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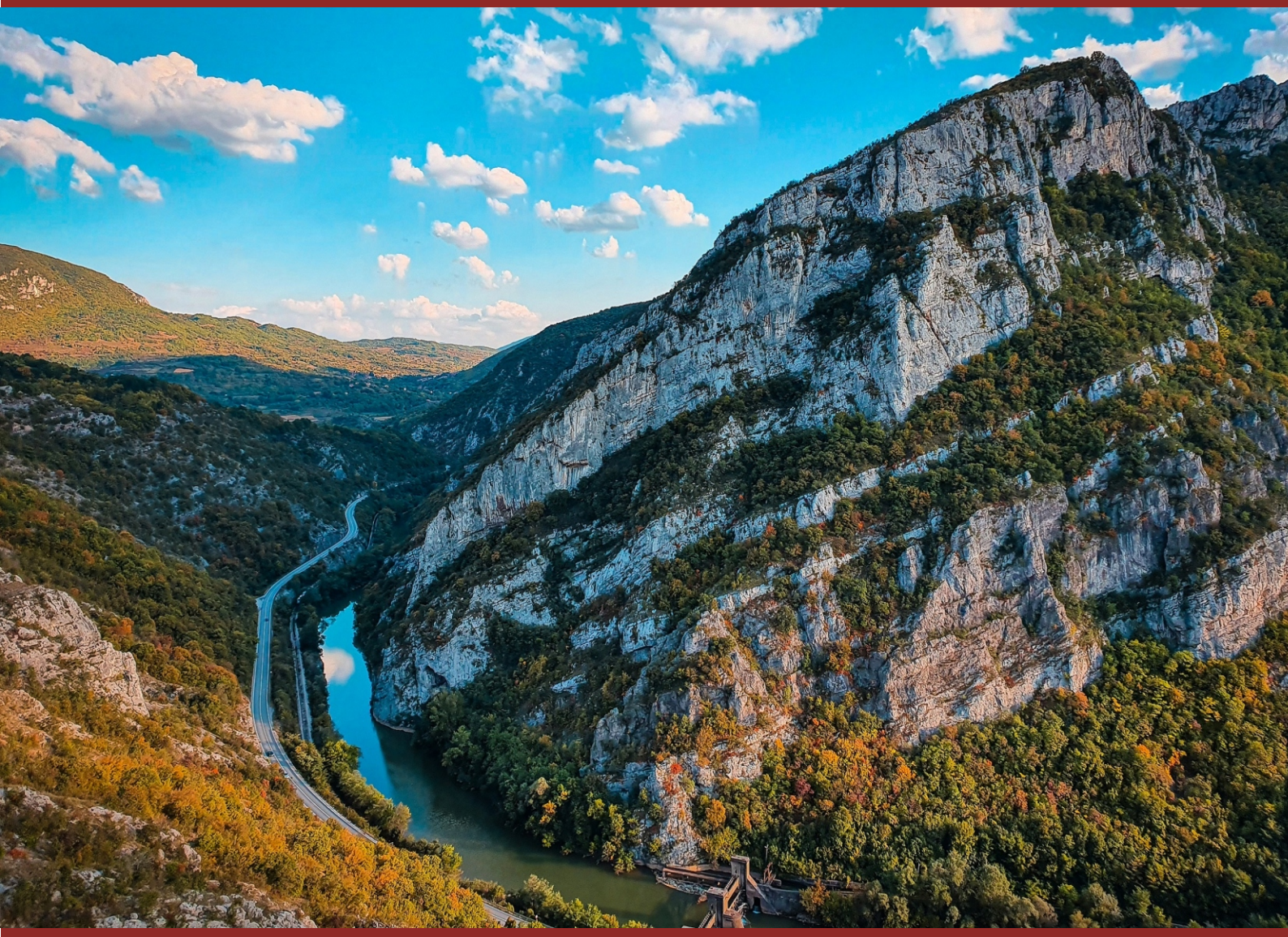

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AN IMMUNOHISTOCHEMICAL ANALYSIS OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 1 IN HIGH GRADE T1 BLADDER CANCER WITH CONCOMITANT CARCINOMA IN SITU

Ana Ristić-Petrović[1], Dragana Stokanović[2], Dane Krtinić[2,3], Milena Potić-Floranović[1], Slavica Stojnev[1], Ljubinka Janković-Veličković[1]

Vascular endothelial growth factor receptor 1 (VEGFR1) reduces the angiogenic activity of vascular endothelial growth factor (VEGF), acting like decoy receptor for VEGF and limiting its availability for genuine angiogenic receptors. The purpose of this study was to establish the significance of VEGFR1 expression in high grade T1 (HGT1) bladder cancer with concomitant carcinoma in situ (CIS) and to determine possible immunohistochemical marker helpful in the follow-up of "unpredictable" HGT1 bladder cancer patients. The analysis included 137 HGT1 bladder cancer samples. Concomitant CIS was diagnosed in 21 (15.33%) of these patients. Sections of 137 formalin-fixed, paraffin-embedded materials were incorporated in tissue microarrays and then stained with a rabbit monoclonal antibody against VEGFR1 (N-term: Y103/-Epitomics, diluted 1:250). Immunohistochemical reaction was scored as following: negative if ≤ 10% of cells were stained and positive if > 10% were stained. We considered both membranous and cytoplasmic expression and staining intensity was scored using a scale of 0 to 3 (0, no staining; 1, weak; 2, moderate; and 3, intense). After a mean follow-up of 50 months, in 137 patients diagnosed with HGT1 urothelial bladder cancer, we found that patients who had concomitant CIS had worse overall survival ($p < 0.05$), furthermore, those tumour samples had weakly expressed VEGFR1 ($p < 0.05$). Patients with positive VEGFR1 had longer disease-free ($p < 0.01$) and overall survival ($p < 0.01$). Present investigation has revealed that the estimation of VEGFR1 expression could be diagnostic supplement, selecting the HGT1 bladder cancer patients that would require more intensive follow-up, especially if accompanied with CIS.

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Key words: *angiogenesis, VEGFR1, bladder cancer, carcinoma in situ*

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Introduction

Bladder cancer is the ninth most common cancer worldwide, with an estimated 430 000 new cases per year (1). Approximately 75% of bladder cancers are non–muscle invasive (NMIBC), and of these, roughly 20% to 25% invade the lamina propria (T1). Fifty percent of NMIBC patients who are treated with transurethral resection (TUR) have a recurrence of the disease and 5 % to 25% of these patients will progress to muscle-invasive disease after repeated recurrences (2). The most difficult NMIBC category for treatment planning is high-grade T1 (HGT1) bladder cancer. Treatment with bacillus Calmette-Guerin (BCG) risks recurrence, progression and metastases, however, may preserve the bladder. Cystectomy may offer the best opportunity for cure, but is associated with morbidity and a risk of mortality, and considering the heterogeneous nature of HGT1 it may constitute unnecessary over-treatment. The dilemma facing the urologist is how to treat these tumours the best in a timely manner, so that the chances of bladder preservation and cancer control are maximised, while the risks of over-treatment with radical intervention are minimised (3).

