examine correlations and characteristics of clinical rating and self-rating depression among alcoholics in the course of early abstinence.

Methods

Study design

The study was performed prospectively in the 8-week period at the Department for Psychiatry of the Military Medical Academy, Belgrade. The depressive symptoms were assessed at the three following times: on admission (T1), repeated after 4 weeks (T2) and after 8 weeks (T3) of the abstinence period. The patients underwent the period of 4 weeks in-patient and 4 weeks abstinence-focused day integrative program for alcoholics.

Subjects

A total of 100 male alcoholics, aged between 20 and 60 years, consecutively recruited on admission in a closed ward for 4 weeks and in the day program unit for the next 4 weeks were studied. Inclusion criteria were alcohol dependence syndrome diagnosed according to Clasification of Mental and Behavioural Disorders – ICD-10 (World Health Organisation, 1992) [17] and Diagnostic and Statistic Manual of Mental Disorders – DSM-IV (American Psychiatric Association, 1995) [18]. The subjects were primary alcoholics and the timeline metod was used for depression and primary alcoholism distinction [6]. Exclusion criteria were a lifetime history of any DSM-IV Axis I disorder included depressive disorder and any psychiatric comorbidity or additional illegal substance abuse. Medical disorders were excluded by a clinical history, routine blood tests and complete physical exam. Examination was done independently by two physicians. The psychotropic medication, other than benzodiazepines and disulfiram were not allowed. Nine participants were excluded due to relpase and 5 had missed follow-up data resulting in a final sample of 86 alcoholics. Alcohol and drug screen were monitored.

The study protocol was approved by the Local Ethics Board and prior to the investigation written informed consents from all the subjects were obtained. The investigation was carried out according to the principles of good clinical practice and according to the Declaration of Helsinki.

Procedures

Sociodemographic characteristics and the pattern of alcohol use were obtained by the semistructured clinical interwiev on the baseline.

Assessment for depressive symptoms

Depression was evaluated and monitored by the Hamilton Rating Scale for Depression (HDRS) and Beck Depression Inventory (BDI).

The Hamilton Rating Scale for Depression is a clinician-rated semi-structured interview [19]. Severity of depression was assessed by independent trained psychiatrist using the 21-item HDRS. Score sum can range from 0 to 63, and measures a normal range between 0 and 7, mild depression between 8 and 16, moderate depression between

17 and 24 and over 24 indicate severe depression. The 4 HDRS factors were extracted: dysphoric mood, anxiety/agitation, vegetative, and cognitive symptoms according Brown et al. [20].

The BDI is a paper and pencil questions survey which completion by patient require 5–10 minutes [21]. Items are scored on 4-point scale value of 0–3. Score sum indicates degree of severity: 0–9, no or minimal depression; 10–16, mild depression, 17–29, moderate depression and 30–63, severe depression. Factor analysis of BDI simptoms extracted only mood factor interpretable in this sample, so we analized each BDI symptom severity.

Statistical Analysis

Descriptive statistics were calculated for all the variables and all data were expressed as mean ± SD. The difference between depression characteristics was calculated using the Student *t*-test. The *p* values of 0.05 or below were defined as statistically significant. Correlations were calculated using Pearson's correlation coefficient. The analysis of variance (ANOVA) for repeated measures was aplied to examine differences of the mean depression rates at each time point (T1, T2, T3). Data were analysed in Statistical Package for Social Sciences (SPSS) for Windows.

Results

Participant characteristics

Sociodemographical characteristics showed that the average age of male alcoholics was ($\bar{x}$ ± SD) 43.3 ± 7.3 years. They had the mean 13.7 ± 1.95 years of education. The majority of them (87.1%) were employed and were married (83.7%). The following data from the pattern of alcohol use were gathered: the first alcohol related problems occurred 10.3 ± 7.5 years ago, the average alcohol consumption in the month before the assessment was 65.5 ± 27.5 alcohol units *per* day.

Depression characteristics

The average mild-degree depression severity was detected by both scales on admission. The mean scores for HDRS and BDI were 15.16 ± 6.34 and 14.20 ± 9.56, respectively. The mean scores decreased in the course of the study. After 4 weeks they were 7.35 ± 4.18 for HDRS and 8.14 ± 7.35 for BDI. Finally, after 8 weeks the mean scores were 4.23 ± 2.93 for HDRS, and 5.30 ± 4.94 for BDI.

One-way repeated measures ANOVA were conducted to compare the scores for each scale. There were a significant differences between each repeated time points; for HDRS Wilks' Lambda = 0.44, F (84) = 53.71, $p < 0.01$; and for BDI Wilks' Lambda = 0.834, F (84) = 203.82; $p < 0.01$.

Depression was assessed in the majority of alcoholics on admission: in 90.7% on HDRS (mild 51.2%, moderate 31.6% and 7% severe degree) and 59.3% on BDI (mild 22.1%, moderate 29.1% and 8.1% severe degree). After 4 weeks (T2) depressive were 39.5% alcoholics on HDRS (mild 36.0%, and moderate 3.5% degree) and 30.2% on BDI (mild 15.1%, moderate 12.8%, and 2.3 % severe degree). After 8 weeks (T3) there were only 17.4% mild depresive alcoholics on HDRS, and 16.3% on BDI (mild 10.5%, moderate 4.6%, and 1.2 % severe degree).

A significant positive correlation between the mean HDRS and the mean BDI sum scores was detected at all the 3 measuring points: $r = 0.763$(T1), $r = 0.684$ (T2), $r = 0.613$ (T3), respectively ($p < 0.01$ for all corellations).

Figure 1 showed the all 4 HDRS subscales presented in the course of 8 weeks of abstinence that decreased from baseline (T1) to T 2 and T3 time points.

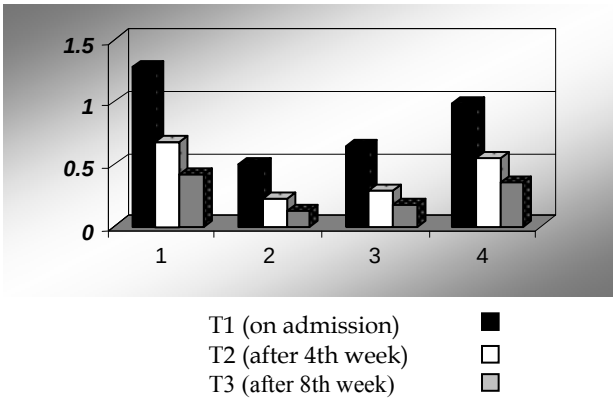

Fig. 1 – Mean values of Hamilton Rating subscale scores at 3-time measurement (T1, T2, T3)
Subsclases: 1. Dysphoric mood; 2. Dysfurctional cognitions; 3. Vegetative symptoms and 4. Anxiety/agitation.

The mean value of BDI symptoms decreased from the time point T1 throughout the study (Figure 2). On admission the most prominent symptoms were self-blame (item 8), punishement (item 6), anhedonia (item 4), agitation (item 11), irritability (item 17), guilt (item 5), insomnia (item 16), sadness (item1), fatigue (item 20).

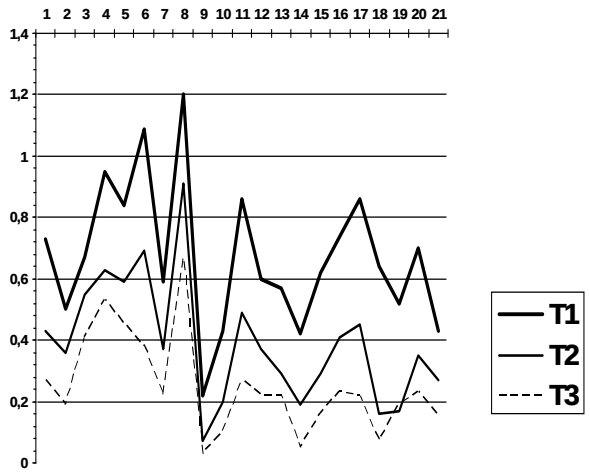

Fig. 2 – Mean values of the Beck Depression Inventory (BDI) items scores at the 3-points of measurement (T1 – on admission; T2 – after 4 weeks; T3 – after 8 weeks.
BDI 21 items: 1. sadness; 2. hopelessness; 3. past failure; 4. anhedonia; 5. guilt; 6. punishment; 7. self-dislike; 8. self-blame; 9. suicidal thoughts; 10. crying; 11. agitation; 12. loss of interest in activities; 13. indecisiveness; 14. worthlessness; 15. loss of energy; 16. insomnia; 17. irritability; 18. decreased appetite; 19. diminished concentration; 20. fatigue; 21. lack of interest in sex.

Discussion

The depression prevalence in the treated alcoholics is quite irregular, which can be partly explained by various evaluation instruments and treatment settings. Major depression was assessed by HDRS among 33.4% of the treated alcoholics on admission and varied from 29% to 53% in different clinical researches [21]. By combination of the cut-off score on HDRS and BDI moderate up to severe depression was detected in 33.3% of outpatient alcoholics [22]. In this paper the average severity of depression at the beginning of abstinence on the upper level of mild degree was detected with a significant decrease of the HDRS sum and BDI sum scores during the 8 week abstinence. Other authors have also reported reducing depression severity within the period of inpatient detoxification and abstinence [23, 24].

Analyzing the frequency of depression severity levels in our sample it was observed that the majority of alcoholics were depressive on admission (HDRS *vs* BDI: 90.7% *vs* 59.3%) with the presence of mild, moderate and severe depression levels. After 4 weeks the HDRS confirmed persistent only mild (36%) and moderate depression (3.5%), and after 8 weeks only mild depression persisted in 17.4% alcoholics. Another researchers found severe depression on HDRS among 25% *vs* 44% in-patient male alcoholics on admission and 11.4% *vs* 6% after the 4-week treatment [25, 26].

Various instruments for clinical evaluation of depression in alcoholics were used by many investigators. HDRS is a golden rule in diagnostics of depression and this observer rating scale was used to minimise influences on the selfrating depression scale. When used for the clinical sample of male alcoholics HDRS showed sensitivity of 100% and specificity of 96%, while BDI showed 67% of sensitivity and 69% of specificity and HDRS and BDI correlation was significant $r = 0.29$ [27]. This paper determined statistically significant positive correlation for BDI and HDRS in all the three points of measurements with r1 = 0.763(T1), r2 = 0.684 (T2) and r3 = 0.613 (T3).

Depressive syndrome represents a constellation of symptom groups, but except for the screening and evaluation of depression severity, attention is not sufficiently paid to some symptoms, so plenty of the obtained items is left unused for exploration of depression content. In this study the most prominent HDRS subscale through all the 3 measurements was dysphoric mood, followed by anxiety, than vegetative and cognitive subscale and it was found that each of them decreased in T2 and T3 reassesments. Another author found that the more prominent were anxiety and vegetative subscale among male alcoholics after 4 weeks of abstinence [20]. Our results with less prominent vegetative and anxiety subscales suggested that it was unlikely that the association of depression is highly influenced by alcohol withdrawal syndrome.

Factor analysis of BDI symptoms was performed, but except depressive mood, other factors were not found in this study. Other authors reported inconsistent findings of the factor model of the BDI in clinical sample of alcoholics [28]. For this reason the BDI items were analyzed in order to recognize depression quality in alcoholics on the basis of their self-rating. After 8 weeks the most persistent and prominent BDI symptoms were: self-blame, anhedonia and guilt, and after 4 weeks the most prominent symptoms together with the aforementioned ones were also punishment and past failure. However, at the beginning of abstinence the following symptoms together with the aforementioned persistent ones were: insomnia, sadness, irritability, agitation and fatigue. The alcohol withdrawal through the psychobiological stress mechanisms and changed neuroadaptation results in marked symptoms of anxiety as well as of vegetative ones which most often withdraw spontaneously within 3 weeks [29]. Depression and outcome of the treated alcoholism are significantly associated, but there is no evidence of the strong, direct causative correlation [5, 22]. In male alcoholics with more expressed depression at the beginning of abstinence, relapse was more often noticed after 5 months [30]. In our sample mild depression on admission was observed, on average. In clinical practice attention is mostly paid to major depression, but the mild one is often overlooked and underestimated. However, the presence not only of major but also of mild depression in alcoholics has predictive importance concerning the course and outcome of their treatment. In a year follow-up after inpatient treatment, the male alcoholics with mild to moderate depression evaluated on admission by the BDI had 2.9 times and with severe depression 4.9 times higher risk of relapse in comparison to non-depressive alcoholics [31].

Comorbid depression has unfavourable affect upon the outcome of treated alcoholism so that integrative psychosocial and pharmacological treatment of dependence is recommendable together with the combination of antidepressive agents and cognitive-behaviour therapy [32]. The focus is on the treatment of dependence and antidepressive agents show moderate effect [10, 14, 15]. The first step is early diagnosis of depression even on admission [31]. Taking into consideration that antidepressive therapy was not included in this study, partial remission of depression should be attributed to discontinued alcohol withdrawal as well as to the absent toxic effect of alcohol.

This study is limited to data from the small clinical sample of male alcoholics and also to the short follow-up period. Further investigations regarding the course of depression, and the impact on possible therapeutic consequences with large samples of both genders and within the longer period of time are needed.

Conclusion

The majority of male alcoholics were depressive on admission and had a mild-degree of severity both on Hamilton Rating Scale for Depresion and Back Depression Inventory scales. A significant positive correlation between rating (HDRS) and self-rating (BDI) of depression was established. A significant decrease of rating and self-rating of depression severity was detected together with the most prominent and persistent dysphoric mood HDRS subscale. Self-blame, anhedonia and guilt were the most persistent and prominent BDI symptoms among alcoholics in the course of early abstinence. The BDI could be a useful tool not only for routinely screening and reassessment of depression, but also for exploring emotional content during early abstinention and planning tailored integrative therapy and relapse prevention for alcoholics.

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Frequency of REM sleep behavior disorders in patients with Parkinson's disease

Učestalost poremećaja REM faze sna kod bolesnika sa Parkinsonovom bolesti

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Abstract

Background/Aim. Sleep is prompted by natural cycles of activity in the brain and consists of two basic states: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. REM sleep behavior disorder (RBD) is characterized by violent motor and vocal behavior during REM sleep which represents dream enactment. The normal loss of muscle tone, with the exception of respiratory, sphincter, extra ocular and middle ear muscles, is absent in patients with RBD. The origin of RBD is frequently unknown, but can be associated with degenerative neurological disorders, such as Parkinson's disease (PD). PD patients do not necessarily express features of RBD, which is identified in approximately third to a half of them. The aim of this study was to estimate the prevalence of RBD in a cohort of PD patients, as well as to identify risk-factors for its development. **Methods.** In the period from December 2010 to September 2011 we recruited 97 consecutive PD outpatients, treated in the Institute of Neurology, Clinical Center of Serbia, Belgrade. After establishing the diagnosis, all the patients filled out a specially constructed questionnaire with the following items: actual age, sex, age at disease onset, disease duration, form of the disease, type of treatment, duration of treatment, the presence of constipation, lessening of smell sense, and family history of PD. At entring the study, patients disability was scored using the Unified Parkinson's Disease Rating Scale (motor part – UPDRS). Cognitive abilities were assessed by the Mini Mental Status Examination (MMSE) scale, and depression symptoms by the 21-item Hamilton Depression Rating Scale (HDRS). The patients with PD were dichotomized to those with and without RBD using the RBD Questionnaire – Hong Kong (RBDQ-HK) in the manner of an interview. Forms of PD, mode of treatment, sex, constipation and family history were investigated using the Fishers χ^2 test. Symptoms and treatment duration, the presence of smell disturbances, MMSE score, UPDRS motor score and HDRS score were analyzed by implementation of the Z-test. Actual age and age at disease onset were evaluated by the unpaired *t*-test. **Results.** The RBD-positive group contained 15 (15.5%) patients, while in the rest of them (82/97), RBD was not identified (non-RBD group). There was no difference between the two groups considering gender distribution ($p = 0.847$), age ($p = 0.577$), age at disease onset ($p = 0.141$), duration of PD ($p = 0.069$), family history ($p = 0.591$), type of initial symptoms ($p = 0.899$), constipation ($p = 0.353$), olfaction ($p = 0.32$) and MMSE scores ($p = 0.217$). The duration of treatment in the RBD group was longer than in the non-RBD group (9.4 ± 5.3 and 6.3 ± 3.9 years, respectively; $p = 0.029$), and the UPDRS motor score in the RBD group was higher (19.1 ± 9.4 and 12.7 ± 8.2, respectively; $p = 0.013$). Also, HDRS scores were higher in patients expressing RBD (10.1 ± 6.0 and 6.4 ± 4.5, respectively; $p = 0.019$). **Conclusion.** We found that 15.5% of the consecutive PD patients had RBD, and that the patients with RBD differed from the non-RBD ones regarding duration of treatment, disease and depressive symptoms severity.

Key words:
parkinson disease; sleep, rem; sleep disorders; prevalence; risk factors.