Another obstacle in perceiving properly HGT1 patients is the possible presence of carcinoma in situ (CIS) in surrounding mucosa (urothelium). It is often not possible to distinguish whether the tumour is recurrent due to aggressive tumour biology and implantation of floating cancer cells or due to evolution of non diagnosed in situ lesion. Carcinoma in situ (CIS) of the bladder is a small, flat, high grade,

confined to the mucosa, lesion and it can be easily overlooked in the primary procedure (4). The diagnosis of CIS cannot be made with imaging methods. Cytology is useful, particularly as an adjunct to cystoscopy, if HG/CIS malignancy is present. Positive voided urinary cytology can indicate a urothelial tumour anywhere in the urinary tract; negative cytology, however, does not exclude the presence of a tumour (5). CIS is often multifocal and can occur in the bladder but also in the upper urinary tract, prostatic ducts, and prostatic urethra (6). CIS can present as an area indistinguishable from inflammation, or it may not be visible at all (7). For this reason, the strategy of taking biopsies from abnormal urothelium and random/mapping biopsies from normal-looking mucosa (trigone, bladder dome, and right, left, and anterior and posterior bladder wall) is recommended (8). Some studies have reported worse prognosis in concurrent CIS and T1 tumours compared with primary CIS, in extended CIS (9), and in CIS in the prostatic urethra (10). Relative indications for cystectomy include multifocal disease, associated CIS, T1 disease on repeat resection, deep T1 disease abutting the muscle and poor patient compliance. The only precystectomy prognostic predictor of recurrence is the presence of concomitant CIS (11, 12).

The purpose of this study was to establish the significance of vascular endothelial growth factor receptor 1 (VEGFR1) expression in HGT1 bladder cancer with concomitant carcinoma in situ and to determine possible immunohistochemical marker helpful in the follow-up of "unpredictable" HGT1 bladder cancer patients.

Materials and methods

We studied 137 patients with stage T1 urothelial bladder cancer who had undergone transurethral resection (TUR). All cases were diagnosed at the Institute of Pathology, Faculty of Medicine, Niš. The analysis included 137 HGT1 bladder cancer samples. Concomitant CIS was diagnosed in 21 (15.33%) of these patients. The mean patients' age was 68.31 ± 8.92. There were 116 male (84.7%) and 21 female patients (15.3%).

The histological sections were processed from tissue fixed in 10% formalin by standard techniques, and stained with haematoxylin and eosin (H&E). H&E-stained slides were used to assess histological grade (low and high grade), pathologic stage (pT), growth of tumour (papillary/solid), and the presence of CIS, cystitis and squamous differentiation within the tumour, according to the WHO criteria (13). Sections from 137 formalin-fixed, paraffin-embedded materials were incorporated in tissue microarrays and then stained with a rabbit monoclonal antibody against VEGFR1 (N-term: Y103/Epitomics, diluted 1:250). Formalin-fixed, paraffin-embedded tissue samples were deparaffinized in xylene for 10 min, followed by washing in decreasing concentrations of ethanol (95%, 75%, 50%), each for 2 min. After deparaffinization, antigen retrieval was performed by boiling the slides in 0.01 M citrate buffer, pH 6.0, in a microwave for 10 min. The slides were then applied on a semi-automatic IHC diagnostic system (Ventana Inc.) and IHC staining was performed using antigen-specific antibody, as indicated above. Immunohistochemical reaction was scored as follows: negative if ≤ 10% of cells were stained and positive if > 10% were stained. We considered both membranous and cytoplasmic expression and staining intensity was scored using a scale of 0 to 3 (0, no staining; 1, weak; 2, moderate; and 3, intense) according to our previous investigation (Figure 1) (14).

All statistical analyses of the obtained data were performed using Statistical Package for Social Sciences (SPSS version 20.0, Chicago, IL, USA).

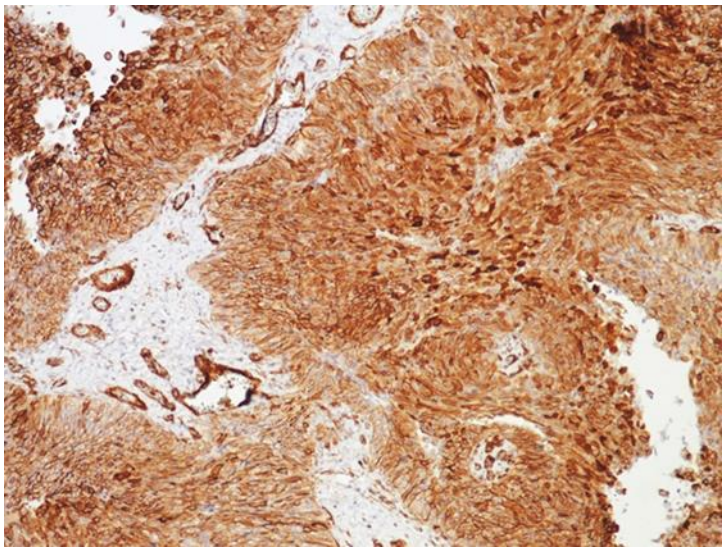


Figure 1. Representative microphotograph of strong, diffuse cytoplasmic and membranous VEGFR1 expression in HGT1 tumour cells and endothelial cells

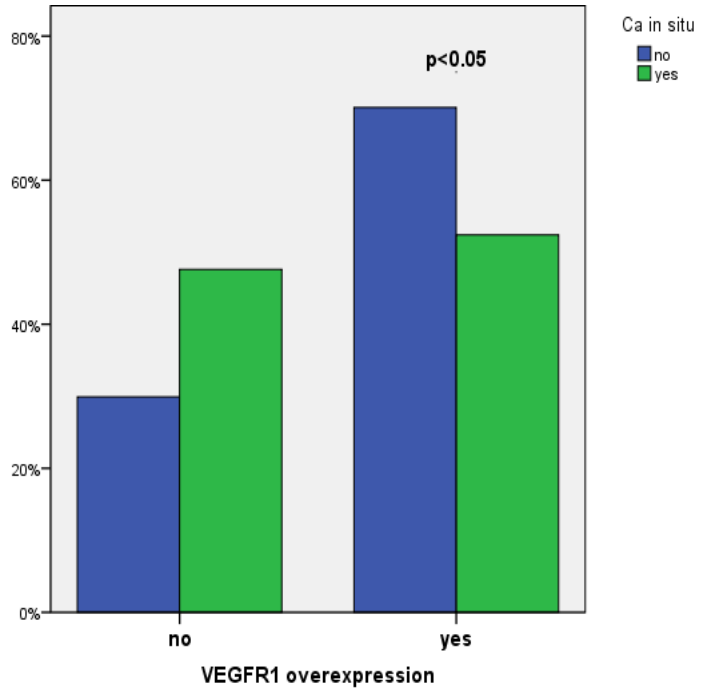
For group comparisons, parametric Student's t-test was performed. The methods of Cox-regression and Kaplan-Meier curves were used to determine survival predictors. The results were considered statistically significant if $p < 0.05$.

Results

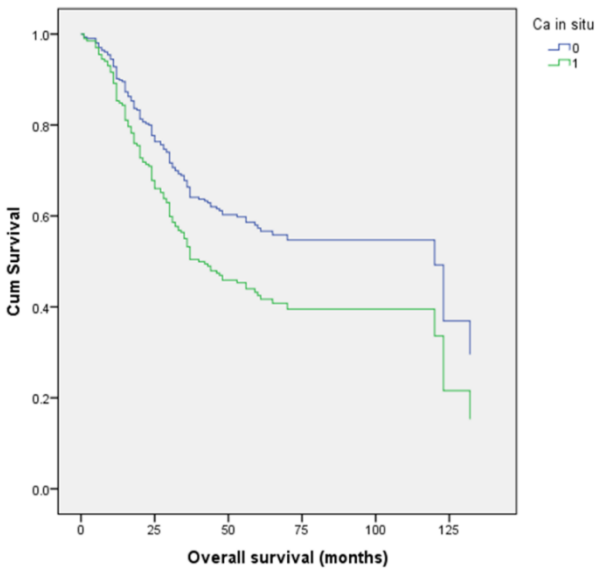
Positive VEGFR1 staining was observed in 11 (52.4%) CIS samples and in 86 (74.1%) samples without CIS (Graph 1), showing that CIS presence in HGT1 tumours was associated with low expression of VEGFR1 ($\chi^2 = 4.072$, $p < 0.05$).

After a mean follow-up of 50 months, mortality was 58.4% (80 patients), and the incidence of relapse was 39.4% (54 patients).

Tumour samples associated with 50 month survival expressed reduced VEGFR1 ($\chi^2 = 4.365$, $p < 0.05$), but no statistically significant association was found between CIS presence and recurrence during the follow-up period ($\chi^2 = 0.000$, $p < 1.000$).

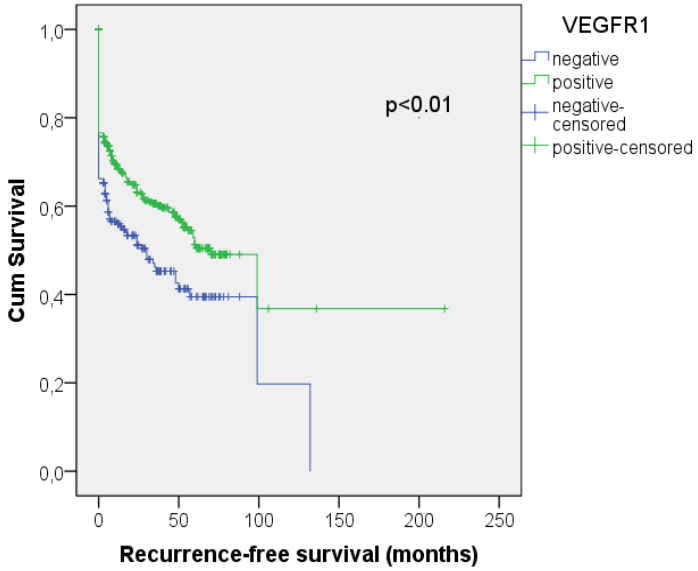


Graph 1. VEGFR1 expression in HGT1 bladder cancer with concomitant CIS

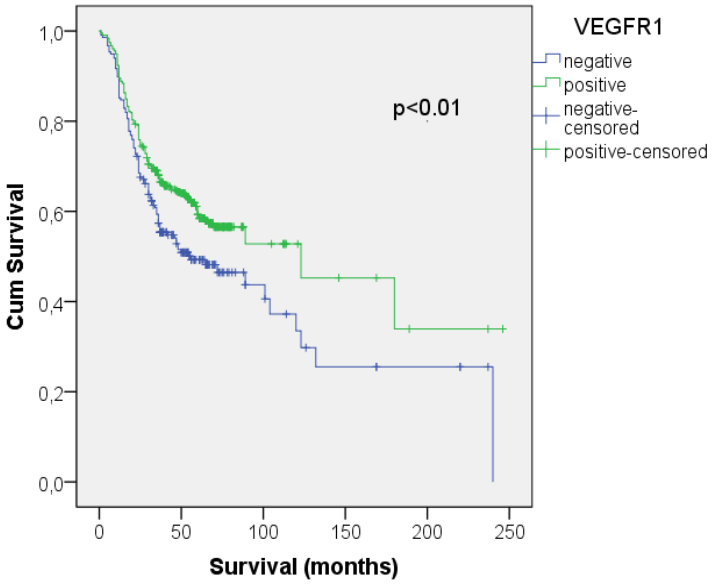


Graph 2. Overall survival in HGT1 bladder cancer associated with CIS

On the contrary, when taking time into the account, we found that patients who had concomitant carcinoma in situ had worse overall survival (HR = 1.537 (1.018-2.320), χ^2 = 4.255, p < 0.05) (Graph 2). Patients with positive VEGFR1 had longer disease-free (HR = 0.663 (0.508- 0.865), χ^2 = 9.305, p < 0.01) (Graph 3) and overall survival (HR = 0.717 (0.565-0.911), χ^2 = 7.258, p < 0.01) (Graph 4).



Graph 3. Recurrence-free survival in VEGFR1 positive HGT1



Graph 4. Overall survival in VEGFR1 positive HGT1

Discussion

Without any treatment, approximately 54% of patients with CIS will progress to muscle-invasive disease (7). The most important prognostic factors for progression are the T category, grade, and the presence of CIS, factors that represent the biological aggressiveness of the disease, and the most reliable