Apstrakt

Uvod/Cilj. San je izazvan prirodnim ciklusima aktivnosti u mozgu i sastoji se od dva osnovna stanja: sna sa brzim kretanjem očiju (REM faza) i sna bez brzog kretanja očiju (NREM faza). Poremećaj REM faze sna (RBD) karakteriše se izraženim motornim i vokalnim manifestacijama u toku REM faze sna koje predstavljaju 'odigravanje' događaja u snovima. Normalan gubitak mišićnog tonusa, sa izuzetkom respiratornih, ekstraokularnih, sfinkternih mišića i mišića srednjeg uva odsutan je kod bolesnika sa RBD. Poreklo RBD često je nepoznato, ali se dovodi u vezu sa degenerativnim neurološkim poremećajima kao što je Parkinsonova bolest (PB). Bolesnici sa PB ne moraju uvek ispoljiti odlike koje prate RBD, koji se identifikuje kod približno jedne trećine do jedne polovine ovih bolesnika. **Metode.** U periodu od decembra 2010. do septembra 2011. ambulantno smo pregledali 97 bolesnika sa PB, lečenih u Institutu za neurologiju Kliničkog Centra Srbije u Beogradu. Nakon što je ustanovljena dijagnoza, svi bolesnici popunili su posebno konstruisani upitnik sa sledećim varijablama: starost, pol, godine života na početku bolesti, dužina trajanja bolesti od pojave simptoma, forma bolesti, način lečenja, dužina trajanja lečenja, prisustvo opstipacije, smanjenje čula mirisa i porodična anamneza PB. Na početku pregleda, motorna sposobnost bolesnika ocenjivana je pomoću skale *Unified Parkinson's Disease Rating Scale* (*motor part*)

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(UPDRS). Kognitivne sposobnosti procenjivane su pomoću skale *Mini Mental Status Examination* (MMSE) (*Mini mental test*), a simptomi depresije bodovani su uz pomoć skale Hamilton-ove *Depression Rating Scale* (HDRS). Bolesnici sa PB podeljeni su na one sa i bez RBD koristeći RBD *Questionnaire* – Hong Kong (RBDQ-HK) upitnik u vidu intervjua. Forma PB, način lečenja, pol, opstipacija i porodična anamneza PD su statistički računate Fišerovim χ^2 testom. Dužina trajanja simptoma i lečenja, prisustvo olfaktivnih promena, MMSE skor, UPDRS skor i HDRS skor analizirani su primenom Z-testa. Starost i godine života na početku bolesti procenjene su neuparenim *t*-testom. **Rezultati.** RBD pozitivnu grupu činilo je 15 (15,5%) bolesnika, dok kod ostalih (82/97) RBD nije bio identifikovan. Između RBD pozitivne i negativne grupe kada je u pitanju pol ($p = 0,847$), starost ($p = 0,577$), uzrast na početku bolesti ($p = 0,141$), dužine trajanja bolesti od pojave simptoma ($p = 0,069$), porodična anamneza ($p = 0,591$), forma bolesti ($p = 0,899$), prisustva opstipacije ($p = 0,353$), smanjenje čula mirisa ($p = 0,32$) i MMSE skor ($p = 0,217$) nije bilo razlike. Dužina trajanja lečenja u grupi RBD pozitivnih bila je značajno duža nego u grupi RBD negativnih ($9,4 \pm 5,3$ i $6,3 \pm 3,9$ godina, respektivno; $p = 0,029$), što je utvrđeno i za UPDRS motorni skor ($19,1 \pm 9,4$ i $12,7 \pm 8,2$, respektivno; $p = 0,013$). Takođe, HDRS skor bio je viši kod bolesnika kod kojih je utvrđen RBD, za razliku od bolesnika u grupi u kojoj je ovo oboljenje bilo odsutno ($10,1 \pm 6,0$ i $6,4 \pm 4,5$, respektivno; $p = 0,019$). **Zaključak.** Dobijeni rezultati pokazuju da 15,5% bolesnika sa PB ima i RBD, kao i da se bolesnici sa RBD razlikuju od onih bez tog oboljenja po dužini trajanja lečenja, težini bolesti (utvrđenoj UPDRS skorom) i težini depresivnih simptoma.

Ključne reči:
parkinsonova bolest; spavanje, rem; spavanje, poremećaji; prevalenca; faktori rizika.

Introduction

Sleep is prompted by natural cycles of activity in the brain and consists of two basic states: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep, which consists of stages N 1 through 3 [1]. Intense dreaming occurs during REM sleep as a result of heightened brain activity, but paralysis occurs simultaneously in the major voluntary muscle groups. However, fascinating REM sleep behavior disorder (RBD) paradoxically represents acting out of vivid, action-filled dreams and is characterized by violent motor and vocal behavior during REM sleep [2]. In the course of REM sleep there is a loss of muscle tone, with exception of respiratory, sphincter, extraocular and middle ear muscles [3]. In contrast, in the course of RBD muscle activity is preserved. The origin of RBD is frequently unknown (idiopathic RBD), but it can be associated with degenerative neurological disorders mainly classified as synucleinopathies, such as multiple-system atrophy, dementia with Lewy bodies (DLB) and Parkinson's disease (PD) [4]. Expression of RBD is probably due to a lesion of the sleep atonia system, mainly located in the pontomedullary brainstem region.

According to the Braak et al. [5] theory of PD progression, pathological process in PD initially appears not in the substantia nigra, but in the olfactory bulbs and medulla oblongata, which can explain emergence of RBD early in the course of PD, frequently before motor signs. Therefore, RBD may be a prodromal marker for PD, with higher sensitivity in comparison to other proposed markers, such as hyposmia, constipation, excessive daytime sleepiness or depression. However, the PD patients did not necessarily express features of RBD, which was identified in approximately third to a half of them [4].

The aim of this study was to estimate the prevalence of RBD in our cohort of PD patients, as well as to identify risk factors for its development.

Methods

Between December 2010 and September 2011 we recruited 97 consecutive PD outpatients treated in the Institute of Neurology, Clinical Center of Serbia, Belgrade, who gave informed and written consent for their participation. The study was approved by the Ethical Committee of our institution.

Only patients who fulfilled the Brain Bank criteria for clinical diagnosis of PD [6] were included. After establishing the diagnosis, all the patients filled out a specially constructed questionnaire with the following items: actual age, sex, age at disease onset, disease duration, form of the disease [7] (tremor predominant or rigidity predominant), type of treatment, duration of treatment, the presence of constipation, lessening of smell sense (quality of olfaction, i.e. hyposmia and/or anosmia, was assessed using the Pocket Smell test (PST) [8] and anamnestic data concerning common etiologies of possible lessening or absence of smell), and family history of PD. At the study entry, patients disability was scored using the Unified Parkinson's Disease Rating Scale (motor part) (UPDRS) [9]. Cognitive abilities were assessed by the Mini Mental Status Examination (MMSE) scale [10], while depressive symptoms by the 21-item Hamilton Depression Rating Scale (HDRS) [11].

The patients with PD were dichotomized to those with and those without RBD, by using the RBD Questionnaire – Hong Kong (RBDQ-HK) [12] in the manner of an interview. The questionnaire comprises 13 queries with a score ranging from 0 to 100. The questions considered various clinical features of RBD defined by the International Classification of Sleep Disorders [13] (ICSD-II) and derived from clinical observations by the authors of the questionnaire and previous empirical work (i.e. frequent dreams, frequent nightmares, emotional, violent or aggressive or frightening dreams, disturbed sleep, sleep talking, shouting or yelling in sleep, dream-related movements, falling out of bed, sleep-related injuries (SRI), attempts to assault/injure and SRI related to dream content). The cut-off score for disproving/proving RBD was 18/19.

The forms of PD, mode of treatment, sex, constipation and family history were investigated using the Fishers χ^2 test. Symptoms and treatment duration, the presence of smell disturbances, MMSE score, UPDRS motor score and HDRS

score were analyzed by implementation of the Z-test. Actual age and age at disease onset were evaluated by an unpaired *t*-test.

Results

The cohort comprised of 41 female and 56 male subjects. The mean age of our patients was 62.1 ± 8.8 years, with the age at disease onset 54.3 ± 9.4 years. The duration of symptoms was 8.3 ± 4.9 years. Concerning the form of the disease by which the symptoms first manifested themselves, 59 (60.8%) of the patients had the tremor-predominant form, 37 (38.1%) akinetic-rigid form, while only 1 (1%) of the patients presented with mixed symptoms. The duration of treatment was 6.8 ± 4.3 years, with levodopa as the most frequently used drug. Other centrally active medications included levodopa, amantadine, ropinirole, clonazepam, pramipexole, diazepam, clozapine, alprazolam, lorazepam, fluoxetine, and selegiline. Therapy-wise, 11 (11.3%) of the patients had undergone monotherapy, while 86 (88.7%) used more than one medication (polytherapy). In regards to constipation, 62 (63.9%) of the patients had, and 35 (36.1%) of them did not have this symptom. The sense of smell was normal, reduced or absent in 11 (11.3%), 19 (19.6%) and 67 (69.1%) of the examinees, respectively. A family history of PD was positive in 6 (6.2%), negative in 87 (89.7%), and unknown in the rest of the patients – 4 (4.1%). The MMSE score was 27.2 ± 3.8, UPDRS motor score 13.7 ± 8.6, Hamilton Depression Rating Scale score 7.0 ± 4.8, and the RBDQ-HK score was 8.8 ± 9.5.

The RBD-positive group contained 15 (15.5%) patients, while in the rest of them (82/97), RBD was not identified (non-RBD group) (Table 1).

There was no difference between the two groups considering gender distribution ($p = 0.847$), age ($p = 0.577$), age at disease onset ($p = 0.141$), duration of PD ($p = 0.069$), family history ($p = 0.591$), type of initial symptoms ($p = 0.899$), constipation ($p = 0.353$), olfaction ($p = 0.32$) and MMSE scores ($p = 0.217$).

The duration of treatment in the RBD group was longer than in the non-RBD group (9.4 ± 5.3 and 6.3 ± 3.9 years, respectively; $p = 0.029$), and the UPDRS motor score in the RBD group was higher (19.1 ± 9.4 and 12.7 ± 8.2, respectively; $p = 0.013$). Also, HDRS scores were higher in the patients expressing RBD (10.1 ± 6.0 and 6.4 ± 4.5, respectively; $p = 0.019$).

Discussion

In this study we detected RBD in 15.5% of the patients with PD, using RBDQ-HK [12], without detailed neurophysiological studies. Briefly, the patients with RBD did not differentiate from the non-RBD patients according to sex, age, age at disease onset, duration of symptoms, family history, form of the disease, constipation, olfaction, mode of treatment and MMS, but they were statistically different regarding duration of treatment, disease severity and the presence of depression.

Our results are quite similar to the results of Vibha et al. [14], with the prevalence of RBD of 19.4% in their PD cohort, and Comella et al. [15] whose estimates were even closer (15%). Overall, the reported prevalence of RBD in PD population varies from 15 to 60%, most probably due to different methods of patient selection and disorder ascertainment (spouses' interview, nocturnal video, etc.) [16].

Cross-sectional studies failed to find the difference in

Table 1

Comparison of the patients with Parkinson's disease with (n = 15) and without REM sleep behavior disorder (RBD) (n = 87)

Variables	RBD	Non-RBD	*p*
Age (years), $\bar{x} \pm SD$	60.9 ± 8.6	62.3 ± 8.9	0.577
Sex (m/f), n (%)	9 (60)/6 (40)	47 (57.3)/35 (42.7)	0.847
Age at disease onset (years), $\bar{x} \pm SD$	51.0 ± 10.9	54.9 ± 9.1	0.141
Symptoms duration (years), $\bar{x} \pm SD$	10.6 ± 5.6	7.8 ± 4.7	0.069
Form of the disease, n (%)			0.899
tremor-predominant	9 (15.3)	50 (84.7)	
akinetic-rigid form	6 (16.2)	31 (83.8)	
mixed		1 (1)	
Mode of treatment, n (%) (Monotherapy / polytherapy)	3 (27.3)/12 (14)	8 (72.7)/74 (86)	0.250
Constipation (Yes/No), n (%)	8 (12.9)/7 (20)	54 (87.1)/28 (80)	0.353
Sense of smell, n (%)			0.32
normal	1(9.1)	10 (90.9)	
reduced	2(10.5)	17 (89.5)	
absent	12 (17.9)	55 (82.1)	
Family history (Yes/No), n (%)	0(0)/15(17.2)	6 (100)/72 (82.8)	0.591
Duration of treatment (years), $\bar{x} \pm SD$	9.4 ± 5.3	6.3 ± 3.9	0.029*
MMSE scores, $\bar{x} \pm SD$	25.0 ± 6.8	27.6 ± 2.9	0.217
UPDRS motor scores, $\bar{x} \pm SD$	19.1 ± 9.4	12.7 ± 8.2	0.013*
HDRS scores, $\bar{x} \pm SD$	10.1 ± 6.0	6.4 ± 4.5	0.019*
RBDQ-HK scores, $\bar{x} \pm SD$	27.5 ± 5.6	5.4 ± 4.9	N/A

REM – rapid eye movement; MMSE – Mini Mental State Examination; UPDRS – Unified Parkinson's Disease Rating Scale; HDRS – Hamilton Depression Rating Scale; RBDQ-HK – REM Sleep Behavior Disorder Questionnaire–Hong Kong; *statistically significant.

age at disease onset between RBD and non-RBD PD patients [17]. In our study, initial Parkinson's disease symptoms started earlier in the patients with RBD than in those without RBD, but the observed difference did not reach statistical significance.

In contrast with our and the study of Arnulf [17], other authors reported a striking male predominance in chronic RBD patients [18]. In the first series of RBD patients described in 1985 by Schenck et al. [19] more than 90% of them were males. Other studies also found male predominance (87% and 83.3%) [20]. One of the possible explanations for such gender predominance is that milder forms of RBD occurs in females as subclinical, non-aggressive behaviors, and therefore are not reported.

In several studies dealing with smaller cohorts of PD patients, RBD was more frequent in patients with akinetic-rigid form of the disease [17]. Romenets et al. [21] reported a relationship between RBD and non-tremor predominant subtype of PD ($p = 0.04$). However, in this study tremor was an initial symptom in 31% of the patients, while in our study PD started with tremor in 60% of the cases. Lack of association of RBD to any type of PD we found in our study was in accordance with the data obtained from a larger cohort of PD patients [22].

Concerning family history, no difference was obtained between the RBD and non-RBD groups of PD patients, in accordance to previous reports [18, 23]. Lack of difference was also noted for constipation and olfactory function.

We assessed cognition with robust instrument, MMSE, and found no statistically significant difference between RBD and non-RBD group. However, some cross-sectional studies gave evidence of mild cognitive impairment in PD patients with RBD, as opposed to those without this sleep disorder [24] (the instrument we used was not sensitive enough to detect mild cognitive impairment in our patients). In a study of PD patients with RBD progression to dementia was documented in 48% of them during a period of 4 years, compared to none in the non-RBD group [24]. Cognition was lower in PD patients with RBD than those without it. Also, the existence of RBD had strong anticipatory value for the development of hallucinations over a 12-year follow-up [25]. However, careful follow-up is needed for a suggestion that the presence of RBD predisposed patients for developing dementia [26].

Contrary to some previous data [16] the presence of RBD in PD in our study correlated with the disease severity (UPDRS motor scores), and HDRS scores. Also, the longer the treatment of PD patients was, the chances they had to develop RBD were larger. Our data are in partial accordance with several cross-sectional studies where RBD was associated with older age, longer disease duration, higher Hoehn and Yahr score, lower amplitude of response to their medication, more frequent falls, more fluctuations, more psychiatric comorbidity, and higher doses of levodopa [22, 26, 27–32]. A population-based prevalence study that evaluated 231 PD patients for RBD during a follow-up period of 8 years found an increased prevalence of probable RBD from 14.6% to 27% during the study period.

In our work, definitive validation of RBD in the patient cohort remains with the RBD-HK questionnaire. Although 10 patients underwent polysomnographic testing (PSG), a larger number is needed in order to yield more precise results, which would make this the main limitation of our study.

Behaviors during RBD were complex and sometimes dramatic. They included arm and leg movements (93.3%), falling out of bed (20%), assaulting the bed-partner (26.6%) etc., and occasionally resulted in trauma of the patient or the partner (26.6%). In some patients violent behavior was associated with variable forms of vocalization (93.3%). Finally, all of our patients remembered the content of dreams.

Conclusion

In our study, we found that 15.5% of the consecutive PD patients had RBD, and that the patients with RBD differed from the non-RBD ones regarding duration of treatment, disease and depressive symptoms severity.

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GENERAL REVIEW

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Benign prostatic hyperplasia and prostate–specific antigen

Benigna hiperplazija prostate i prostata specifični antigen

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Key words:
prostatic hyperplasia; prostate-specific antigen; prognosis.

Ključne reči:
prostata, hiperplazija; prostata, specifični antigen; prognoza.

Androgens and prostate function

Testosterone (T) and 5α-dihidrotestosterone (DHT) play a crucial role in the fetal prostate development. These androgens stimulate mesenchyme, while mesenchyme induces the proliferation of the epithelial buds from the urogenital sinus. This process, called "mesenchyme-epithelial interaction", starts in the 10th gestational week and continues in the adult age [1, 2]. Testosterone induces the development of the seminal vesicles and Wolffian ducts, while DHT induces the development of the prostate, penis and scrotum.

Prostatic tissue is composed of stroma and epithelium. Prostatic stroma is composed of stromal cells (fibroblasts, endothelial capillary cells, lymph vessels and smooth muscle cells), neuroendocrine (NE) cells, neural cell axons, intercellular liquid and collagen fibers[3]. Prostatic epithelium is composed of secretory, basal, intermediary and NE cells. Secretory cells synthesize and secrete various proteins, like prostate specific antigen (PSA), prostatic acid phosphatase (PAP), androgen receptor (AR) and make the greatest part of the prostatic epithelium. It is believed that NE cells induce growth, differentiation, and secretory functions of the prostatic epithelium[4].

Numerous factors regulate prostatic growth: endocrine, neuroendocrine, paracrine, or growth factors (GF), autocrine and intracrine factors. However, the action of the endocrine factors is the best known. Testosterone is the most important serum androgen in the male, with the average serum concentration of 611 ± 186 ng/dL, while the average DHT serum concentration is 56 ± 20 ng/dL. However, the average concentration of active, free T is only 12.1 ± 3.7 ng/dL, while the rest is bound to the globulins and albumins. The major androgen in the prostatic tissue is DHT, with the average tissue concentration of 2.4–5.1 ng/g. The average tissue concentration of T is 3–5 times lower and measures 0.9 ng/g [5–7].

Only free T molecules can enter the prostatic cell, by diffusion. In the cytosol, one part of T molecules transforms into DHT. Both T and DHT bind to AR and form androgen-AR complexes. Subsequently, those complexes make pairs, entering the nucleus and bind to androgen-responsive elements (ARE) on DNA. After the information was transcripted from DNA to mRNA, mRNA leaves the nucleus and comes on ribosomes, where the information is translated into protein. Enzyme 5-alpha reductase (5αR) performs the conversion of T to DHT. There are two isoforms of 5αR: type 5αR-2 is dominant in the prostatic stroma and accessory genital tissues. Type 5αR-1 is present in the skin and prostate epithelium.

Benign prostate hyperplasia

Benign prostate hyperplasia (BPH) denotes progressive prostatic enlargement, associated with the symptoms of impaired emptying of the urinary bladder and followed with gradual progression of the symptoms and complications. The most common BPH-related complications are chronic urinary infection, urinary bladder stones, chronic and acute urinary retention. The prevalence of BPH is very high: BPH is the fourth most common disease, after coronary disease, hypertension and diabetes. In 2010, 210 million of men, or 6% of general population, suffered from BPH [8, 9]. According to Pan European Expert Project "Triumph", the prevalence rate of urinary symptoms suggestive for BPH is the lowest among males 45–49 years old (2.7%) and increases with age, until a maximum at 80 years (24%) [10]. On the other hand, the prevalence of histological BPH found on autopsy material is

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much higher: it is 10% for men in IV decade, 20% for men in V decade, 50–60% in men in VII decade and 80–90% in men in VIII and IX decade [11].

Etiology of benign prostatic hyperplasia

The most common factors that induce the development of BPH are steroid hormones, growth factors, interactions between stroma and epithelium, and the regulation of apoptosis (Figure 1). The gradual decrease of T concentration is characteristic for an aging man; however, DHT concentration increases in prostatic tissue, or remains unchanged. The role of estrogens and estrogen receptors (ER) in the etiology of BPH is very possible. It is proved that dog prostate contains large amounts of ER and that estrogen administration induces stromal growth in dogs. In humans, epithelial proliferation is stimulated by fibroblast growth factors (FGF) and inhibited by transforming growth factors (TGF). The concentration of TGF-β is decreased in BPH [12]. In brief, the development of BPH requires androgenic influence in young age and long-term androgen stimulation in adult age. In old age, characteristic events are the decrease of T concentration and the increase of DHT and estrogen concentration, increased FGF activity, decreased TGF-β activity and the decreased apoptosis.

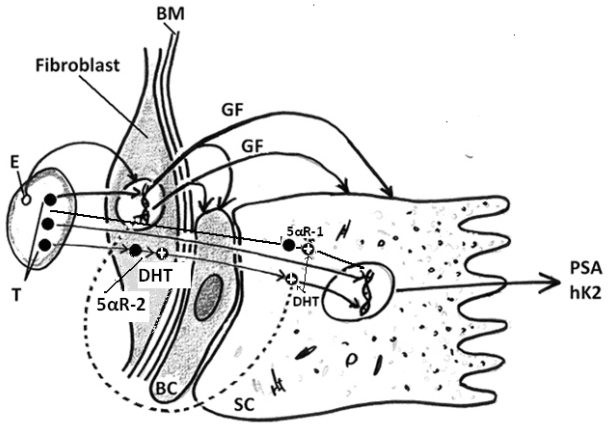

Fig. 1 – Stromal-epitelial interaction (Autor T. Pejčić)
E – estrogen; T – testosterone; DHT – dihydrotestosterone; 5αR-1 – 5-alfa reductase type-1; 5αR-2 – 5-alfa reductase type-2; BM – besement membrane; GF – growth factor; BC – basal cell; SC – secretory cell; PSA – prostate-specific antigen; hK2 – human kalikrein 2.

Benign prostatic hyperplasia is common among members of Western civilization; however, there is an increasing incidence of BPH in Asian countries, with traditionally low prevalence of BPH [13]. It is believed that the increase in incidence is associated with the lifestyle of the modern man. In fact, today's man is fed differently than his ancestors, live longer and retains sexual activity long after the generative period. Humans have drastically changed diet about 15,000 years ago, when domesticated animals and, of obligate herbivores, became carnivorous [14]. Excessive intake of meat, fried and baked foods and obesity, lead to hormonal disturbances and oxidative DNA damage [15]. In developed countries, the average human life span is today over 80 years, while the length of human life in the Neolithic period was only 20 years [16, 17]. In addition, the man retains sexual activity for a long period: 20–35% of men aged 60–69 years have one intercourse *per* week [18]. Unlike most of primates, man's sexual activity does not have seasonal variations. Therefore, long-term hormonal stimulation of the prostate, oxidative stress and increased incidence of genetic changes associated with aging are all important combined etiological factors for the development of BPH.

Pathology and pathophysiology of benign prostatic hyperplasia

Prostatic hyperplasia increases urethral resistance, which leads to a compensatory increase in detrusor pressure and the reduction in bladder capacity. It is believed that the capsule of the prostate plays a very important role in the development of lower urinary treat symptoms (LUTS), because it transmits the pressure of the hyperplastic tissue on the urethra.

The important characteristic of BPH is the increase of the total number of cells, not only an increase in cell size. McNeal has shown that early periurethral nodules have a stromal structure, while the early nodules in the transition zone (TZ) represent the proliferation of the glandular tissue. Glandular nodules rise from the newly formed small ducts, arising as buds on existing ducts; these ducts grow and branch out, creating an entirely new ductal system within the nodule. During the first 20 years, the development of BPH is characterized by an increased number of slowly growing nodules. Thereafter, in the second stage, major nodules show significant growth [18].

Clinical characteristics of benign prostatic hyperplasia

Common characteristics of BHP are progressive enlargement of the prostate, voiding symptoms and increased PSA. Total prostate volume (TPV) increases from 25 mL in men aged 30–35 years, to 45 mL in men over 70, while the transition zone (TZ) volume increases from 15 mL to 25 mL. Transrectal ultrasound (TRUS) provides the most accurate measurement of TPV. The Olmsted study revealed that TPV grows 0.4 mL per year in men aged 40–59, and 1.2 mL *per* year in men aged 60–79. The overall TPV growth was 0.6 mL, or 1.9% *per* year.

Lower urinary tract symptoms are typical for BPH [20]; however, LUTS is also common in men with the stenosis of the urethra, or the weakness of the detrusor muscle. That was the reason for the introduction of the new terms, like “bladder outlet obstruction” (BOO), "benign prostatic obstruction" (BPO) and "benign prostatic enlargement” (BPE). In the Serbian literature, symptoms of urinating are commonly classified as "irritative" (urgency, pollakiuria, nocturia) and "obstructive" (waiting for the beginning of urination, straining to urinate, interruption of the urinary stream). The severity of the symptoms can be expressed using the International Prostate Symptom Score (IPSS). The symptom score ranges

from 0–35. However, the obstruction can be assessed objectively by the measurement of the urinary flow, or Uroflow. It is accepted that the maximum urine flow, Qmax < 10 mL/sec, carries a high probability for the presence of the obstruction, while Qmax > 15 mL/sec carries a low probability. Some authors tried to express urine flow through the single number, Qi. Index Qi is the result of multiplying Qmax and average flow, Qave: Qi = Qmax × Qave. Pejčić et al. [20] found that 71% of men with IPSS > 7 had Qi < 100, while 75% healthy men with IPSS < 7 had Qi > 100.

Prostate – specific antigen

The main secretory proteins of the prostate gland are prostate-specific antigen (PSA), human glandular kalikrein (hK2), prostatic acid phosphatase (PAP) and prostate-specific protein (PSP-94). Molecular weight of PSA is 34 kDa; PSA molecule consists of one chain with 240 amino-acids and four carbohydrate lateral chains (Figure 2).

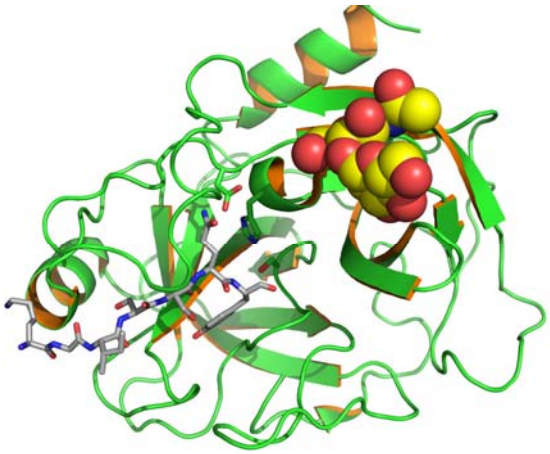

Fig. 2 – Human prostate-specific antigen (PSA/KLK3) with bound substrate from complex with antibody [21].

Prostate-specific antigen was isolated in seminal plasma in 1966; for a long time, PSA has been used in forensic evidence of rape [22]. It has been estimated that the PSA concentration in seminal plasma was very high (1.5 mg/mL) and lower in urine (250 ng/mL). However, Wang et al [23] were the first to predict the possible use of PSA determination in the blood in the diagnosis of the prostate diseases. The average PSA concentration in the prostatic tissue ranges from 10,000 ng/mg of tissue to 76,000 ng/mg of tissue [24, 25]. In other words, prostate gland weighing 20 mL contains 0.2–1.5 mg PSA. The expression of PSA is high in benign epithelial cells, low in malignant cells and progressively decreases with the degree of anaplasia [26–31].

Synthesis and secretion of prostate-specific antigen

The intensity of PSA synthesis largely depends on the concentration of DHT in the prostatic tissue [32]. DHT molecules bind to AR and form DHT-AR complexes. Those complexes enter the nucleus and bind to ARE on the DNA. The following is a transcription to mRNA; after that, mRNA leaves the nucleus and goes to the ribosomes, where the translation and PSA synthesis take place [33, 34]. Alternative PSA synthesis pathway was demonstrated in the tissue culture. This pathway goes *via* the membrane steroid receptor; it is considerably faster than the genomic process and lasts 1–30 minutes [35].

The first product of the synthesis is the preproPSA molecule, with a leading sequence of 17 amino acids. PreproPSA molecules are placed in a number of prostate secretary granules (PSGs), which migrate towards the apical part of the cell. During the following biochemical process, leading sequence of 17 amino acids is separated from the preproPSA molecule. This product is now referred to as proPSA, and it is ejected as an inactive proenzyme from a cell *ie* secreted into the lumen of the acinus. In the lumen of the acini, hK2 separates another seven amino acids from the proPSA molecule, which results in the creation of active enzyme PSA. The last reaction which happens in the acini is very important: acinar enzymes change the conformal structure of the PSA molecule, after which it becomes inactive as an enzyme. In normal acini, 25–30% of the PSA molecules are inactivated and thereafter diffuse into the systemic circulation. The remaining PSA molecules enter blood as active enzymes, where they rapidly form complexes with heavy plasma proteins. One fraction of PSA molecules does not enter blood, but leaks down the acini and ducts, as a part of prostatic secretion. Secretion is moving towards prostatic urethra due to the difference in hydrostatic pressure and stromal smooth muscle tone.

The prostatic urethra is closed most of the time, due to the muscle tone of the proximal and distal urinary sphincter. During this time, prostatic secretions are constantly entering the urethra, through a number of holes around the verumontanum. Secretion accumulates in the urethra until the first voiding, when the flow of urine ejects it out of the body. All PSA molecules detected in the urine were made in the prostate and urethra: PSA molecule is too large to pass the glomerular membrane of the kidney [36]. Therefore, it should be named "secreted PSA", rather than "urinary PSA". PSA molecules detected in urine are all in the free form, with the weight of 32.9 kDa and are chemically and structurally identical with the PSA molecules of seminal plasma [37]. The normal healthy prostate gland secretes 0.01–0.02 mg PSA *per* day, while hyperplastic prostate secretes ten times larger amounts of PSA. During sexual inactivity, prostatic secretion rich with PSA molecules leak slowly to the prostatic urethra, so around 10–100 ng PSA gather in the urethra between two urinations. However, during the sexual act, parasympathetic nerves stimulate the increased PSA discharge from the cell; meanwhile, stromal muscles squeeze the secretion from the acini and inject it into the urethra in a large quantity. Before ejaculation, 5–6 mg of PSA is collected in the prostatic urethra. The PSA concentration in seminal plasma is 0.3–3 mg/mL [38–40]. Only in the ejaculated semen, PSA molecules perform their function: they split the proteins of the seminal clot and thus allow active sperm motility [41].

Prostate-specific antigen in serum

Under normal conditions, 25–30% of the inactive PSA molecules and 70–75% of active PSA molecules enter blood stream. As soon as the active PSA molecules enter blood, they are immediately bound by protease inhibitors, such as antichymotrypsin (ACT) and alpha 2-macroglobulin (alpha-2M). This process is fast and efficient because the molar concentration of the inhibitors exceeds molar PSA concentration over 100,000 times. PSA-ACT complex is the most common PSA form in serum, and is formed in the 1 : 1 molar ratio, in the irreversible reaction. These molecules can be detected in blood tests and they are named "complexed PSA". On the other hand, inactive PSA molecules can be detected as the fraction of free molecules and they are named "free PSA". In the presence of prostatic acinar lesion, smaller fraction of active PSA molecules completes the transformation to an inactive PSA; that is the explanation why free PSA fraction is lower in patients with prostate cancer (PCa).

It is not yet exactly known how PSA molecules enter blood. However, it is known that PSA molecules have to cross the so-called "prostatic blood-barrier", consisting of prostatic basal cells, basement membrane of the duct, the extracellular space, the capillary basement membrane and the layer of capillary endothelial cells. All processes which lead to damage of the prostatic blood-barrier enable massive PSA transfer in blood [42, 43].

Determination of PSA in serum in the diagnosis of BPH

Clinical application and research related to PSA over the last 25 years have been so extensive, that this period of urology is called the "PSA era". What is even more important, the occurrence of PSA strongly influenced the tremendous changes in the diagnostics and treatment of prostate cancer (PCa) and the development of new strategies and technologies for the treatment of this disease. However, the phenomenon of elevated PSA in BPH patients has always been regarded as the "artifact", which complicates the diagnosis of localized PCa.

It has long been thought that normal PSA level is below 4.0 ng/mL. However, subsequent studies have shown two confusing facts. First, it became clear that a significant number of patients with localized PCa had PSA < 4.0 ng/mL; soon, the new PSA cut-off value of 2.5 ng/mL was established [44, 45]. Second, it has been proven that 70–80% of people without PCA after biopsy, had PSA within the "gray zone" i.e., 4.0–10.0 ng/mL. It became clear that the PSA level in each subject depends on many factors. In the first place, the synthesis of PSA depends on the concentration of DHT, as well as the presence of BPH. Then, the level of PSA depends on the hormonal status, obesity, body weight, total blood volume and so on. In patients with localized PCa, the size and location of the tumor also influence the PSA level. Finally, serum PSA level depends on the concentration of PSA in prostatic tissue surrounding the growing tumor [46].

The so-called "PSA derivatives" were introduced in order to distinguish the patients with BPH and PCA, with the PSA in gray zone. PSA density (PSAD) was introduced with the aim to reduce the impact of prostate size on the interpretation of the PSA. For the threshold is taken PSAD = 0.1, i.e., patients with PSAD > 0.1 are more likely to have PCa [47]. Similarly, subjects with PSA velocity (PSAV) > 0.8 ng/mL *per* year, have greater risk for the presence of PCa. Those with PSAV ≤ 0.8 ng/mL *per* year are more likely to have BPH. A derivative called "PSA doubling time" (PSADt) expresses the increase of PSA in time (t) more precisely; it is calculated using the formula: PSADt = log 2t / log final PSA – log initial PSA. Prostate cancer has shorter PSADt than BPH; in addition, the more aggressive the tumor is, PSADt is shorter [48, 49]. Free/total PSA ratio (f/t PSA) is frequently used to distinguish the persons with BPH and PCa having PSA in the gray zone and normal digital rectal examination (DRE). Normal values of f/t PSA are 0.18 to 0.22 [50]; however, the patients with f/t PSA < 0.1 have 56% chance to have PCa [51].

Today, it becomes quite clear that BPH is the main reason for the PSA values from 4.0–10 ng/mL, or 2.5–10 ng/mL. The American Urological Association (AUA) states that a very high risk for the presence of PCa, about 90%, is present in PSA > 20 ng/mL. It is not difficult to conclude that the increase in PSA, caused by the presence of BPH, was the main reason for the unnecessary diagnosis of a large number of clinically insignificant PCa. This is one of the reasons why the AUA reduced the range of PSA screening for men aged 55 to 69 years in 2013.

On the other hand, in the field of BPH, the situation is far less complicated and PSA is a precise parameter of disease progression. Several large multicenter studies have defined the precise parameters for monitoring the growth and progression of BPH. The most well-known studies are: Proscar Long-Term Efficacy and Safety Study (PLESS), Medical Therapy of Prostatic Symptoms (MTOPS), "Olmsted County Study of Urinary Symptoms and Health Status Among Men" and The Combination of Avodart and Tamsulosin (CombAT).

MTOPS study included 3,047 patients, who were followed for 4.5 years. Factors that indicated the progression of BPH were TPV ≥ 31 mL, PSA ≥ 1.6 ng/mL, Qmax < 10.6 ml/s, residual urine, RU ≥ 39 mL and the age ≥ 62 years [52]. In patients who were taking finasteride, the average reduction in TPV for 4.5 years was 19%. However, men with TPV > 40 mL had an average reduction in TPV by 25% [53, 54]. CombAT study included 4,844 men aged over 50 years with a clinical diagnosis of BPH, IPSS > 12, TPV > 30 mL, PSA in the range of 1.5–10 ng/mL and at least two urinations with Qmax of 5–15 mL/s [55].