in patients with HGT1 tumours is the presence of concomitant CIS (15). In HGT1 patients without CIS, the probability of progression is 10% in one year and 29% in five years; in HGT1 patients with CIS, the corresponding numbers are 29% and 74%, respectively. Patients with deep lamina propria invasion (T1b/T1c) should be managed more aggressively, especially those with associated CIS (15). For all cases of newly diagnosed HGT1 transitional cell carcinoma (TCC), a secondary TUR 4–6 weeks after the primary TUR is strongly recommended (16). Repeated resection of the previously resected site 4–6 weeks after the initial resection (along with any other cystoscopically suspicious areas) will provide more accurate staging information. This is particularly important because the probability of understaging a HGT1 tumour ranges from 20% to 70%, depending on the presence of muscularis propria in the sample and concomitant CIS obscured by inflammation (17). Molecular markers such as p53, Ki-67, NMP22, and Cox-2 have some promise; however, they have not been sufficiently validated to be used day to day at this time (18). Vascular endothelial growth factor (VEGF), an important protein for triggering and regulating angiogenesis, effects cellular responses by binding to VEGF receptors on the cell surface. Vascular endothelial factor receptor 2 (VEGFR2) mediates most of the known cellular responses to VEGF. VEGFR1 negatively regulates VEGFR2 via high-affinity binding of VEGF, which consequently becomes unavailable for VEGFR2 (19). VEGFR2 expression has been correlated with increasing disease stage and tumour invasion into the muscle, and may be an important determinant for prediction of nodal metastasis in TCC patients (20). Several studies pointed that expression of VEGF and its receptors VEGFR1/VEGFR2 is associated with invasiveness of bladder cancer (21). In this study we found that tumour samples with VEGFR1 overexpression were associated with better recurrence-free and better overall survival, and, explanation lies in decreased angiogenesis. By binding VEGF, soluble VEGFR1 reduces the angiogenic activity of VEGF, acting like decoy receptor for VEGF and limiting its availability for genuine angiogenic receptor VEGFR2. Although tumour cells produce tremendous amounts of VEGF, angiogenic inhibitor VEGFR1 will block angiogenesis disabling the sprout of microvessels and indirectly further nourishment and spreading of tumour cells (14, 22). When observed in tumour associated macrophages, VEGFR1 expression can strongly indicate metastatic potential of the tumour, since VEGFR1 in tumour associated macrophages is required for metastatic tumour outgrowth. This was demonstrated in the breast cancer, but not in TCC (23). However, this new concept of microenvironmental regulation of metastasis through immune cells that express a high level of VEGFR1 points that VEGFR1 is the marker beyond the angiogenic pathway.

Conclusion

Present investigation has revealed that the estimation of VEGFR1 expression could be diagnostic supplement, selecting the HGT1 bladder cancer patients that could require more intensive follow-up, especially if accompanied with CIS. Vascular endothelial growth factor receptor 1 is potentially reliable marker and could be a trustful prognostic predictor of surveillance and recurrence of the disease. Diverse angiogenic pathways occurring in tumours with and without accompanying CIS lead to unequivocally different expression of VEGFR1. Increased VEGFR1 expression was associated with HGT1 tumours without CIS, and longer disease-free and overall survival and vice versa, HGT1 tumours accompanied with CIS were associated with decreased VEGFR1 expression, and worse overall survival in those patients.

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The authors declare no conflict of interest.

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IMUNOHISTOHEMIJSKA ANALIZA RECEPTORA 1 ZA VASKULARNI FAKTOR RASTA KOD T1 KARCINOMA MOKRAĆNE BEŠIKE VISOKOG GRADUSA I KONKOMITANTNIM *IN SITU* KARCINOMOM

Ana Ristić-Petrović[1], Dragana Stokanović[2], Dane Krtinić[2,3], Milena Potić-Floranović[1], Slavica Stojnev[1], Ljubinka Janković-Veličković[1]

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Receptor 1 za vaskularni faktor rasta (VEGFR1) smanjuje angiogenetsku aktivnost vaskularnog faktora rasta (VEGF), ponašajući se kao lažni receptor za VEGF i ograničavajući njegovu dostupnost za prave angiogene receptore. Cilj rada je da se ustanovi značaj VEGFR1 ekspresije kod T1 karcinoma mokraćne bešike visokog gradusa (HGT1) sa konkomitantnim *in situ* karcinomom (CIS) i da se odredi imunohistohemijski marker koristan za praćenje "nepredvidivih" HGT1 bolesnika. Analizirano je 137 HGT1 karcinoma mokraćne bešike. Konkomitantni CIS dijagnostikovan je kod 21 bolesnika (15,33%). 137 parafinskih uzoraka uklopljeno je u tkivne mikroareje i obojeno zečjim monoklonalnim antitelom na VEGFR1 (N-term: Y103/Epitomics, razblaženje 1 : 250). Imunohistohemijska reakcija procenjivana je na sledeći način: negativna ekspresija ukoliko je obojeno ≤ 10% ćelija, a pozitivna ukoliko je obojeno > 10% ćelija. Procenjivana je i membranska i citoplazmatska ekspresija i skorirana prema skali od 0 do 3 (0 - nema bojenja; 1 - slabo bojenje; 2 - umereno; i 3 - intenzivno). Nakon praćenja u trajanju od 50 meseci, utvrđeno je da bolesnici sa HGT1 karcinomom mokraćne bešike i konkomitantnim CIS imaju ukupno manju stopu preživljavanja ($p < 0,05$), kao i manju ekspresiju VEGFR1 ($p < 0,05$). Bolesnici sa pozitivnim VEGFR1 imali su duži vremenski period bez recidiva ($p < 0,01$) i duže ukupno preživljavanje ($p < 0,01$). Istraživanje je pokazalo da procena VEGFR1 ekspresije može biti dijagnostička dopuna za odabir bolesnika sa HGT1 urotelnim karcinomom mokraćne bešike, kojima je neophodno intenzivnije praćenje, posebno ukoliko je prisutan i CIS.

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Ključne reči: *angiogeneza, VEGFR1, karcinom mokraćne bešike, carcinoma in situ*

Original article

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ADAPTED MILK FORMULAS IN THE NUTRITION OF CHILDREN

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From the evolutionary perspective, human milk represents a biological standard, that is, a gold standard in terms of nutrition for newborn babies, while adapted formulas represent an effective replacement for the nutrition of infants.

Based on a survey, the aim of this paper was to examine the extent to which adapted milk formulas are used as substitutes for human milk in infant nutrition, as well as the reasons for using and selecting adaptive milk formulas.

A total of 309 respondents, divided into three age categories, were interviewed: those aged from 20 to 25; from 25 to 30; and those over 30.

Of the total number of respondents, 62.13% fed their infants with human milk, 29.13% used adapted milk formulas, while 8.74% fed infants with human breast milk with the addition of adapted milk formulas. In our data analysis, the average values of baby weight were obtained, depending on whether they were fed with human milk or adapted milk formulas after three months and it was found that there was a statistically significant baby weight difference between the women who were breastfeeding and those who fed the self-administered dairy formulas ($p < 0.05$). When asked about the source of the recommendation on the use of adapted milk formulas, the largest number of respondents answered that they got the recommendation from their doctors (46.15%), while as the reason for use of adapted milk formulas instead of human milk, the majority of respondents (64.10%) stated that the reason was the absence of milk secretion.

The research shows that the infants fed with adaptive dairy formulas gain weight more quickly than breastfed infants, which is a tendency that can serve as the hypothesis that artificially fed children will have obesity problems later on in life. The benefit of the study itself was that the decision of using adaptive dairy formulas by the examined women regardless of their level of education was a result of consultation with their pediatricians and that fact gives special importance to the proper development of infants.

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Key words: *adapted milk formulas, nutrition, infants*

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Introduction

From the evolutionary perspective, human milk represents a biological standard, that is, a gold standard in terms of nutrition for newborn babies. The health benefits of human milk have been documented in many studies that have shown that human milk reduces the risk of developing infections, allergies, asthma, diabetes, obesity, cardiovascular diseases and various carcinomas both in childhood and in adulthood (1). A recent study has confirmed that human milk alleviates the onset of late metabolic disorders and provides protection against obesity and type 2 diabetes, while the World Health Organization recommends breastfeeding as mandatory in the first six months of an infant's life (2). The American Academy of Nutrition and Dietetics confirms that breastfeeding provides optimal nutrition and health care in the first six months, and that breastfeeding with a complementary diet from 6 to 12 months of age is an ideal nutritional approach for infants (3). In addition to its nutritional benefits, breastfeeding is convenient and inexpensive and it also represents an inseparable bond between the mother and the baby. The decision on breastfeeding is personal and it is often influenced by many factors. In many situations, breastfeeding is impossible or inadequate, so mothers decide for another type of infant nutrition. On a global level, only 38% of babies are exclusively breastfed with human milk (4). Human milk has a unique chemical and biochemical composition. It consists of water, proteins, lipids, carbohydrates, mineral substances and vitamins. Water is the main

component, and it makes about 87%. Lipids make up about 3.8% and provide 50% of the total energy value of milk. The protein content is about 10%, whereas about 70% of the protein is provided by whey protein (5). Milk changes throughout the day, as well as throughout the entire lactation period, and therefore we may distinguish between colostrum, transitional and mature mother's milk. Colostrum is the first food to be given to a baby, it is secreted in small quantities and is rich in immune components such as IgA, lactoferrin, leukocytes, as well as epidermal growth factor (6, 7, 8). Colostrum also contains relatively low lactose concentrations, indicating that its primary functions are immunological and trophic rather than nutritional. The levels of sodium, chloride and magnesium are higher, and the levels of potassium and calcium are lower in colostrum than in transitional and mature milk (7, 8). Transitional milk starts forming from the fifth day to two weeks after giving birth, whereas mature milk is considered to be the milk that is formed from the third week after labor. Human milk contains various bioactive factors (living cells, antibodies, cytokines, growth factors, oligosaccharides, hormones). Bioactive factors are the elements that affect biological processes and thus affect the bodily functions and ultimately our health. Mother's milk contains various growth factors that have a significant effect on the intestines, blood vessels, nervous and endocrine system of infants (9). Human milk contains many different living cells (white blood cells, stem cells). In early lactation, the infant may receive up to 10 white blood cells from the mother every day (10). Secretory IgA is the most important class of antibodies in breasts and its role is to protect the mucosal surface. There are many other anti-infective proteins, such as lysosomes and lactoferrin. The composition of mature milk is not constant, but it changes depending on the time of day, length of the breastfeeding and needs of an infant. If the infant is fed by human milk in large quantities, it meets the needs for water and, in that way, the kidneys are relieved and the baby does not retain excess fluid. At the end of breastfeeding milk is different in color, much brighter due to high fat content (9). While science has yet to discover the functions of all bioactive components, it is safe to say that breastfeeding is more than just nutrition.

Adapted formulas represent an effective replacement for the nutrition of infants. Although the production of an identical product is not possible, efforts have been invested to make a product that would be similar to human milk by its performance and which would allow normal growth and development. Cow or soy milk is most commonly used as the base, and other ingredients are added to complement the composition and make the profile of the milk that is most similar to human milk. Vitamins, minerals, fatty acids – arachidonic (AA) and docosahexaenoic (DHA) – as well as probiotics and other mostly genetically engineered compounds are usually added to adapted formulas. Providing optimum nutrition for infants is very important because the consequences of inadequate nutrition can hardly be overestimated (11). Children fed on adapted milk formulas have lower immune protection due to the lack of immunological factors provided by colostrum, as well as other bioactive factors that help protect the child during the first two years of its life when the immune and nervous systems are poorly developed (1). Apart from nutritive, the advantages of adapted milk formulas are also optimal levels of iron, iodine and vitamin D, the content of easily digestible proteins, the content of prebiotics that have a positive effect on the intestinal flora and the appropriate ratio of whey and casein proteins. The formulas themselves maintain an optimal concentration of nutrients as well as an optimal amount of essential fatty acids necessary for the proper development of infants.

Research aim

Based on a survey, the aim of this paper was to examine the extent to which adapted milk formulas are used as substitutes for human milk in infant nutrition, as well as the reasons for using and selecting adaptive milk formulas.

Methodology

Our descriptive study included the patients 20 to 40 years of age, who were randomly selected in the DONA FARM pharmacy in Niš during 2017. and who agreed to be interviewed. The survey was conducted using an anonymous questionnaire containing open-ended and closed-ended questions. The respondents completed it independently in the presence of a pharmacist, who was available in case of difficulties in understanding certain concepts. The respondents were informed about the objectives of the research, and the percentage that declined to participate in the study was 10%. Three hundred and nine correctly completed questionnaires were selected and they were the subject of further analysis. The study was conducted in accordance with the Declaration of Helsinki (12). The questionnaire consisted of several parts: socio-demographic characteristics of mothers (age, educational level), questions about the length of breastfeeding, as well as the use of adapted milk formulas. All the questions in the questionnaire offered the answers from among which the respondents had to choose (closed questions), except for the question on the reason for use of adapted milk formulas.

The statistical analysis of the data was performed by using the SPSS 20 software. A statistically significant difference was the value of $p < 0.05$, using the Student's T-Test.

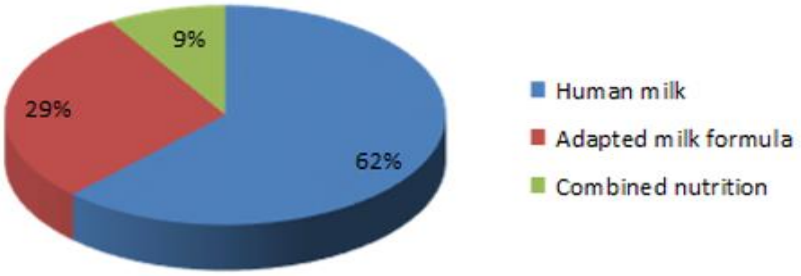
Results

From the total number of respondents, 192 (62.13%) fed infants with human milk, 90 (29.13%) used adapted milk formulas, while 27 (8.74%) fed infants with human breast milk with the addition of adapted milk formulas (Graph 1).

The demographic characteristics of the respondents are summarized in Table 1. In the upper part of the table, it can be clearly seen that the largest

number of respondents, 165 (53.4%), were over 30 years of age. In the lower part of the table, the interviewed patients were categorized according to their education. Most of them had secondary education – 186 (60.19%).