Average TPV was 43 mL, and the mean PSA, 3.6 ng/mL. It was concluded that a combination therapy was better for patients with TPV < 43 mL and PSA < 3.6 ng/mL, and that in the patients with higher values, dutasteride was as effective as the combined therapy. After 24 months, the decrease of TPV was 30.5% (combination therapy), or 28.6% (dutasteride). These studies conclude that the enlarged prostate and elevated PSA are good predictors of complications such as acute urinary retention and need for surgery, while the severity of symptoms and lower flow often behave paradoxically [56–58].

Determination of PSA in the urine in the diagnosis of BPH

Determination of PSA concentration in urine (uPSA) has never been used in the diagnosis of BPH and monitoring of BPH progression. It is interesting that even in 1987, Tremblay et al. [59] found that the average uPSA concentration was 216 ng /mL and that people with BPH had higher uPSA values than young men. However, over the following years, researches have focused mainly on the ability to distinguish BPH and PCa and to detect early relapse after radical prostatectomy (RP). From 1994 to 2000, few works on this topic concluded that uPSA was higher in BPH than in PCa, but that it cannot help in differentiating those two diseases [60–63]. The hope that uPSA will become a marker of the early recurrence after RP, was closed when Iwakiri et al. [64] demonstrated that PSA was normally present in urine in all patients after RP and that it originated from the urethral glands [64]. In some studies, it has been found that men with alopecia had higher values of urethral PSA after RP [65].

All researchers agree that uPSA is highly androgen-dependent marker for monitoring of the hormonal treatment, in both men and women [66, 67]. Also, uPSA can be used as an early noninvasive marker of the appearance of puberty in boys [68, 69]. In most primates, the seasonal uPSA increase indicates the beginning of the breeding season [70]. Except the determination in fresh urine, uPSA can be determined in the dried urine, on filter paper, where it remains stable over a long period of time [71]. However, most researchers agree that the methodology of PSA determination in urine is still inconsistent [72].

In recent years, several papers that trigger the clinical use of uPSA were published. In a group of patients with PSA of 2.5–10.0 ng/mL, Bolduc et al. [73] found a significant difference in mean uPSA in BPH (123.2 ng/mL) and PCa (52.6 ng/mL). With the uPSA threshold > 150 ng/mL, the sensitivity of the test was 92.5%. The authors believe that subjects with PSA of 2.5–10.0 ng/mL and uPSA > 150 ng/mL, could be exempted from prostate biopsy, in the absence of suspicious lesions on DRE and TRUS. In some studies, it has been found that larger tumors had lower uPSA than smaller tumors, probably due to the obstruction of the drainage of secretions [74–76].

However, only one paper described the methodology of uPSA usage as a prognostic marker of BPH [77]. In a group of 265 patients without PCa, uPSA, PSA, TPV and patients` age were determined. According to MTOPS criteria, TPV ≥ 31 mL, PSA ≥ 1.6 ng/mL and age ≥ 62 years were used as cutoff values of BPH progression. Persons with TPV < 31 mL had significantly lower uPSA, than patients with TPV ≥ 31 mL (119.3 ± 124.5 and 255.5 ± 204.9 ng/mL, respectively; $p < 0.0001$). In addition, persons in the so-called "non-progressive BPH" group (TPV < 31 mL, PSA < 1.6 ng/mL, age < 62 yrs) had significantly lower uPSA than patients from the "progressive BPH" group (86.8 ± 82.4 ng/mL and 274.9 ± 208.3 ng/mL, respectively; $p < 0.0001$). Urinary PSA correlated significantly with TPV ($r = 0.32$, $p < 0.0001$).

The urinary PSA cutoff level of 150 ng/mL discriminated the patients with non-progressive BPH and progressive BPH with specificity of 0.83 and sensitivity of 0.67. In that issue, Pejčić et al. [77] conclude that uPSA reflects prostatic hormonal activity and correlates with TPV, PSA and age. Therefore, uPSA level ≥ 150 ng/mL can be used as an additional predictive parameter of BPH progression.

Conclusion

Testosterone and 5α-dihidrotestosterone play a crucial role in the prostate fetal development, growth and function. Testosterone is the most important serum androgen in the male, but the major androgen in the prostatic tissue is 5α-dehidrotestosterone.

Benign prostatic hyperplasia is the fourth most common disease and affects 6% of general population. Biochemical characteristics of benign prostatic hyperplasia are decreased testosterone, increased 5α-dehidrotestosterone and estrogen concentration, increased fibroblast growth factor and decreased transforming growth factor-beta activity.

Prostate-specific antigen is the main secretory product of the prostate gland; its synthesis largely depends on the 5α-dehidrotestosterone concentration in the prostatic tissue. Normal healthy prostate gland secretes 0.01–0.02 mg prostate-specific antigen per day, while hyperplastic prostate secretes ten times larger amounts of prostate-specific antigen. Secreted prostate-specific antigen is washed out from the urethra during voiding and can be detected in the urine.

However, the phenomenon of elevated prostate-specific antigen in benign prostatic hyperplasia patients has always been regarded as the "artifact", which complicates the diagnosis of localized prostate cancer. Nevertheless, recent studies precisely established that serum prostate-specific antigen ≥ 1.6 ng/mL is suggestive for benign prostatic hyperplasia progression in men with prostate volume ≥ 31 mL and age ≥ 62 years. In addition, urinary prostate-specific antigen concentration is significantly higher in subjects with benign prostatic hyperplasia; urinary prostatic antigen level ≥ 150 ng/mL can be used as additional predictive parameter of benign prostatic hyperplasia progression.

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CASE REPORTS

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Stent dislodgement in the distal left main coronary artery and its successful management with balloon crushing technique

Zaglavljivanje stenta u distalnom segmentu glavnog stabla leve koronarne arterije i uspešno rešavanje tehnikom gnječenja balonom

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Abstract

Introduction. Stent entrapment and dislodgement in the coronary arteries is a rare but potentially fatal complication of percutaneous coronary intervention. Different retrival techniques of dislodged stents have previously been reported with high success rate but all of them are time-consuming, so as not quite useful in hemodinamically unstable patient. **Case report.** A 59-year old female patient with acute ST-elevation myocardial infaction of anterior wall was admitted for primary percutanous coronary intervention. Unexpectedly, during intervention stent entrapment and dislodgement in the distal left main coronary artery occured followed by occlusive coronary dissection and compromisation of the coronary flow in the left descending coronary artery with a rapid hemodinamic deterioration. In order to reestablish coronary flow as soon as possible, the dislodged unexpanded stent was crushed against the wall with a balloon in the distal left main. It immediately restored coronary flow in the left descending coronary artery and rapidly improved the patients hemodinamics. Intervention was successfuly completed with totally four stents implanted in the left main, the osteo-proxymal circumflex coronary artery and the osteo-proxy-medial left descending coronary artery. Later post-interventional hospital course as well as the clinical and angiographic six month follow-up was uneventful. **Conclusion.** This case shows that percutaneous baloon crushing technique can be a safe and effective first option in management of dislodged and unexpanded stent in the left main coronary artery, particularly for a hemodynamically unstable patient.

Key words:
coronary artery disease; angioplasty, transluminal, percutaneous coronary; stents; treatment outcome.

Apstrakt

Uvod. Zaglavljivanje i zadržavanje stenta u koronarnim arterijama veoma je retka ali potencijalno fatalna komplikacija perkutanih koronarnih intervencija. Različite tehnike izvlačenja zaglavljenog stenta prikazane su do sada sa visokim stepenom uspešnosti, ali sve zahtevaju dosta vremena za izvođenje, zbog čega nisu najpogodnije kada je bolesnik hemodinamski nestabilan. **Prikaz bolesnika.** Bolesnica, stara 59 godina, sa akutnim anteriornim infarktom miokarda sa ST elevacijom, primljena je radi primarne perkutane koronarne intervencije. Neočekivano, tokom intervencije, došlo je do zaglavljivanja i zadržavanja stenta u distalnom segmentu glavnog stable leve koronarne arterije, uz okluzivnu disekciju i kompromitovanje koronarnog protoka u levoj descedentnoj koronarnoj arteriji, praćeno brzim pogoršanjem hemodinamskog statusa. U cilju što bržeg uspostavljanja koronarnog protoka u levoj descedentnoj koronarnoj arteriji, učinjeno je gnječenje zaglavljenog i neekspandiranog stenta balonom uz zid distalnog segmenta glavnog stabla. Ovim je odmah uspostavljen koronarni protok u levoj descedentnoj koronarnoj arteriji, što je bilo praćeno i brzom stabilizacijom hemodinamskog statusa. Intervencija je zatim uspešno završena sa ukupno četiri stenta implantirana na glavno stablo, ostioproksimalnom segmentu cirkumfleksne koronarne arterije i ostioproksimomedijalnom segmentu leve descedentne koronarne arterije. Postinterventni bolnički tok, kao i šestomesečna klinička i angiografska kontrola bili su uredni. **Zaključak.** Ovaj prikaz pokazuje da perkutana tehnika gnječenja balonom može biti bezbedna i efikasna prva opcija za rešavanje komplikacije zaglavljenog neekspandiranog stenta u glavnom stablu leve koronarne arterije, posebno kod hemodinamski nestabilnog bolesnika.

Ključne reči:
koronarna bolest; angioplastika, translumenska, perkutana, koronarna; stentovi; lečenje, ishod.

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Introduction

Stent entrapment and dislodgment in the coronary arteries is a rare but life-threatening complication of percutaneous coronary intervention (PCI), occuring with the incidence of 0.02% [1]. It may cause coronary or systemic embolisation, thrombus formation, myocardial infarction, and eventually death [2].

Different retrival techniques of dislodged stents have previously been reported which include the use of balloon catheters, basket devices, loop snares, twisted wires, etc, with a high success rate [3]. In emergency cases when time is crucial and because percutaneous retrival is a time-consuming procedure, the crushing of an entrapped and dislodged stent against the wall has been proposed as alternative option [4]. However, the crushing technique in the treatment of dislodged stent in the left main still has not been widely accepted due to the increased risk of stent thrombosis and restenosis. Finally, a surgial removal of a dislodged stent should be the last option in case when percutaneous treatment fails [5].

Case report

A 59-year-old female patient was admitted to our hospital with the symptoms and signs of acute myocardial infarction with ST-segment elevation (STEMI) of anteroseptal localization, six hours after the chest pain onset. She was a smoker and diabetic, and reported previous treatment for high blood pressure and dyslipidaemia. An electrocardiogram at admitance showed the sinus rhythm with Q-waves and persistent ST-elevation in the leads V2-V6. Physical examination showed hypotension 95/75 mmHg and pulmonary congestion (Killip class II). A bedside transthoracic echocardiography revealed akinesis of the distal half of anterior wall, anterior septum and apex of the left ventricle, with severely depressed global systolic function (left ventricular ejection fraction 35%) and mild ischaemic mitral regurgitation (MR) 1-2+. She was imediatelly referred to the Cardiac Catheterization Laboratory for primary angioplasty. Her coronary angiogram revealed thrombotic occlusion of the medial left anterior descending coronary artery (LAD), whereas the dominant circumflex coronary artery (CX), the obtuse marginal (OM) branch and the right coronary artery (RCA) were without significant disease (Figure 1A).

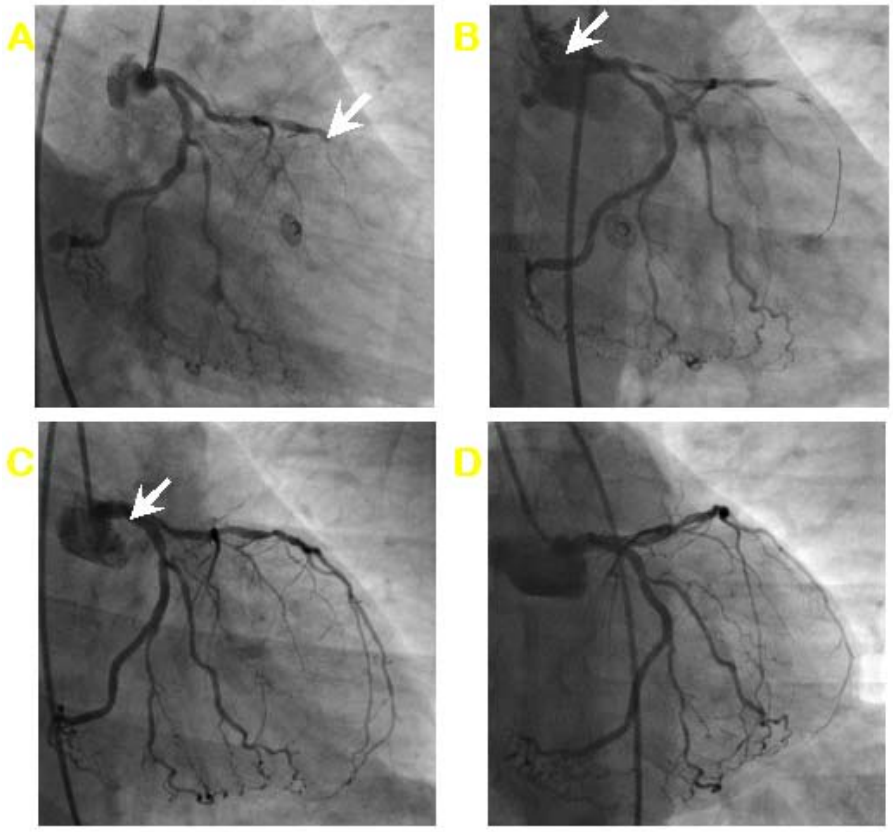

Fig. 1 – Angiographic images: A) Thrombotic occlusion in the medial left descending coronary artery (LAD) (arrow); B) Entrapped stent in the distal left main and proximal LAD (arrow) with occlusive dissection in the proximal LAD: C) Final positive angiographic result after successful management of the complication; D) Control coronary angiogram after six months with only insignificant diffuse in-stent restenosis.

After a bolus of heparin (100 IU/kg), left coronary system was engaged with EBU 3.5–6 Fr guiding catheter and coronary wire BMW was advanced through the thrombotic occlusion and placed in the distal segment of LAD. Several balloon predilatations (Sprinter Legend 2.0 × 15 mm at 16 atmosphere) in the mid LAD restored the TIMI-III flow, but created a non-occlusive dissection type B in the medial segment of LAD

proximally to the culprit lesion. A Micro-Driver bare metal stent 2.5 × 20 mm was deployed over the culprit lesion in the mid LAD, but the dissection was not fully covered. Another stent Micro-Driver 2.5 × 20 mm was advanced but it could not be overlapped with the previous one, so we decided to pull it back and perform additional predilatation. However, during pull-back resistance was felt in the ostial LAD, and eventually the whole system consisting of the guiding catheter, wire and stent was completely stucked and could not be further manipulated (Figure 1B). The patient compained on intense chest pain and suddenly developed severe bradycardia (30 beats *per* minute) with a drop in blood pressure to 60/40 mmHg. As we could not pull back the stent-balloon system which was stucked with the guiding catheter and the wire, we decided to forcefully and rapidly pull out the whole system, e.g. stent-balloon together with the wire and the guiding catheter. It was followed immediately by application of atropine and saline infusion which resulted in hemodynamic stabilization of the patient. However, the stent was dislodged and lost in the distal left main and ostial LAD, with angiographic signs of occlusive coronary dissection in the proximal LAD. Immediately, we introduced a new guiding catheter EBU 3.5–7 Fr, and rewired LAD with a new BMW wire. Subsequently, balloon Sprinter Legend 2.5 × 15 mm was advanced in the distal LAD, confirming that a wire was not between the stent struts, and thereafter the dislodged stent was crushed against the wall in the distal left main and ostioproximal LAD with the same balloon, inflating it up to 16 atmosphere. This resulted in a rapid blood flow restoration in LAD (TIMI-III), thought there was some plaque shifting in the ostial CX. We performed also additional predilatation in the medial LAD with the same balloon. Stent MicroDriver 2.5 × 20 mm was overlapped with the previously implanted stent in the medial LAD, covering the dissection at the same time. Another coronary wire BMW was advanced in the distal CX. Over the crushed stent in the distal left main and ostioproximal LAD additional stent Driver 3.0 × 26 mm was depolyed, as well as over the ostial CX stent Driver 3.0 × 15 mm, with final balloon kissing. The final angiographic result (Figure 1C) was optimal with uneventful later inhospital course. The patient was discharged on the sixth day.

A follow-up during the next six months showed good patient health with the absence of ischemic simptoms. Coronary angiography was performed after six months which showed only a non-significant diffuse in-stent restenosis (Figure 1D).

Discussion

The incidence of stent entrapment and dislodgement during PCI has significantly decreased over the last twenty years, from 8.3% to currently 0.02% [1]. This is largely due to improvements in stent design, such as the use of factory premounted stents instead of previous manually crimped stents [6]. However, having in mind that stents are used in contemporary practice in more than 98% of PCIs, along with increasingly complex interventions, this serious and potentially fatal complication of PCI will stay an important issue in interventional cardiology.

Stent entrapment and dislodgement in the left main coronary artery is an extremely rare but a serious and life-threatening complication which may produce hemodynamic instability, intracoronary thrombosis, stent embolization, myocardial infarction and sudden death.

According to the literature data, the most common cause of stent entrapment and dislodgement during PCI was attempt to deliver a stent though a previously deployed stent and pull-back [7]. Other common causes include poor support of the guiding catheter, vessel tortuosity and sharp angle proximal to the lesion, as well as severe vessel calcifications and use of longer stents [8]. In our case, there was a combination almost all of previously mentioned causes such as sharp angle of ostial LAD with tortuosity of proximal segment, poor and inadequate preparation of a calcified lesion in the medial LAD, previously implanted stent, and finally a longer stent. Probably, the most important causes of stent loss in our case were poor predilatation of the calcified lesion in the mid LAD and a sharp angle between the left main and ostial LAD.

Management of stent dislodgement in the coronary arteries depends on several issues. The most important issue is hemodynamic state of the patient as well as the coronary flow in the vessel with entrapped and unexpanded stent. If the patient is hemodynamically unstable with compromisation of the coronary flow, as in reported case, it is crucial to stabilize the hemodynamics first and promptly reestablish the coronary flow. Furthermore, in certain cases stent entrapment may be followed by getting stuck of the whole system of guiding catheter, stent-balloon and the wire, as happened in our case. In this situation, manipulation with the stent-balloon system can be completely blocked, caused by an entrapped stent within the angulated segment of the coronary artery, as in our case the stent was entrapped within the angle of the distal left main and ostial LAD. So, in this situation the only way to go further with the procedure was to forcefully pull back the whole system of the guiding catheter with the stucked stent-balloon and the wire. Although it led to the loss of the wire from the distal segment of LAD and it potentially could have further compromised the coronary flow, it seemed as the best management option in a hemodynamic unstable patient. Furthermore, the next step in the emergent management was to restore the coronary flow as quickly as possible, with the most simple and fastest way, which was balloon crushing technique. However in these settings, before crushing an unexpanded and dislodged stent, it is necessary to make sure that the guide wire is outside of the stent to be crushed, in order to prevent entrapment of the wire. So before crushing the stent, one should pass a balloon distally to the stent and withdrawing partially inflated balloon indicating that the wire did not pass though the stent struts. Then a procedure of crushing the unexpanded stent against the wall can be safely deployed, as we showed. However, this technique has not been widely accepted for the left main and proximal LAD because it may pose later an increased

risk for both stent thrombosis and restenosis due to excess metal layer [9].

For hemodynamically stable patients, the first option would definitely not be the balloon crushing technique, but the percutaneous retrieval of entrapped stent. Several techniques for retrieval of entrapped unexpanded stents from the coronary artery have been previously described with the success rate as of 86%, but all of them are time-consuming [1–3], so as not suitable for hemodynamically unstable patients. The most simple and most commonly used is low-profile balloon inflation up to 4–6 atmospheres within an unexpanded stent or distally to the lost stent [10]. Deployment of other techniques depend on operators experience and skills as well as locally available equipment, and it may include the use of myocardial biopsy or biliary forceps, two twisted guide wires, basket devices, loop snares and other devices [11].

Surgical intervention should be definitively the last option for the removal of entrapped stent [12], so as before transferring a patient to the surgery all alternative percutaneous options should be attempted cautiously and in consultation with the senior and more experienced interventional cardiologist, including different attempts for percutaneous removal or balloon crushing technique.

Finally, the management of entrapped and dislodged stent in the coronary arteries includes also the full heparinization of a patient in order to prevent thrombus formation around an undeployed stent [1].

Conclusion

The presented case demonstrated that balloon crushing of entrapped and dislodged stent in the distal left main and ostial left anterior descending coronary artery in a hemodynamically unstable patient is a feasible, safe and effective option for management of this life-threatening complication. Interventionalists should always keep in mind that angulated and tortuous segments of the coronary arteries as well as the heavily calcified lesions may reduce the possibility and success of stenting. Every effort should be made to prepare lesions adequately and to avoid stenting of extremely angulated and tortuous vessels in order to prevent possible complications such as entrapment, dislodgement and loss of a stent.

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CASE REPORT

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Eagle's syndrome – A report of two cases

Iglov sindrom

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Abstract

Introduction. Eagle's syndrome is defined as elongation of the styloid process or the stylohyoid ligament mineralization complex which consist of styloid process, stylohyoid ligament and lesser horn of hyoid bone. It is a rare entity, is not commonly suspected in clinical practice. It is characterized by recurrent facial and throat pain, dysphagia, odynophagia, parapharingeal foreign body sensation, otalgia and neck pain. Eagle's syndrome can be treated conservatively (lacing local anesthetic into the styloid process and stylomandibular ligament attachment) or surgically. Its pathogenesis and threatment modalities are still being debated while different theories have been presented. **Case report.** The two traditional surgical approaches to styloidectomy (removal of the elongated portion of the styloid process) were presented the intraoral approach and the extraoral approach. We presented two cases (49 years and 34 years old males), with bilateral and unilateral elongated styloid process. The surgical treatment included unilateral right side stiloidectomy by intraoral approach in the first case and right styloidectomy by extraoral approach in the second case. In both eases postoperative course passed regularly with no complaints at regular postoperative control. **Conclusion.** Surgical techniques for treatment of Eagle's syndrome have many advantages and disadvantages. We believe that the length of the styloid process or the calcified ligament is a decisive parameter for the selection of techniques and approach.

Key words:
temporomandibular joint disorders; diagnostic techniques and procedures; oral surgical procedures.

Apstrakt

Uvod. Iglov sindrom se definiše kao skup simptoma nastao usled elongacije stiloidnog nastavka ili mineralizacije stilohioidnog kompleksa koji se sastoji od: stiloidnog nastavka, stilohioidnog ligamenta i malog roga hioidne kosti. To je redak entitet koji obično se u kliničkoj praksi slučajno dijagnostikuje. Karakterišu ga simptomi u vidu rekurentnog bola u predelu grla i lica, disfagija, odinofagija, osećaj stranog tela u grlu, otalgija i bol u vratu. Iglov sindrom leči se konzervativno (ubrizgavanje lokalnog anestetika u stiloidni nastavak i stilomandibularni ligament) ili hirurški. Sama patogeneza i lečenje ovog sindroma uzrok su mnogobrojnih debata, i ostavljene su različite teorije. **Prikaz slučaja.** Prikazali smo dva bolesnika sa slučajno dijagnostikovanim Iglovim sindromom. Prikazana su dva tradicionalna hirurška pristupa stiloidektomije (uklanjanja izduženog dela stiloidnog nastavka): intraoralni pristup i ekstraoralni pristup. Bolesnici su bili muškarci, starosti 49 i 34 godine sa bilateralnim i unilateralnim produženim stiloidnim nastavkom. Kod prvog bolesnika urađena je unilateralna desna stiloidentomija intraoralnim pristupom, a kod drugog desna stiloidektomija ekstraoralnim pristupom. U oba slučaja postoperativni tok bio je uredan, bez navođenja tegoba sa redovnim postoperativnim kontrolnim pregledom **Zaključak.** Obe hirurške tehnike (intraoralnim i ekstraoralnim pristupom) imaju mnogo prednosti i nedostataka. Veruje se da je dužina stiloidnog nastavka ili kalcifikovani stilohioidni ligament odlučujući parametar za izbor tehnike i pristupa.

Ključne reči:
temporomandibularni zglob, poremećaji; dijagnostičke tehnike i procedure; hirurgija, oralna, procedure.

Introduction

Eagle's syndrome is a group of symptoms characterized by recurring pain in the region of the pharynx and the face, most often in the retromandibular region. Symptoms occur as a result of an elongated styloid process or ossificated stylohyoid ligament. It was first described in the literature in 1937 by Eagle [1] who connected the length of styloid process with atypical facial neuralgia.

Styloid process and stylohyoid ligament are in close contact with parapharyngeal anatomical space in which beside the common carotid artery (CCA) the internal jugular

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vein, glossofaringeal, facial, vagal and hypoglossal nerve are located.

Stylohyoid complex, which is formed by styloid process, stylohyoid ligament and lesser horn of hyoid bone (Figure 1), is embriologically derived from Reichert's cartilage of the second branchial arch.

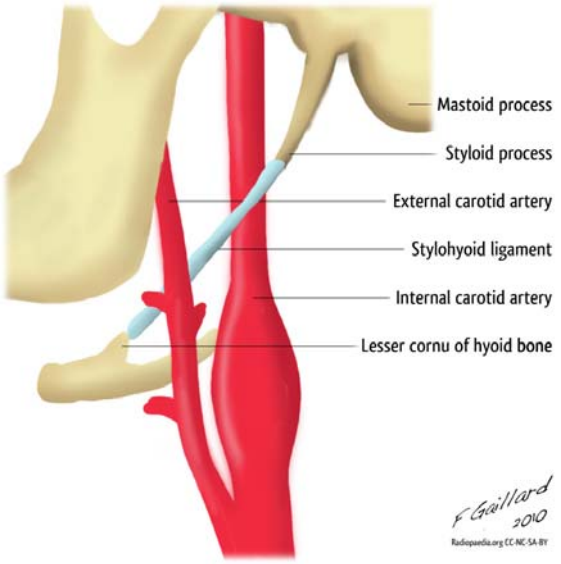

Fig. 1 – Stylohyoid complex anatomy.

Eagle defined physiological length of the styloid process to be 2.5–3.0 cm.

Diagnostic procedures used for the diagnosis of this syndrome are mainly used for the diagnosis of other diseases, and the diagnosis of an enlarged styloid process or calcification of the stylohyoid ligament is mostly the incidental finding. The radiologic diagnosis of the elongated styloid process is usually established during diagnoses of injuries and/or disease of the cervical spine. It is also evident that the styloid process can be clearly shown on the orthopantomography (OPT).

Definite diagnostic is performed with computered tomography (CT) scan radiography with 3D reconstruction.

Elongated styloid process and/or ossified stylohyoid ligament can be found in only 4% of total population, of which only 4% to 10.3% has clinical symptoms of recurring pain in the region of the pharynx and the face. It is more common in females in the ratio 3 : 1 [2].

Case report

Case 1

A 49-year-old male patient was sent to maxillofacial surgeon after examination of neurologist with symptoms of pain in the neck on the right side and painfull swallowing, and pain was intensified during the movements of the neck. The case history noted that he had the same pain several years earlier but it spontaneously stopped and he did not contact the doctor. Using careful clinical examination and analysis of radiography, CT scan and OPT images the existence of elongation of the styloid process bilaterally was established without involvement of the ligaments, and surgical treatment by intraoral approach was planned. Surgical treatment included entailed resection of elongated styloid process on the right side under general anesthesia. After the usual preoperative preparation unilateral right side styloidectomy by intraoral approach was made (Figure 2). Antibiotic therapy was prescribed. The postoperative period passed regularly, the wound healed without any signs of infection. The patient was discharged on the third postoperative day. Regular postoperative control showed no complaints.

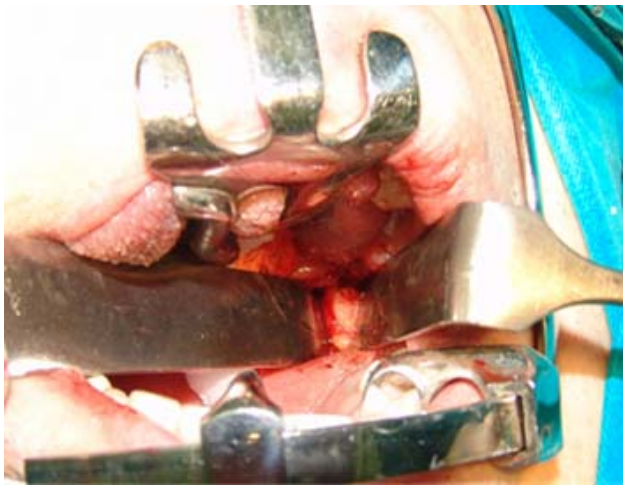

Fig. 2 – Intraoral approach to styloid process.

Case 2

A 34-year-old male patient was sent to the maxillofacial surgeon from the dermatologist. The OPT was made as a part of the dental diagnostics performed during the procedure of finding the causes of alopecia that occurred a few months earlier. OPT showed elongated styloid process on the right side (Figure 3).

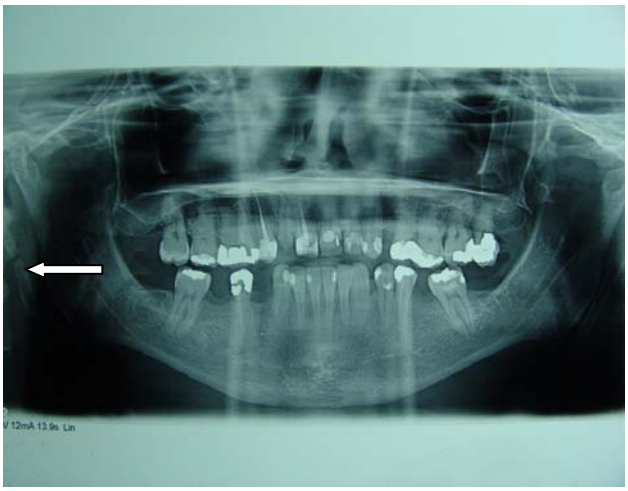

Fig. 3 – Ortopantomography of the patient with Eagle's syndrome.

The patient was sent to the maxillofacial surgeon. The patient complained of pain in the neck on the right side and difficult swallowing, earlier on both sides and at the moment

of examination more on the right. The patient denied earlier surgical intervention. CT scan with 3D reconstruction revealed the elongated right styloid process (62 mm length, 5 mm wide, with slight angulation of 20º right stylohyoid ligament) while the length of the left styloid process was 49 mm and the width of about 4 mm (Figure 4). The treatment plan included right styloidectomy under general anesthesia, and because of the length of the process and involvement of stylohyoid ligament, it was decided to approach it extraorally (Figures 5 and 6). Antibiotic therapy was administered preoperatively. Surgery under general anesthesia and postoperative period passed regularly, and the patient was discharged on the third postoperative day. At regular postoperative examinations the patient was subjectively without complaints.

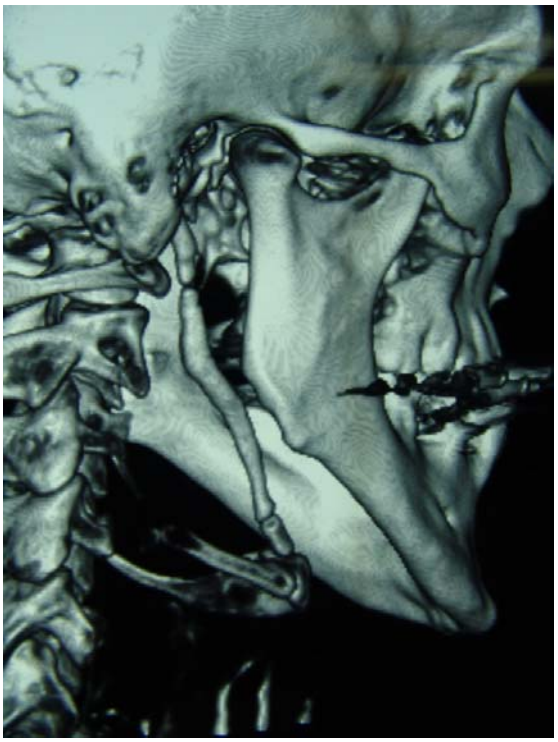

Fig. 4 – Computed tomography scan with 3D reconstruction in Eagle's syndrome.

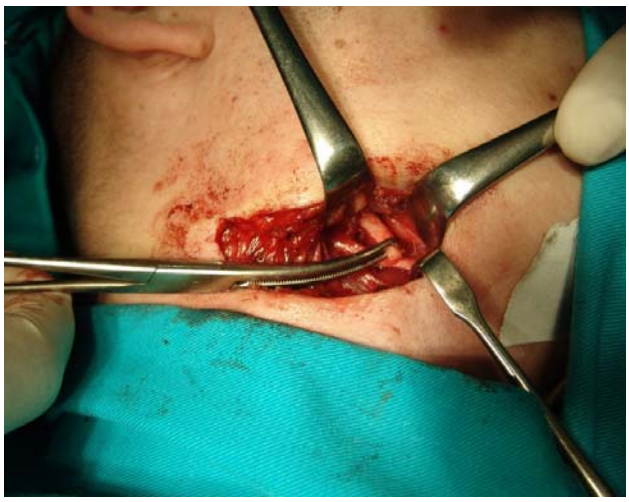

Fig. 5 – Extraoral approach to styloid process.

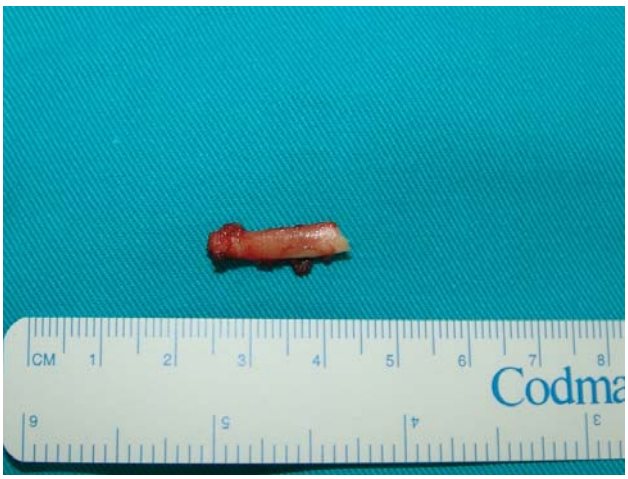

Fig. 6 – The resected styloid process.

Discussion

The styloid process, stylohyoid ligament and lesser horn of hyoid bone make stylohyoid complex, which is embriologically derived from Reichert's cartilage of the second branchial arch. The styloid process is located on the basis of the temporal bone behind the mastoid, it is positioned anteroinferiorly relative to the lower aspect of the temporal bone. It is placed between the parotid gland laterally and the internal jugular vein medially, passes between the external and internal carotid arteries and reaches the lateral wall of the pharynx. Cranial nerves: *n. hypoglossus, n. vagus* and *n. glossopharingeus* are placed medially to the styloid process. On the styloid process *m. stylohyoideus, m. styloglossus* and *m. stylopharingeus*, stylohyoid and stylomandibular ligament are attached [3].