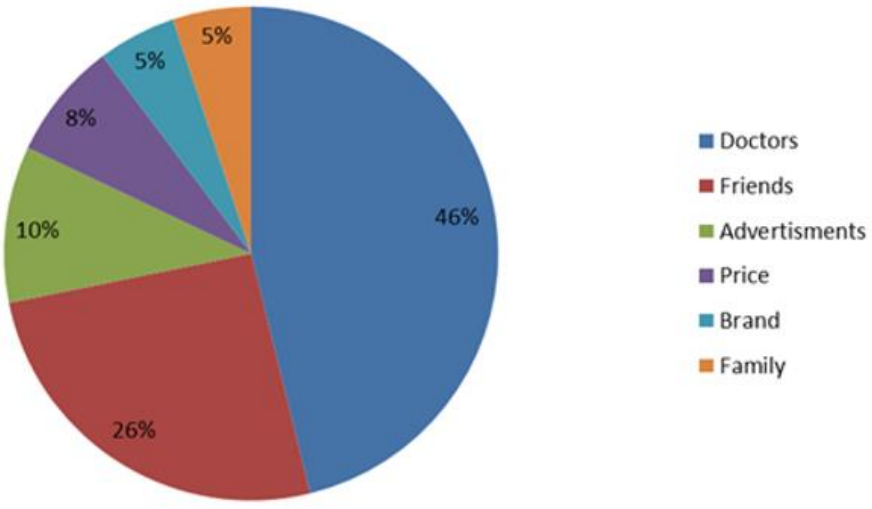
When asked about the source of the recommendation on the use of adapted milk formulas, the largest number of respondents answered that they got the recommendation from the doctor 54 (46.15%), whereas a somewhat smaller number of respondents got the recommendation from a friend 30 (25.64%). Some of the respondents were influenced by advertising 12 (10.26%), some were influenced by the price of adapted milk 9 (7.69%), whereas only a small number of respondents were influenced by the milk's brand 6 (5.13%), as well as by their family 6 (5.13%) (Graph 2).



Graph 1. Infant nutrition model

Table 1. Demographic characteristics of respondents

WOMEN	AGE			TOTAL
	20 - 25	25 – 30	> 30	
	24	120	165	309
EDUCATION	AGE			TOTAL
	20 - 25	25 – 30	> 30	
Primary	4	3	5	12
Secondary	11	83	92	186
Post-secondary	6	19	46	71
Higher	3	15	22	40



Graph 2. Factors that influence the purchase of an adapted milk formula

When asked about the brand of adapted milk they used, most mothers said that they used Novalac brand 36 (30.77%). On the other hand, Humana was used by 27 (23.08%), Aptamil by 24 (20.51%), Nestle by 15 (12.82%), Hipp by 9 (7.69%), whereas a much smaller number of respondents decided to use Bebelac 6 (5.13 %). When asked about the reason for use of adapted milk formulas instead of human milk, the majority of respondents 75 (64.1%) said that the reason was the absence of milk secretion, while a smaller number of respondents 27 (23.08%) said that the reason was insufficient milk secretion, while 15 (12.82%) did not want to state the reason. The largest number of respondents bought the milk formula in a pharmacy, as many as 90 (76.92%), a slightly lower number in a super market, 27 (23.08%), while no one responded positively to the question about online shopping. One of the questions in the survey was whether the patients had used some medications on their own initiative during the pregnancy. The answers we received from the respondents were that they most often used analgesics on their own initiative due to headache, stomachache, toothache and in cases of cold and flu.

Discussion

According to the results of our research, the respondents with a higher level of education and older age have a more positive attitude towards breastfeeding, which can be explained by a more responsible attitude toward the condition in which they are. This study showed that a large number of women (62.13%) breastfed their children with human milk. Many studies have shown that educated women have a higher awareness of breastfeeding and that they support breastfeeding more than women with lower levels of education (13). The promotion and support of breastfeeding and the awareness of the community as to the implementation of breastfeeding recommendations represent a significant step in our health care system. The World Health Organization (WHO) and United Nations International Children's Emergency Fund (UNICEF) recommend that breastfeeding should be continued until the second year of life, and even later. A particular emphasis on the continuation of breastfeeding is placed in the countries where hygiene is poor and where infection rates are high (14, 15) The American Academy of Pediatrics recommends breastfeeding for at least 12 months, while European countries, such as Denmark and the UK, recommend breastfeeding for 6 months (16). According to the UK guidelines, if there is no possibility of breastfeeding, the most appropriate option for the nutrition of babies is an adapted formula for babies (17, 18). Adapted formulas are an effective substitute for human milk and they are formulated in such a way as to imitate the nutritional composition of human milk. All adapted formulas must meet certain standards that would affect the normal growth and development of infants (19). The production process itself is highly regulated and supervised in order to meet the national and international quality criteria (20, 21). Over the next five years, baby food in the form of adapted formulas is expected to be the fastest growing category of food products (22). Formulas for babies must have an adequate amount of water, carbohydrates, proteins, fats, vitamins and minerals. The composition of the formula for babies is strictly regulated and each manufacturer must follow the prescribed guidelines. The required range of nutrients must be maintained throughout the entire shelf life of the product (23, 24). For amino acids, it is only allowed to add L-shaped amino acids, while D-shapes are not allowed as they can cause D-acidosis (25). Fructose should be avoided due to fructose intolerance, as well as hydrogenated fats and oils that are not generally permitted in the product. The World Health Organization (WHO) has sent an announcement that cow's milk and goat's milk should not be used in infants due to various harmful effects to their health. In addition to the WHO guidelines, local agencies from different countries control and follow the regulations on children's formulas, including the requirements for quality of production practices in their countries. From the manufacturers' perspective, it is in their best interest to continuously improve their products in order to obtain a formula that would be identical to human milk. There are three main classes of milk for babies: formulas based on cow's milk, soybeans and specialized formulas (23, 24). According to the American Academy of Pediatrics, young children of one year of age should not be fed with raw, unmodified or unpasteurized cow milk as a substitute for human milk or formula (26). Recent studies have shown that high protein content in the formula is associated with high body weight in childhood, which can lead to a 20% increased risk of obesity later in life (27), whereas cow's milk is one of the most common causes of food allergy (28). Soybean protein formulas are the effective options for infants with galactosemia or congenital lactose deficiency, and they can help with colic allergies. However, it rarely happens that children allergic to milk are also allergic to soy (29). Soy-based products should not be used in infants under the age of six months with food allergies (30). Further, the addition of probiotics to the formula represents a key strategy for reducing the incidence and severity of diarrhea in infants. Specialized formulas are prescribed by the doctor because they are intended for nutrition when there are certain problems (31).

Conclusion

While science has yet to discover the functions of all the bioactive components of both human milk and adapted milk formulas, it can be said with certainty that adapted milk formulas are considered the best choice in the cases of insufficient secretion of mother's milk, as well as a supplement to breastfeeding of babies that do not progress adequately in terms of their body weight. This research showed that the decision on the use adaptive milk formulas was the result of consultation with the pediatricians, and this fact gives a special contribution to the appropriate development of infants.