The normal length of the styloid process may vary. Eagle [5] defined normal length of styloid process in the range from 25 to 30 mm. Kaufman et al. [6] believe that the normal length is up to 30 mm while in some other papers 40 mm was mentioned as the upper limit [7, 8]. From the radiological point of view the normal styloid process length is 25 mm (the length along the posterior aspect of the styloid process from the base to peak is measured) [9].

Elongated styloid process can be classified into three types according to Langlias et al. [10]: uninterrupted styloid process; styloid process with pseudoarthrosis between the styloid process and stylohyoid ligament; segmental interrupted stylohyoid ligament that gives the appearance of multiple pseudoarthrosis.

Eagle's syndrome is most common in the third and fourth decade, slightly more often in women (ratio women : men is 3 : 1) and more often bilateral than unilateral although bilateral symptoms do not necessarily occur. We described a case of bilateral and a case of unilateral elongated styloid process, both patients were men in the fourth and fifth decade with unilateral (right side) symptoms.

Patients can develop different symptoms because of elongation of styloid process or calcification of stylohyoid ligaments, in the form of non-specific neck pain, pain in the

ear and mastoid region that is amplified during the movements of the neck, dysphagia or odynophagia with the feeling of a foreign body in the throat, vertigo and tinnitus. Eagle [4] described two syndromes which are related to the elongation of the styloid process. Normaly, classic Eagle's syndrome, which is seen in patients after pharyngeal injuries during tonsillectomy, followed by constant dull pain, a patient as the epicenter pain alleges the tonsillar region, pain spreads to the ear when patient rotates the head. Pain increases with pressure in tonsillar region. Among other symptoms, there is pain when swallowing, feeling of a foreign body in the mouth (pharynx), ringing in the ears (tinnitus) or pain spreading to the face and neck region. The other form of Eagle's syndrome, also called stylocarotid syndrome, is not associated with pharyngeal injuries, and symptoms such as pain are the result of mechanical irritation and compression of perivascular sympathetic nerve fibers in the wall of the external and internal carotid arteries. Pain spreads along the blood vessel in the neck, during the rotation or pressure in the neck, and extends in the supraorbital region and the parietal region (because of pressure on the internal carotid artery), or to the infraorbital region (because of the pressure on external carotid artery).

The etiology of this syndrome is still causing debate. Eagle [1, 5] believed that surgical injury (tonsillectomy) leads to osteitis, periostitis or tendinitis of stylohyoid complex with subsequent ossification. The presence of mesenchymal elements in the stylohyoid complex (Reichert's cartilage) may under the influence of trauma and mechanical stress lead to metaplasia of the bone. There are different theories of the cause of ossification of stylohyoid ligament (reactive hyperplasia, reactive metaplasia, both based on post-traumatic response of the body, and the theory of anatomical variations, based on anatomical variations without previous injury) [11]. Bafaqeeh [12] considers that ossification is associated with the endocrine disorders in menopausal women, which lead to ligament ossification. Elevated serum calcium, phosphorus and vitamin D metabolism disorder encountered in end-stage renal disease can lead to calcification of stylohyoid ligament.

Although Eagle [5] believed that tonsillectomy is responsible for the appearance of ossification of styloid process, Eagle's syndrome occurs in people who had no surgical procedures in the region.

The diagnosis is based on detailed anamnesis, clinical examination and radiological examination (OPT, lateral radiography by Eissler, CT scan with 3D reconstruction). The elongated styloid process and its deviation can be seen clearly on OPT, but for the diagnosis of Eagle's syndrome CT with 3D reconstruction is most significant, because it allows measurement of the length of styloid process and determination of its relationship with other structures of the head and neck, which is most important for surgical planning [13].

Based on the density of calcification the elongated styloid process can be radiologically classified into: marginally calcified (calcification occurs in the outer part while in the center of process lightening is seen that occurs in most cases); partially calcified; nodular complexes; completely calcified [10].

The differential diagnosis includes: diseases of the temporomandibular joint, hyoid bursitis, glossopharyngeal and sphenopalatinal neuralgia, esophageal diverticulum, migraine, temporomandibular arteritis, myofascial pain syndrome, cervival arthritis, otitis, diseases of the salivary glands, impacted third molar, tumors, etc. [14–16].

Treatment of Eagle's syndrome can be a conservative and surgical.

Our experience presented in this paper is based on the surgical treatment.

Conservative treatment advocated by Evans and Clairmont [17] (symptomatic therapy similar for treatment of trigeminal neuralgia) involves the use of non-steroidal anti-inflammatory drugs, corticosteroids (corticosteroid and anesthetic injection in the region of the lesser horn of hyoid bone or the lower aspect of the tonsillar lodge), anticonvulsants, antidepressants, physical treatments and exercises for the neck.

Surgical treatment involves styloidectomy (removal of elongated styloid process) extraorally or intraorally. The success of both surgical techniques is 93.4% [13].

Intraoral technique is simpler, takes less time and avoids the surgical scar, but can lead to infection of deep neck spaces, injury of the blood vessels and one of the disadvantage is poor visualization of the operative field. It is not recommended to made styloidectomy for both sides intraorally in the same act due to high postoperative discomfort for the patient [18, 19].

Extraoral technique involves access through cervical incision. This approach allows better visualization of the surgical field. This technique, however, takes longer time, there is a risk of injury of the facial nerve, the patient postoperatively recovery is longer and the postoperative scar is visible. Extraoral approach is reserved for patients who have extreme ossification, practically the entire ligament – from the styloid process to the hyoid bone. It is considered reasonable in such cases since it avoids the risk of intraoral access and iatrogenic injury to the neurovascular structures [20–22].

It is estimated that the success of treatment of Eagle's syndrome (both conservative and surgical) is more than 80%. It is believed that treatment failure is associated with the presence of the other factors involved in the pathogenesis of this syndrome.

Conclusion

In patients with orofacial pain, neck pain, dysphagia and pain in the temporomandibular joint area it is needed to pay attention to the existence of elongated styloid process. Careful clinical examination, palpation of the mastoid region and tonsillar fossa and additional imaging methods (orthopantomography and computered tomography diagnostics) can confirm the agnosis of elongated styloid process.

Surgical techniques for treatmet of Eagle's syndrome have many advantages and disadvantages. It is considered that the length of the styloid process or the calcified ligament is a decisive parameter for the selection of techniques and approaches.

If the styloid process is augmented (extended) without involvement of the ligament, it is considered that is better and easier to use intraoral approach. If the stylohioid ligament is calcified, and it is necessary to remove a structure that extends to the hyoid bone, it is considered that it is safer to use the extraoral approach.

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C A S E R E P O R T S

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Overlooked retained intraocular foreign body

Previđeno zaostalo intraokularno strano telo

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Abstract

Introduction. The most common cause for litigation against ophthalmologists in a trauma case is a missed intraocular foreign body (IOFB). IOFBs cause internal eye damage, but some will come to rest in the posterior segment of the eye. **Case report.** We presented a 57-year-old male who was referred to the ophthalmologist due to decreased visual acuity in his left eye. Slit lamp examination of his left eye showed no pathological findings. Goldmann contact lens examination showed IOFB which was lying in the vitreous body in the inferior-temporal region. Retinal rupture was noticed at 7 o'clock. The optical coherence tomography (OCT) examination was performed and it showed atrophic macular area as well as decreased peripapillar retinal fiber layers thickness. Ultrasound showed the IOFB in vitreous body cavity. History revealed that the patient had an accidental trauma, 48 years ago, when an old bomb from World War II (WWII) exploded. Due to the decrease in visual acuity and fibrosis of the vitreous body surgical intervention was performed on his left eye (phacoemulsification with intraocular lens implantation, *pars plana* vitrectomy and instrumental extraction of foreign body). **Conclusion.** The intraocular foreign body (IOFB) was asymptomatic for 48 years. Symptoms depend on material and localization of the foreign body and the type of injury.

Key words:
eye foreign bodies; diagnosis; ophtalmologic surgical procedures; treatment outcome.

Apstrakt

Uvod. Najčešći uzrok sudskih sporova protiv oftalmologa u slučaju traume je previđeno strano telo. Intraokularna strana tela izazivaju unutrašnja očna oštećenja, ali neka od njih mogu da ostanu mirna u zadnjem segmentu oka. **Prikaz bolesnika.** Prikazan je 57-ogodišnji muškarac koji je upućen oftalmologu zbog sniženja vidne oštrine na levo oko. Pregled levog oka procepnom lampom nije pokazao patološke nalaze. Pregled Goldmanovom lupom pokazao je intraokularno strano telo, koje je ležalo u staklastom telu u donjetemporalnom segmentu. Ruptura retine, uočena je na 7 časova. Pregled optičkom koherentom tomografijom ukazao je na atrofičnu makularnu oblast i smanjenu debljinu peripapilarnih retinalnih nervnih vlakana. Ultrazvukom levog oka ustanovljeno je strano telo u staklastom telu. Istorija lečenja bolesnika ukazala je na traumu, pre 48 godina, kada je stara bomba iz Drugog svetskog rata eksplodirala. Zbog sniženja vidne oštrine i fibroze staklastog tela urađena je hirurška intervencija (fakoemulzifikacija sa ugradnjom intraokularnog sočiva, *pars plana* vitrektomija i instrumentalno odstranjenje stranog tela). **Zaključak.** Intraokularno strano telo bilo je asimptomatsko 48 godina. Simptomi zavise od materijala i lokalizacije stranog tela i od tipa povrede.

Ključne reči:
oko, strana tela; dijagnoza; hirurgija, oftalmološka, procedure lečenje, ishod.

Introduction

The frequency of ocular traumas varies between 7 and 9.22% in regard to a total number of all body injuries. The most common cause for litigation against ophthalmologists in a trauma case is a missed intraocular foreign body (IOFB). Commonly injured structures include the cornea, the lens and the retina. IOFBs cause internal eye damage, but some will come to rest in the posterior segment of the eye [1].

Ocular injuries with foreign body are highly associated with severe ocular damage requiring extensive surgical repair or evisceration/enucleation. IOFB is a common cause of poor anatomical (atrophic eyeball) and visual outcome (amaurosis) [1, 2].

IOFB could be easily overlooked if there are no symptoms such as inflammatory sign, diplopia, pain, etc. The identification of IOFB can be quite challenging clinically.

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Case report

We presented a 57-year-old male who had an explosive injury in the right side of the body (right eye and right hand) long ago, but at the same time he had an injury in the left eye caused by a metallic foreign body, too. He lived for 48 years with an asymptomatic IOFB in his left eye. History revealed that he had an accidental trauma 48 years ago when an old bomb from World War II (WWII) exploded. In that accident, he got numerous face injuries, including the left eye.

He was referred to ophthalmologist due to decreased visual acuity in the left eye and unpleasant flashing lights. Test results were as follows: visual acuity VOD = amaurosis, VOS = cc 0.3; ocular pressure TOD = N (dig), TOS = 18 mmHg. Slit lamp examination showed that there was a big corneal scar in the anterior segment of the right eye, but deeper areas were unreachable and the right eye was atrophic. Slit lamp examination of his left eye showed no pathological findings. There were no scars in eyelids or in the anterior segment of the left eye. Goldmann examination showed IOFB unnoticed until then in the vitreous body of the left eye. Photo-fundus (45°) of his left eye was normal, but ophthalmoscopic examination showed an IOFB (Figure 1) lying free in the vitreous body in inferior and temporal parts near to optic nerve head (at 7 o'clock).

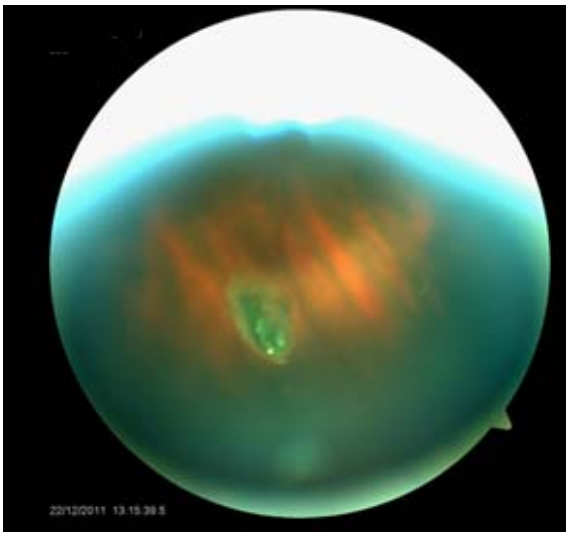

Fig. 1 – Intraocular foreign body (IOFB) in vitreous body of the left eye.

Retinal rupture was noticed at 7 o'clock. Optical coherence tomography (OCT) examination was performed and it showed atrophic macular area as well as decreased peripapillar retinal fiber layers thickness (Figures 2 and 3). Ultrasound of the left eye showed the IOFB in vitreous body cavity (Figure 4). The patient had complaints on persistent discomfort in his left eye since then. He was examined several times by ophthalmologists during the past period, but nobody noticed an IOFB. Due to the decrease in visual acuity and fibrosis of the vitreous body surgical intervention was performed on his left eye (phacoemulsification with intraocular lens implantation, *pars plana* vitrectomy and instrumental extraction of IOFB). The extracted foreign body was 1.8 mm in length. Endolaser photocoagulation was performed for a retinal rupture. Postoperative visual acuity in the left eye was VOS = 0.7.

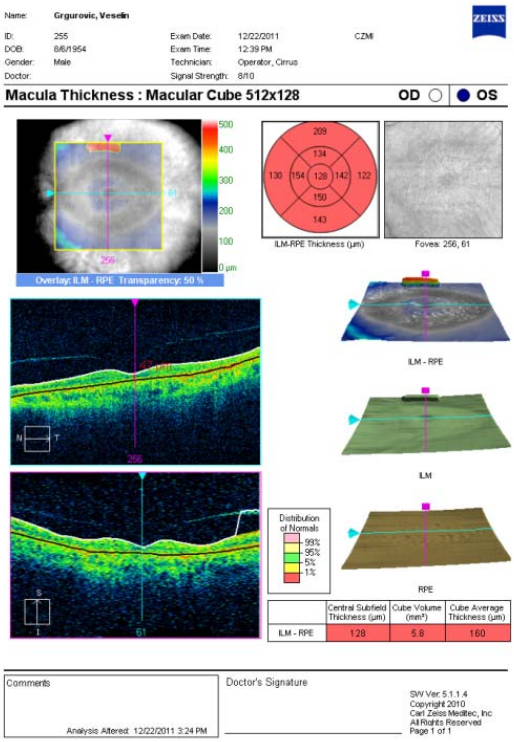

Fig. 2 – Optical coherence tomography (OCT) findings of the left eye macula.

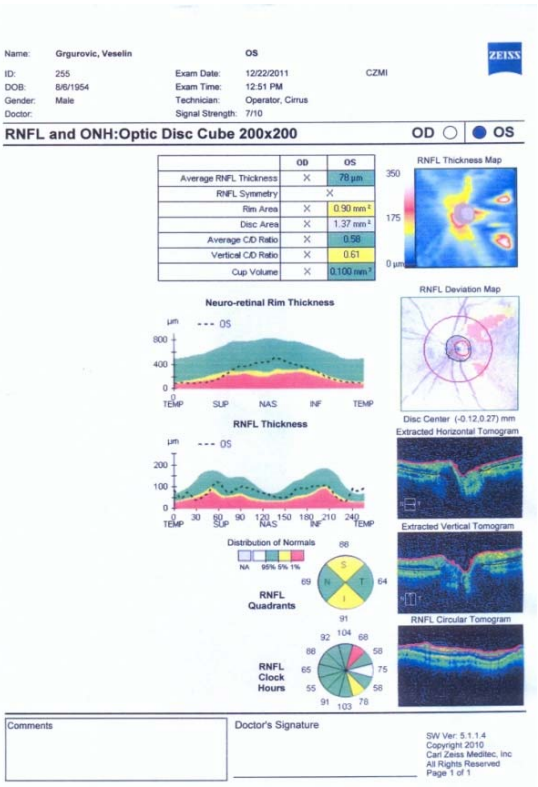

Fig. 3 – Optical coherence tomography (OCT) finding of the left optic nerve head.

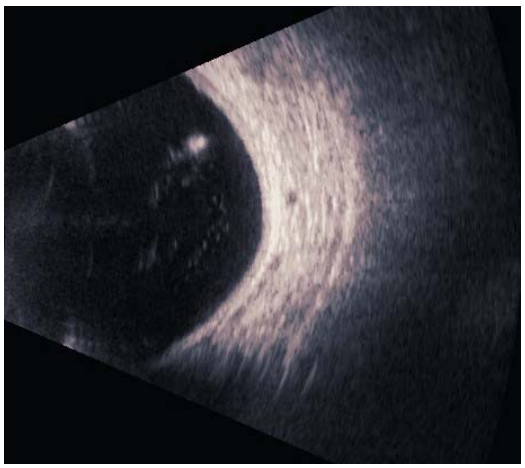

Fig. 4 – Ultrasound of the left eye with intraocular foreign body.

Discussion

Careful history examination will help in finding asymptomatic IOFB. Simptomatology of IOFB can be various. Symptoms depend on the material a foreign body consists, localization of the body and type of injury. Rarely, when an IOFB completely enteres the eye, its presence can be asymptomatic [3].

It is well-known that computed tomography (CT) scans or X-rays should represent initial imaging tests performed for metallic IOFB because magnetic resonance imaging (MRI) testing on these patients may result in blindness.

Reviewing the literature of diagnosis and management of traumatic IOFBs is presented together with the schematic ''flight plan'' to assist in clinical decision making when confronted with the IOFB [4].

There was a study of demographic profile, causes, type of ocular injuries, severity, complications and final visual outcome following the Deepawali festival fireworks in India. Factors associated with poor visual outcome included poor initial visual acuity. Poor visual outcome was associated with poor initial visual acuity and retinal detachment, as in our case, too, if analyzing the state of the right eye [5].

Many authors reported injury patterns, management strategies and outcomes for eye injuries in British Armed Forces in Iraq and Afghanistan. Primary repair can be safely delayed beyond 24 hours in patient's best interests, in order to optimize the conditions for treatment, the opinion we also share [6].

Ranking among the most severe combat damages, war-related open-globe injuries (WROGI) are not uniform, so the treatment approaches are unclear. The essential issue is to define exact indications for time and resource - intensive vitreoretinal surgery (VRS) known to be an effective procedure for severe posterior segment injuries. The authors studied WROGI structure and summarized experience of specialized ophthalmologic care management during local armed conflicts. Treatment should be determined by the diagnosis, because this damage determines only the choice of enucleation/evisceration of the eye [7].

Eye injuries represent one of the biggest problems in eye surgery throughout the world, including our country. Vitreoretinal operations are the most prevailing in treatment of serious eye injuries. *Pars plana* vitrectomy is the most common method of surgical treatment of eye injuries with IOFB [8]. War eye injuries are a specific group of injuries. A relatively low percentage of posttraumatic endophthalmitis is definitely worth attention, especially in comparison with peacetime eye penetrating injuries with IOFB [9].

In our case IOFB was asymptomatic (overlooked) for several decades, due to the nature of material, location and shape of the foreign body.Diagnostic capabilities before 48 years were limited to diagnostic X-rays, and today they are facilitated with the use of ultrasound diagnostic for comprehensive and easier localization and verification of IOFBs.

Conclusion

In the presented case, intraocular foreign body was asymptomatic for 48 years. Nobody had noticed intraocular foreign body when examining the patient because of flashing lights. This symptom was a sign of retinal rupture. In our case, intraocular foreign body was clearly presented in ultrasound and additional imaging tests were unnecessary. Surgical treatment was performed due to the decrease in visual acuity and fibrosis of vitreous body. *Pars plana* vitrectomy is the most common method of surgical treatment of eye injuries with intraocular foreign body.

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CASE REPORT

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Specificity of treatment is mandatory in very old patients with hairy cell leukemia

Specifičnost lečenja je neophodna kod vrlo starih bolesnika sa leukemijom vlasastih ćelija

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Abstract

Introduction. There are only a few available data about hairy cell leukemia (HCL) in very old patients. We presented three very different cases of HCL in very old patients diagnosed in a single center and discussed some epidemiological and therapeutical issues in such patients. **Case report.** The first patient, 89-year-old, had symptomatic cytopenia and achieved sustained complete remission after cladribine treatment. The second patient, 89-year-old, had asymptomatic disease with stable full blood counts during a 3-year follow-up period in which watch-and-wait policy was adopted. The third patient, 82 years old, had two malignancies (HCL and presumably metastatic colorectal carcinoma) and his only treatment were occasional red blood cell transfusions and symptomatic therapy. **Conclusion.** The presented illustrative examples confirm individualization of treatment is mandatory in very old patients with HCL.

Key words:
leukemia, hairy cell; aged, 80 and over; therapeutics; prognosis.

Apstrakt

Uvod. U literaturi postoji vrlo malo podataka o leukemiji vlasastih ćelija (*hairy cell leukemia* – HCL) kod vrlo starih osoba. Prikazali smo tri vrlo stara bolesnika sa vrlo različim tokom HLC dijagnostikovanim u jednom centru i razmotrili epidemiološke i terapijske aspeke ove bolesti u grupi vrlo starih bolesnika. **Prikaz bolesnika.** Prvi bolesnik, star 89 godina, imao je simptomatsku citopeniju i postigao kompletnu remisiju nakon lečenja kladribinom. Drugi bolesnik, star 89 godina, imao je asimptomatsku bolest sa stabilnom krvnom slikom tokom trogodišnjeg perioda praćenja, zbog čega je primenjena "*watch-and-wait*" strategija lečenja. Treći bolesnik, star 82 godine, imao je dva maligniteta (HCL i metastatski kolorektalni karcinom) i njegovo lečenje je uključivalo samo povremenu primenu transfuzija koncentrovanih eritrocita i simptomatsku terapiju. **Zaključak.** Prikazani ilustrativni primeri bolesnika potvrđuju neophodnosti individualnog terapijskog pristupa kod vrlo starih bolesnika sa HLC.

Ključne reči:
leukemija vlasastih ćelija; stare osobe, 80 i više godina; lečenje; prognoza.

Introduction

Hairy cell leukaemia (HCL) is an indolent neoplasm of small mature B lymphoid cells with oval nuclei and abundant cytoplasm with irregular projections (hairy) involving peripheral blood and diffusely infiltrating the bone marrow and splenic red pulp [1]. HCL is characterized by splenomegaly, pancytopenia, and infiltration of the bone marrow with lymphocytes that have irregular cytoplasmic projections when identified in the peripheral blood [1]. At the time of diagnosis, most patients manifested symptoms related to anemia, neutropenia, thrombocytopenia, or splenomegaly. Approximately 25% of patients manifested fatigue or weakness, 25% manifested infection, and 25% were present because of the incidental discovery of splenomegaly or an abnormal peripheral blood count [2]. Blood film and bone marrow examination are essential for the diagnosis of HCL. Immunohistochemistry of the marrow trephine specimens should include CD20 and DBA44 [3].

There are no available data about the incidence of HCL in very old patients. We presented three very different cases of HCL in very old patients and discussed some epidemiological and therapeutical issues in such patients.

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Case report

The first 89-year-old patient had the right neck femur fracture in January 2011 and was treated surgically. After the surgery, there were no complaints. Physical examination showed cardiac arrhythmia and arterial hypertension. His complete blood counts (CBC) showed pancytopenia: hemoglobin 121 g/L (normal range 130–180 g/L), white blood cells (WBC) 1.1×10^9/L (normal range 4–10 $\times 10^9$/L), absolute neutrophil count (ANC) 0.6×10^9/L and platelets 37×10^9/L (normal range 1.6–7.2 $\times 10^9$/L). Biochemistry findings were normal. Ultrasonography of the abdomen revealed enlarged spleen (165 × 61 mm). He was admitted to the Hematology Department and trephine bone marrow biopsy was performed. Bone marrow examination revealed diffuse/interstitial infiltration with lymphoid cells (Figure 1). The cells were CD20+, with oval, round nuclei with small nucleolus and abundant light-grey cytoplasm. The marrow reticular fibrosis was gradus III. Morphologically, the diagnosis of HCL was established. The patient was treated in March 2011 with 2-chlorodeoxyadenosin (cladribine 0.14 mg/kg/day) *iv* infusion over two hours for 5 consecutive days. During and after the treatment period he had no infections, nor other complications. The complete remission was achieved. On the occasion of his last follow-up (January 2013), the patient felt well, without any complains and with normal hematological findings.

The third 82-year-old patient was admitted to the Hematology Department in November 2011 with the history of pancytopenia in his blood count from February 2011 when he had a surgical intervention due to acute appendicitis. He complained of weight loss, malaise and dizziness. He also suffers from hypertension and cardiomiopathy. Laboratory evaluation showed bycytopenia (hemoglobin 64 g/L, WBC 1.4×10^9/L, ANC 0.3×10^9/L, platelets 154×10^9/L) and elevated sedimentation rate (120 mm/h; normal range < 30/h). The trephine marrow biopsy showed infiltration with hairy cells with following imunnophenotype: TdT-, CD34-, CD117-, MPO-, lysosime-, glycophorine A-, PAX5+, CD20+, CD3-, BCL2+, DBA.44 +, CD43-, IgD-, CD68-. Biochemistry findings were normal. Ultrasonography of the abdomen showed the enlarged spleen (180 mm in diameter) and the enlarged liver (180 mm in diameter) with numerous secondary deposits (about 25 mm in average diameter). These findings were confirmed by computed tomography (CT) scan. CT scan also showed the thickness of the right colon wall. The patient refused colonoscopy and biopsy of the liver tumor. Regarding such decision of the patient, we decided not to treat him with specific anti-HCL therapy. He occasionally received red blood cell transfusions. On the occasion of his last follow-up (December 2012), the patient was still alive.

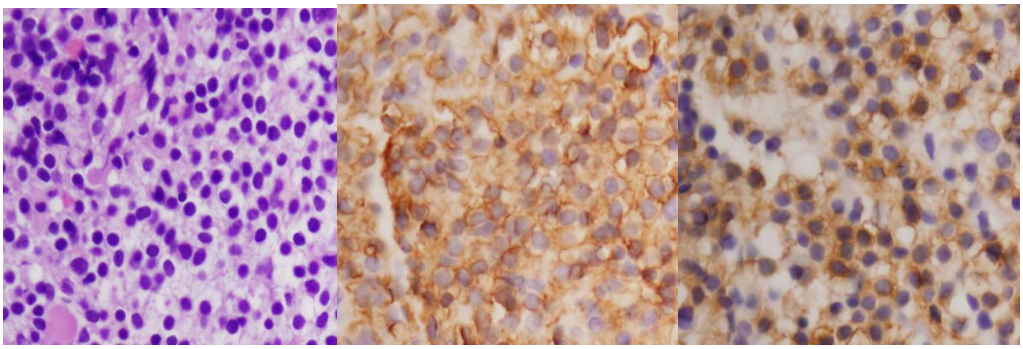

Fig. 1 – Histopathological finding of hairy cell leukemia in the bone marrow: hematoxillin-eosin, ×400 (left); CD20, ×400 (center); DBA, ×400 (right).

The second 89-year-old patient showed up in March, 2011, with the one year history of pancytopenia. He had been suffered from diabetes mellitus type II for 10 years, without complications. His complete blood counts showed: hemoglobin 102 g/L, WBC 4.0×10^9/L, ANC 1.1×10^9/L and platelets 109×10^9/L. Other laboratory findings revealed elevated blood glucose (8.2 mmol/L – normal range 3.3–6.1 mmol/L). Ultrasonography of the abdomen showed enlarged spleen, 190 mm in diameter. Morphological examination of the bone marrow specimen led to the diagnosis of HCL, with the following immunophenotype: CD20+, CD79alfa+, CD3, CD5-, CD43+, CD23-, cyclin D1-, DBA44+, CD34-, CD117-, CD10-, BCL2+, BCL6-, MUM1-. Ki-67 was positive in 20% of the cells. The marrow reticular fibrosis was gradus II-III. Bearing in mind that the patient's CBC remained almost unchanged in the course of a 3-year follow-up, we opted for the watch-and-wait approach. On the occasion of his last follow-up (January 2013), the patient was in a very good condition, without any complaints, still requiring no therapy.

Discussion

HCL is one of the rarest types of leukemia. The overall incidence is around 3 cases in million people *per* year, with a marked male preponderance (4 : 1) [4]. Over the past 30 years the incidence of this disease has not changed according to the largest population based study carried out on 3,104 HCL patients identified from 1973 through 2002 through the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) Program [5]. The median age at diagnosis is about 50 years [3], although some national HCL registries reported higher median age value for onset of the disease [6, 7]. However, there are no precise literature data about incidence

of HCL in elderly persons. In the largest study of HCL in Serbia so far [8], which included 46 patients, there were no patients older than 60 years. According to the report of Population Division DESA, United Nations, the patients older than 80 years belong to the so-called "oldest old" population, which is now about 1% of the total human population [9].

Therefore, we were very surprised when diagnosed three patients older than 80 years in our hospital in the last few years. We believe that this was only a coincidence, not a change in epidemiological pattern of the disease in our country. Beside older age, clinical presentation of HCL in our patients was common: men with pancytopenia, splenomegaly and infiltration of the bone marrow with CD20+/DBA.44+ lymphocytes.

There is no widely agreed system for staging HCL, as well as for assessing prognostic factors. Heavy bone marrow infiltration and a large spleen will result in maximal degrees of cytopenia. Anemia (hemoglobin < 100 g/L), neutropenia (ANC $< 1.0 \times 10^9$/L) and thrombocytopenia (platelets $<100 \times 10^9$/L) in any combination are associated with a relatively poor prognosis [10, 11]. An assessment of prognosis should include response to purine analogue therapy [3]. The main indications for treatment are symptomatic cytopenias or painful splenomegaly. If a patient is asymptomatic and cytopenia is minimal, it is reasonable to adopt watch-and-wait policy [3]. In our three patients we strictly followed current guidelines for the HCL management [3]. The first patient with symptomatic cytopenia received specific therapy (cladribine) and achieved complete remission. In contrast, since the second patient appeared to be quite stable CBC during a 3-year follow-up period, we decided that active monitoring is the most appropriate clinical approach. In addition, this patient was currently 92 years old. The third patient had two malignancies (HCL and presumably metastatic colorectal carcinoma) and, therefore, his treatment was only supportive (occasional red blood cells transfusions) and symptomatic therapy. HCL has an increased risk of second tumors [5]. The relative risk of second cancers reported in various series of HCL patients ranged from 0.95 to 4.33, but simultaneous presentation of HCL and solid malignancies is very rare. Simultaneous presentation of HCL and solid malignancy is exceptional and only a few cases have been described [12–14]. It has been described synchronous occurrence of HCL with neuroendocrine colon carcinoma, HIV-negative Kaposi's sarcoma and signet ring carcinoma of the stomach [12–14].

Conclusion

The presented cases are illustrative examples that individualization of treatment is mandatory in very old patients with hairy cell leukaemia.

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CASE REPORTS

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Massive necrotizing fasciitis following bellow-knee arterial surgery – A therapeutic challenge

Masivni nekrotizujući fasciitis posle hirurškog zahvata na arteriji potkolenice – terapijski izazov

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Abstract

Introduction. Necrotizing fasciitis is a rare, progressive bacterial infection of superficial fascia followed by secondary subcutaneous tissue necrosis. We pressented a patient with massive fulminant lifethreatening necrotising fasciitis after bellow-knee femoro-popliteal vein bypass grafting successfully treated by antibiotics, surgical debridement and final skin reconstruction using the Tierch method. **Case report.** A 61-year-old patient was admitted to the Vascular Surgery Clinic for below-knee femoropopliteal bypass grafting. He complained of intermittent claudication in the left leg after 50 m, ankle brachial indexes were 0.45 on the left and 1.0 on the right side. Femoropopliteal below-knee bypass grafting was done using the autologous great saphenous vein. In the very next day, initial signs of skin infection appeared including local inflammation, erythema, swelling and cellulitis restricted to saphenectomy site. These changes had rapidly spread in the following days on the deep tissue of the whole upper and lower leg, including the groin and with clinical signs of lifethreatening systemic infection. Immediate surgical debridement was done followed by extensive wound packing and wide spectrum antibiotics administration for the next 33 days when final skin reconstruction by the Tierch method was performed. Interesting point is that this entire time wound swab was sterile. **Conclusion.** In the presented case immediate surgical debridement, wide spectrum antibiotics administration and consistent wound packing gave satisfactory results in this life-threatening systemic infection. Wound swab is not always a reliable indicator of the infection while clinical findings and surgeons' experience are of great significance in rapid reaction to this rare surgical complication.

Key words:
vascular surgical procedures; treatment outcome; fasciitis, necrotizing; anti-bacterial agents.

Apstrakt

Uvod. Nekrotizujući fasciitis je retka, progresivna bakterijska infekcija površinske fascije, praćena sekundarnom nekrozom potkožnog tkiva. Prikazali smo bolesnika primljenog radi famorodistalne rekonstrukcije venskim graftom, koja je u neposrednom postoperativnom toku bila komplikovana fulminantnim, nekrotizirajućim faciitisom. Bolesnik je uspešno tretiran ekstenzivnim, dnevnim debridmanom inficirane rane uz parenteralnu primenu antibiotika širokog spektra. Lečenje je završeno uspešnom rekonstrukcijom kože Tiršovim graftom. **Prikaz bolesnika.** Bolesnik, star 61 godinu, primljen je radi planirane hirurške revaskularizacije venskim femoropoplitealnim *bypass*-om. Žalio se na intermitentnu klaudikaciju u levoj nozi, posle pređenih 50 metara. Brahijalni indeks gležnja iznosio je 0.45 levo i 1.0 desno. Drugog dana po rekonstrukciji pojavili su se prvi znaci infekcije kože na mesu salenektomije, uz ubrzano širenje na dublja tkiva i mišiće cele noge praćeno sistemskim znacima sepse. Ukupno 33 dana primarna terapija bila je hirurška debridman rane podržan parenteralnom primenom antibiotika. Bris rane uziman je na drugi dan i konstantno je bio sterilan. Sticanjem adekvatnih uslova urađena je rekonstrukcija kože Tiršovom metodom. **Zaključak.** Hitan opsežan hirurški debridman uz adekvatnu primena antibiotika dali su zadovoljavajuće rezultate. Samo bris rane nije uvek pouzdan indikator prisustva infekcije. Kliničko iskustvo hirurga i lokalni status rane ključni su faktori uspeha.

Ključne reči:
hirurgija, vaskularna, procedure; lečenje, ishod; fasciitis, nekrotizujući; antibiotici.

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Introduction

Necrotising fasciitis (NF) is rare, progressive, rapidly spreading, inflammatory infection, primarily involving the superficial fascia and usually followed by secondary necrosis of the subcutaneous tissues. [1–7]. The infective process leads to thrombosis of subcutaneous blood vessels, resulting in gangrene of the overlying skin. We presented a patient with massive fulminant life-threatening NF after bellow-knee femoropopliteal vein bypass grafting successfully treated by antibiotic, surgical debridement and final reconstruction by skin transplant using the Tierch method. The Institutional Ethics Committee approved the manuscript.