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ADAPTIRANE MLEČNE FORMULE U ISHRANI DECE

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Iz perspektive evolucije, humano mleko predstavlja biološku normu, odnosno zlatni standard kada je u pitanju ishrana novorođene dece, dok se adaptirane formule koriste kao efikasna zamena za ishranu odojčadi.

Cilj ovog rada bio je da se na osnovu ankete ispita u kojoj meri se adaptirane mlečne formule koriste kao zamena za humano mleko u ishrani odojčadi, kao i da se ispitaju razlozi za korišćenje i odabir adaptiranih mlečnih formula.

Intervjuisano je ukupno 309 ispitanica podeljenih u tri starosne kategorije: one starosti od 20 do 25 godina; od 25 do 30; i preko 30 godina.

Od ukupnog broja ispitanica, 62,13% hranilo je odojčad humanim mlekom, 29,13% adaptiranim mlečnim formulama, dok je 8,74% dojilja uz humano mleko dohranjivalo odojčad adaptiranim mlečnim formulama. Analizom podataka dobijene su prosečne vrednosti težine beba, u zavisnosti od toga da li su hranjene humanim mlekom ili adaptiranom mlečnom formulom, nakon tri meseca i utvrdjeno je da je postojala statistički značajna razlika u težini beba između žena koje su dojile i koje su hranile odojčad apatiranim mlečnim formulama ($p < 0,05$). Na pitanje o izvoru preporuke o korišćenju adaptiranih mlečnih formula, najveći broj ispitanica odgovorio je da je preporuku dobio od stane lekara (46,15%), dok je kao razlog korišćenja adaprtirane mlečne formule najveći broj naveo izostanak lučenja mleka (64,10%).

Istraživanje pokazuje da odojčad hranjena adaptiranim mlečnim formulama dobija na težini brže od dojene odojčadi, što je tendencija koja može da posluži kao hipoteza da će veštački hranjene bebe kasnije u životu biti gojazne. Benefit same studije bio je da je odluka o korišćenju adaptiranih mlečnih formula, bez obzira na nivo obrazovanja ispitanica, bila rezultat konsultacije sa pedijatrom, a ta činjenica daje poseban značaj kvalitetnom razvoju odojčadi.

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Ključne reči: *adaptirane mlečne formule, ishrana, odojčad*

Original article

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PREVALENCE OF DEPRESSIVE SYMPTOMS IN MEDICAL STUDENTS

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Depression, anxiety and stress symptoms are common in medical students. The objective of the paper was to assess and to compare the prevalence of depressive symptoms in the first and fourth year medical students. Methods. The cross-sectional study based on the Patient Health Questionnaire-9 (PHQ-9) was done.

The response rate was 83% (331 of 400). Overall, 48% of the students (both first and fourth year) had symptoms of depression. The average PHQ-9 score in first year students was significantly higher than in fourth year students, 6.75 ± 4.60 vs. 5.03 ± 4.67, $p < 0.05$. The most prevalent were mild depresive symptoms and they were observed in almost every third medical student. The female students had significantly higher average PHQ-9 score compared with the male students 6.37 ± 4.88 vs. 4.89 ± 4.27, $p < 0.01$. The significant negative correlation between depressive symptoms in medical students and their everyday achievement was observed ($\rho = 0.610$; $p < 0.001$).

More than a half of all the examined students did not have signs of depression and 48% of them did. Depressive symptoms were more prevalent in the first year students than in the fourth year students and also among the female compared with the male students. Depressive symptoms had a significantly negative impact on daily activities of the students. During medical studies students experience high levels of stress and they should be screened for the symptoms of depression.

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Key words: *depressive symptoms, prevalence, medical students, PHQ-9 questionnaire*

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Introduction

It is well known that depression, anxiety and stress symptoms are common in medical students (1, 2). Stress and depression during the medical school may predict later mental health problems in physicians (2, 3, 4) and the stress that began in medical school tends to continue throughout the years of practicing medicine.

According to the results of the study from 2016, medical students in the United States of America (the USA) have two to five times more prevalence of depression than the general population; their depression prevalence ranged from 9% to 56%. The authors determined that 27% of medical students had depression or symptoms of depression (5).

The percentage of medical students with depression or depressive symptoms ranged from 20% in Europe to 31.8% in the Middle East. Medical students in the North America had the second highest prevalence at 30.3% (6).

Depression of medical students is associated with poorer quality of life, impaired academic productivity and an increased usage of some medicines like benzodiazepines, the use of alcohol, tobacco, illicit substances, and self-harming behavior to help cope with negative affects (7, 8).

In the study of Mackenzie (2011), depression was associated with a number of health issues such as unwanted sexual experiences, and other forms of victimization or violence (8). Furthermore, increased severity of depression is associated with suicidal ideation and suicide attempts (9, 10). Cross-country, cross-ethnic and cross-cultural differences may contribute to depression prevalence (11).

In general, depression is one of the most prevalent mental disorders (12), but it is hard to document real prevalence of depression among medical students. To our best knowledge, only a few similar studies among medical students in Serbia have been conducted to date.

The objective of the paper was to determine and compare the prevalence of depression among the first and fourth year medical students at the Faculty of Medicine, University of Niš.

Method

The cross-sectional study based on the Patient Health Questionnaire-9 (PHQ-9) was carried out. All first and fourth year medical students of the Integrated Medical Studies at the Faculty of Medicine in Niš were involved in the study. The study was conducted from October to November 2016.

The city of Niš is the third largest University City in Serbia. It is the centre of the Nišava's region and the population of the region makes 5.1% in comparison to the population of the central Serbia (the population figures from the middle of 2016). The Faculty of Medicine of the University of Niš is the only Faculty of Medicine in the city of Niš and it is the state Faculty of Medicine. No private faculty of medicine exists either in the city of Niš or in the southeast of Serbia. Integrated Medical Studies are perceived as being prestigious among pupils of high schools. Integrated Medical Studies last for six study years at the Faculty of Medicine in Niš.

Each year, pupils with the best marks from the fourth year high schools from the city of Niš and from other parts of southeast Serbia try to pass an entrance exam and become students at the Faculty of Medicine in Niš.

This study is a part of an internal project at the Faculty of Medicine in Niš which is called: "The quality of life and the prevalence of depression among university students at the University of Niš". The Number of the project is 28/2016 and this project is approved by the Council of the Faculty of Medicine in Niš and the Ethical Committee of the Faculty of Medicine in Niš and the data presented in this paper are the first findings.

All participants were given the self-administrated Patient Health Questionnaire (PHQ-9). The total of 400 questionnaires (Serbian version of anonymous, validated PHQ-9) was distributed. Sixtynine questionnaires were incomplete and we decided not to include them in the study.

The PHQ-9 Questionnaire

Depression is a common mental disorder, charracterized by sadness, loss of interest or pleasure, feelings of guilt or low self-esteem, disturbed sleep or appetite, feelings of tiredness and poor concentration (12).

The PHQ-9 questionnaire is short and self-completed and it is a validated tool used to screen for depression based on the standard Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition) (13).