Case report

A 61-year-old male patient was admitted to the Vascular Surgery Clinic for below-knee femoro-popliteal bypass grafting. He complained of intermittent claudication in the left leg after 50 m of walking. The ankle–brachial index (ABI) was 0.45 on the left and 1.0 on the right side. Previously, multislice computed tomography (MSCT) (a General Electrics light speed VCT 64) discovered left superficial femoral artery occlusion as well as popliteal artery above knee with a good run-off of the crural arteries. As it was, below-knee bypass was indicated. The preoperative medical history of the patient included hypertension and hyperlipoproteinemia. All laboratory findings were within referent values [white blood cells (WBC) 5.1×10^9/L (normal range 4.5–10.0 $\times 10^9$/L); haemoglobin (Hgb) - 9.8 g/dL (normal range 13.5–17.5 g/dL); platelets count 405×10^9/L) (normal range 150–400 $\times 10^9$/L)].

The femoro-popliteal below-knee bypass procedure was performed using an autologous great saphenous vein graft from the left leg. Ceftriaxone [2 g/intravenous (*iv*)/24 h] was administrated initially as usual postoperative antibiotic. Immediately after the surgical reconstruction, ABIs for the left foot were 0.82 and 0.77.

In the very next day, initial signs of skin infection were noted with accompanied local inflammation, erythema, swelling and cellulitis restricted to the saphenectomy site.

In the meantime, body temperature rase over 39 ºC and local necrosis rapidly progressed with associated ecchymosis, vesicles and bullae all around the incision sites. As soon as possible, more aggressive antibiotic therapy was introduced with ceftazidime (1 g/*iv*/12 h) and clindamycin (400 mg/*iv*/8 h), with concomitant *iv* crystalloid infusions. Still, the patient had a high fever followed by general malaise and unclear confusion.

Regardless a newly introduced therapy, as time went by skin lesions became deeper, accompanied by microvascular thrombosis and large surrounding areas of necrosis including the groin and calf. Superficial fascia was patchy with yellowish-green colour followed by diffuse superficial muscle necrosis (Figures 1a and 1b). The first wound swab was sterile. At the moment, there was no imbalance concerning electrolytes, renal function, and glucose values. Blood and urine culture samples were tested, as well.

On the second day, extensive necrotic tissue excision and debridement removing the smelling parts of necrotic fascia and muscles were performed. Open wounds quoted bandage three to four times a day. All wound swabs taken daily were sterile. The whole leg was swollen, painful to touch, with limited mobility.

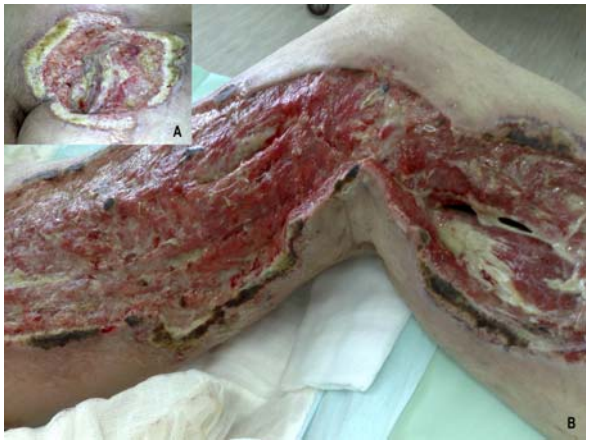

Fig. 1 – A) Massive necrosis of the left groin; B) thig and lower leg after femoro-popliteal bypass application.

The next few days, despite daily debridement, adequate wound toilets and vigorous antibiotic therapy, the patient was still highly febrile (38.2–40.2ºC) and extremely prostrated. The laboratory value of C-reactive protein was 150 mg/L (normal range < 10 mg/L), procalcitonin level > 0.25 ng/mL (normal level < 0.1 ng/mL), WBC 40×10^9/L, erythrocyte sedimentation rate > 100 mm/h. The immune status was examined in detail, and we were not endorsed more important immunity disorders.

The plain x-ray films of the left leg revealed no gas in the involved tissues, thus the third time, we changed antibiotic therapy, introducing the most powerful combination with vancomycin (10 mcg/mL) and imipenem (500 mg/*iv*/6 h). Sixteen days later the body temperature dropped down on subfebrile level (37.5 ºC). Computed tomography (CT) did not identify pus collections or gas in the examined leg muscles. Several tissue biopsies and smear cultures did not isolate causative bacteria clearly. Also, pathogenic bacteria were not isolated from blood or urine.

After 33 days of extensive wound packing and antibiotic therapy, the local wound status was significantly better (Figure 2).

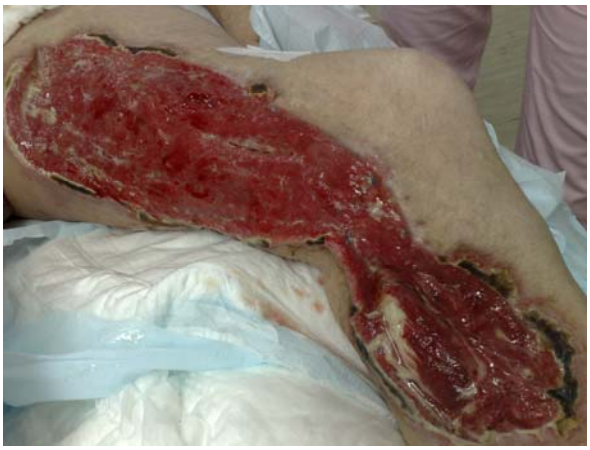

Fig. 2 – Local wound status 33 days following the surgery.

Inflammation factors had a significant tendency to fall. When the wound looked clean enough with pearly grey looking fascia, without new signs of necrosis, skin transplantation was performed using the Tierch method with skin transplant taken from the other thigh (Figure 3). The skin transplant was well received, with no signs of rejection, nor reinfection. In the later stages of the treatment, the patient went to the everyday treatment in a hyperbaric chamber.

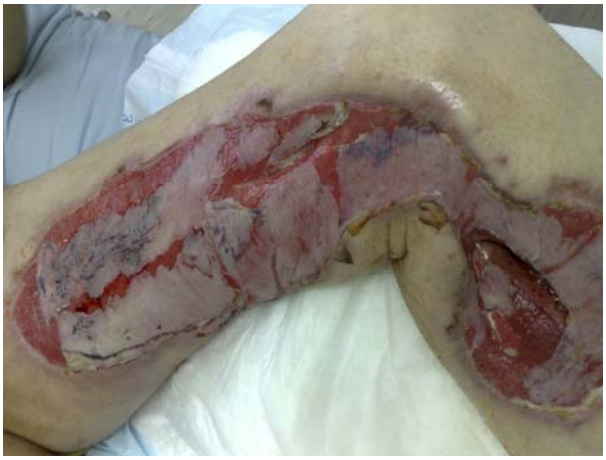

Fig. 3 – Local status after skin transplantaton (Tierch method).

The patient was dismissed from the hospital on the postoperative day 45 in good general condition with locally well healed and cicatrized wounds (Figures 4a and 4b). After a 6-month follow-up, local status of wounds was satisfactory (Figure 5). Although control CT angiography showed femoro-popliteal bypass occlusion, conservative treatment was indicated since the patient had claudication after 200 m and no signs of threating vascular ischemia were noted.

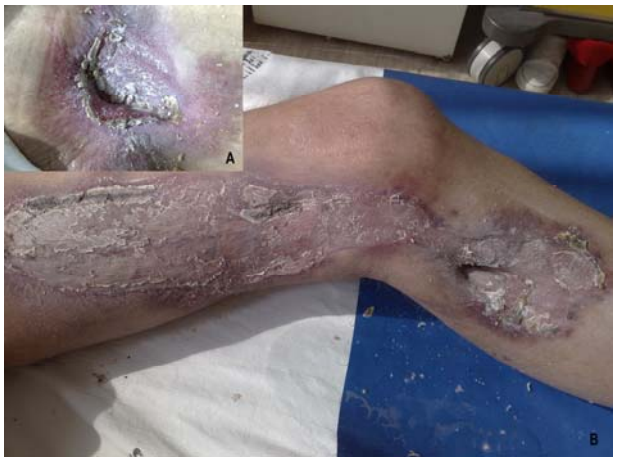

Fig. 4 – A) left groin; B) thigh and lower leg after 45 days.

Discussion

The NF is a life threatening bacterial infection with reported mortality rate of 25–60% [1–7]. The NF clinical manifestations range from a fulminant presentation to a subtle and insidious development [6–14]. The underlying pathogenesis of idiopathic NF is basically unknown, occurs in the absence of a known or identifiable etiologic factor and difficult to recognize in early stages.

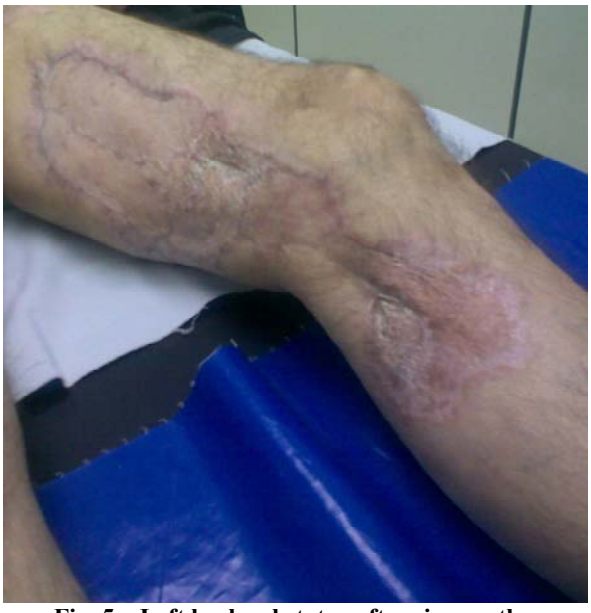

Fig. 5 – Left leg local status after six months.

On the other side, a form of the group A streptococcal invasive disease (GAS) could be NF. Sometimes, the culture of tissue involved by NF also yields a mixture of other non-anaerobic bacterial agents such as *Pseudomonas*, *Esherichia coli*, *Klebsiella*, *Clostridium* and *Staphylococci*. The underlying conditions or diseases of NF are certainly some kind of an immunological failure mostly provoked by diabetes mellitus, malignant disease, renal failure, malnutrition, radiotherapy, hepatic disease, chronic skin ulcers and others.

The nature of infection involves a rapid progression of tissue necrosis. Possible causes are unknown aerobic or anaerobic bacteria and the expected clinical course is individual. This paper illustrates the evolution of rapidly spreading massive NF following below-knee femoro-popliteal autologous vein graft bypass. We believe that the condition associated with NF was probably operative trauma. Early recognition of the problem, persistent wound debridement and intensive antibiotic therapy prevented possible loss of the limb and other severe life-threating postoperative complications such as respiratory failure and sepsis due to released toxins into the bloodstream.

When discussing NF, in addition to surgical debridement and antibiotic therapy hyperbaric oxygen therapy (HBOT) and vacuum-assisted closure (VAC) system might be very helpful [15–18]. Some authors [15] report that HBOT could significantly improve the effectiveness of necrotizing soft tissues infection healing by bactericidal and bacteriostatic effects and increasing oxygen supply up to the cellular level. In contrast to this, the authors [16] of a recently published review concluded that none of the patients with NF had benefit from HBOT. In the presented case, HBOT was introduced as soon as infection was verified, still its effect remained questionable having in mind aggressive antibiotic

therapy and persistent surgical debridement. Likewise, the use of VAC system has been reported with favorable outcome in managing non-healing limb wounds in patients suffering from acute NF [17, 18]. Unfortunately, at the time VAC system had not yet been introduced to our Clinic. When NF is identified, all of these mentioned treatment modalities should be applied to contribute to faster wound healing. As for the diagnosis, prompt investigation such as ultrasonography and CT might be very helpful in recognising the problem timely and start immediate surgical excision of all necrotic tissue until a full recovery of the wound [12–14].

Conclusion

The presented case suggests that immediate surgical debridement, wide spectrum antibiotics administration and consistent wound packing give satisfactory results in fulminant necrotizing fasciitis treatment.

Acknowledgements

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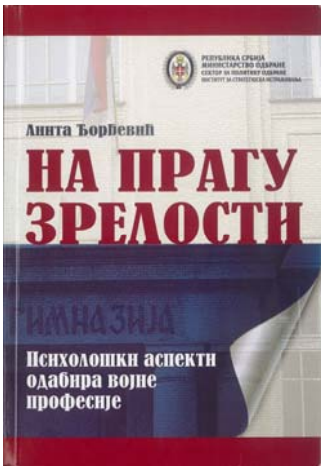
B O O K R E V I E W

Coming of Age: Psychological Aspects of Choosing the Military Profession



Origninal title: Na pragu zrelosti. Psihološki aspekti odabira vojne profesije
Author/autor: Anita Djordjević
Publisher: Republic of Serbia, Ministry of Defence, Defence Policy Sector, Strategic Research Institute, and Media Center "ODBRANA"
Published: Belgrade, 2014.

This book is the result of a significant scientific research that the author, MSc Anita Đorđević, did in the study on psychological aspects of choosing the military profession in adolescence. The problem of choosing the military profession is the access in a specific way, by researching determinants belonging to the family environment (parents' educational styles, patterns of family affective attachment and socioeconomic characteristics of the family). By highlighting the importance and the influence of the family as the primary environment for the development of the individual, the author addresses some of the psychological dispositions associated with the choice of the military profession, particularly stressing the importance of affective attachment.

In the empirical part of the study, the connection between dominant parental styles, patterns of family affective attachment, socioeconomic characteristics of families on one side, and professional maturity and choice of secondary school on the other side is examined. These are the basic questions explored in this book, supported by the results of the survey on a sample that consisted of 356 male subjects from the Military High School and civil high schools from Aleksinac and Niš.

It is a 167-page book and it has six chapters: Introduction (9–10), Theoretical Framework of the Problem (11–80), The Methodological Approach (81–92), The Results of Research and Discussion (93–144), The Conclusion in Serbian (145–148), French and English Language (149–155), and Bibliography (157–167).

The first chapter provides an overview of relevant theories of career choice and their social aspect, with the emphasis on contemporary (developmental) theories of professional choices. Theoretical concepts of the Ginsberg's and Super's theory, Theory of family climate, Holland's theory of vocational personalities and Attachment theory are discussed here. A special attention is payed to the Attachment theory (in the second chapter), with the subchapters stating the founder, nature and the origin of the term, the application of the basic assumptions in practice, as well as a review of the affective attachment theory and emotional development of personality, family affective attachment and organization. In the next (third) chapter the author deals with the family and parents' educational styles, function and role of the family through time, but also with the importance of the family for the education and development of personality. This section presents the two-dimensional model of parental care, with special emphasis on the emotional tone and parental control. The fourth chapter deals with the factors that influence the choice of profession. In the subchapters the author discusses about psychological gender differences and the impact of communities, families, parents' education, social background, school, skills, peer groups and mass media on the choice of profession. All the above is the theoretical basis for the empirical part of the research, which gives the book originality in the thematic sense. The fifth chapter tells about methodological approach to the examined problem. It has the necessary structure that follows the scientific research: the problem, significance, goal, variables, instruments, hypotheses, the survey sample and statistical data analysis. This section also describes the instrument for testing the professional maturity designed for this research, representing a significant contribution to Serbian science, i.e. to the enrichment of psychological measurement instruments in the field of vocational guidance and selection. The sixth chapter presents the results of the research and discussion through the subchap-

ters relating to the description of the patterns of family affective attachment, parental educational styles, socioeconomic characteristics of families of high school students and the relations between them, and the description of the connection between selection of the secondary school (military or civilian high school) and professional maturity. Within this chapter the author also presents the results of the relations between independent (patterns of family affective attachment, the dominant parental styles, socioeconomic characteristics of the families) and dependent variables (selection of high school and professional maturity). Discussion about the results obtained in the subheading on the connection between affective family attachment, parental educational styles, socioeconomic characteristics and selecting high school and professional maturity is also presented in this chapter. The last (seventh) chapter presents the concluding observations, i.e. the author's reflections on the most important results of the research, emphasizing once again the role and importance of the family for the professional development of adolescents. The very end of the book offers Conclusion in English and French, as well as a Bibliography (166 references).

As the most important conclusion from the book Coming of Age: Psychological Aspects of Choosing the Military Profession we underline: Military High School students come from families with warm emotional climate and secure patterns of family affective attachment, which is important for the formation of emotionally and socially mature personality. It is also the important precondition for willingness to explore environment and search for the optimal professional direction of students.

The book is useful for experts in the field of professional orientation and selection because it provides them with guidance in their work. It facilitates understanding of important aspects of life, such as the selection of future profession in adolescence. It is also a significant contribution to the field of psychology that deals with the choice of profession and professional interests. Also, the book is important for parents and adolescents (current and future high school students) because it provides them with the answer to the question: What are the determinants and specifics concerning the family associated with the selection of military *versus* civilian education?

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C O R R I G E N D U M

In the article by *Vesna Bjegović-Mikanović, Nebojša Lalić, Helmut Wenzel, Ružica Nikolić-Mandić, Ulrich Laaser.* Continuing medical education in Serbia with particular reference to the Faculty of Medicine, Belgrade. Vojnosanit Pregl 2015; 72(2): 160–8. (DOI:10.2298/VSP1310216060B), on the page 168, after the data of receipt, revision and acceptance of the article, should be added:

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Primeri referenci:

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

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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 fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

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.

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

Skraćenice i simboli

Koristiti samo standardne skraćenice, izuzev u naslovu i apstraktu. Pun naziv sa skraćenicom u zagradi treba dati kod prvog pominjanja u tekstu.

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

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Poreski identifikacioni broj (PIB) za ustanove	
Mesto	
Ulica i broj	
Telefon / telefaks	

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1. Lično. Dokaz o pretplati dostavljam uz ovu prijavu.
2. Za pripadnike MO i Vojske Srbije: Dajem saglasnost da se prilikom isplate plata u Računovodstvenom centru MO iz mojih prinadležnosti obustavlja iznos mesečne rate (pretplate).
3. Virmanom po prijemu profakture.

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