As described in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM) (13) the hallmark of major depressive disorder (MDD) is the occurrence of depressed mood (dysphoria) and loss of interest for the activities that were rather pleasurable in the past (anhedonia) for a duration of at least two weeks.

The PHQ-9 questionnaire has 9 items which, on the Likert scale, are designed to establish the diagnosis of depression in accordance with the criteria of the DSM-V. The PHQ-9 as a Measure of Depression severity ranges from 0 to 27, because each of the 9 items can be scored from 0 ("not at all") to 3 ("nearly every day") (14, 15).

The PHQ-9 is a self-administrated and can be used either as a diagnostic test to make a probable diagnosis of MDD or as a continuous measure with scores ranging from 0 to 27 and cut off points of 5, 10, 15 and 20 representing mild, moderate, moderately severe and severe levels of depressive symptoms. MDD should be considered in individuals who endorse ≥ 5 of the 9 symptoms as present "more than half the days" (the 9th item counts if endorsed "several days") and one of the first two symptoms (depressed mood or loss of interest) is endorsed (16).

The PHQ-9 has been used in the student population of the city of Niš with good reliability (Cronbach coefficient α = 0.828). Cronbach's highest reliability was in the second question "feel depressed" (Cronbach coefficient α = 0.756), the sixth question "low self-esteem" (0.796) and the first question– "anhedonia" (Cronbach coefficient α = 0.807).

Statistical analysis

Quantitative analyses were performed using SPSS 18.0 statistical software package (SPSS Inc, Chicago, Illinois).

Cronbach coefficient α test was used for testing the rialbility of the PHQ-9 questionnaire for the examened population. For scoring the average PHQ-9 score, the Student's t-test was used. Chi square test (χ^2) and the Pearson's correlation coefficient were calculated. The difference was cosidered statistically significant when the p-value was below 0.05.

Results

The total number of students who completed the PHQ-9 questionnaire was 331 (112 males and 219 females).The response rate was 83% (331 of 400). There were 161 first year students (48 males and 113 females) and 170 fourth year students (64 males and 106 females). The average age of first year students was 19.08 ± 0.47 (ranged 18-21) and the average age of fourth year students was 22.22 ± 1.07 (ranged 21-30).

The fourth year students were statistically significantly older than the first year students ($t = 34.05$; $p = 0.00$) (Table 1).

There was not statistically significant difference in the structure by gender and by the year of study ($\chi^2 = 2.266$; $p = 0.132$).

The average PHQ-9 score in the first year students was significantly higher 6.75 ± 4.60 vs. 5.03 ± 4.67, $p < 0.05$, than in the fourth year students. There were more fourth year students with the PHQ-9 score of 0-4 than first year students, which means that they did not have signs of depression, 106

(62.4%) vs. 66 (41%). The determined difference was statistically significant (χ^2 = 15.13, p = 0.0001; 95% CI 10.3 to 31.9).

There was statistically significant difference in the prevalence of depresive symptoms of various grades between the students of the first and of the fourth year (χ^2 = 15.370; p < 0.001).

There were more first year than fourth year students with PHQ-9 score of 5-9 (mild depressive symptoms) 57 (35.4%) vs. 41% (24.1%) and it was statistically significant (χ^2 = 5.052, p = 0.02). It was determined that there were more first year students with score of 10-14 (moderate depressive symptoms, 38 (23.6%) than fourth year students 23 (13.5%). The difference was significant statistically (χ^2 = 5.598, p = 0.01).

The PHQ-9 score of 15-19 (moderate severe depressive symptoms) had 8 (4.9%) of the first year students and 6 (3.9%) of the fourth year students. The difference was not statistically significant (χ^2 = 0.197, p = 0.65). The highest PHQ-9 score 20-27 (severe depressive symptoms) had the equal number of the first and fourth year students: 3 (1.8%) vs. 3 (1.8%).

Table 1. Characteristics of the examined medical students

Variable		n	(%)	test	p-value
Sex	**Male**	112	(33.8)		
	Female	219	(66.2)		
Age	$\bar{x}$ ± SD				
First year students	19.08 ± 0.47			t = 34.05	p = 0.00
Fourt year students	22.22 ± 1.07				
Structure by gender					
First year	**Male**	48	(29.8)	χ^2 = 2.266;	p = 0.132
	Female	113	(80.2)		
Fourth year	**Male**	64	(37.6)		
	Female	106	(62.4)		
Average PHQ-9 score	$\bar{x}$ ± SD				
First year students	6.75 ± 4.60				p < 0.05
Fourth year students	5.03 ± 4.67				

Table 2. Distribution of the medical students by various grades of depression according to the PHQ-9 score and by the year of studying

PHQ-9 Total score	Total N = 331		First year students N = 161		Fourth year students N = 170		p-value[1]
0-4	172	52.0	66	41.0	106	62.4	p < 0.001
5-9	98	29.6	57	35.4	41	24.1	p = 0.02
10-14	41	12.4	27	16.8	14	8.2	p = 0.01
15-19	14	4.2	8	4.9	6	3.5	p =0.65
20-27	6	1.8	3	1.9	3	1.8	nonsignificant

[1]Chi-squared test

According to the data presented in Table 2, there were more first year than fourth year students with PHQ-9 score of 5-9 (mild depressive symptoms) 57 (35.4%) vs. 41% (24.1%) and it was statistically significant (χ^2 = 5.052, p = 0.02).

There were more first year students with score of 10-14 (moderate depressive symptoms), 38 (23.6%) than fourth year students 23 (13.5%). The difference was significant statistically (χ^2 = 5.598, p = 0.01).

The PHQ-9 score of 15-19 (moderate severe depressive symptoms) had 8 (4.9%) first year students and 6 (3.9%) fourth year students. Difference was not statistically significant (χ^2 = 0.197, p = 0.65).

The highest PHQ-9 score 20-27 (severe depressive symptoms) had the equal number of the first and fourth year students 3 (1.9%) vs. 3 (1.8%).

According to the results showed in Table 3, there were significantly more females 71 (32.4%) than males 27 (24.1%) with PHQ-9 score of 5-9. The difference was not statistically significant (χ^2 = 2.443, p = 0.1, 95% CI -2.5 to 18.3).

Table 3. Distribution of medical students according to the total PHQ-9 score and by gender

PHQ-9 Total score	Total N = 331		Male N = 112		Female N = 219		p-value[1]
0-4	172	52.0	69	61.6	103	47.0	p < 0.001
5-9	98	29.6	27	24.1	71	32.4	p = 0.11
10-14	41	12.4	14	12.5	27	12.3	p = 0.95
15-19	14	4.2	0	0.00	14	6.4	-
20-27	6	1.8	2	1.8	4	1.8	non-significant

[1]Chi-squared test

It was determined that the PHQ-9 score of 10-14 (moderate depressive symptoms) had 27 (12.3%) female and 14 (12.5%) male students. There was not a statistically significant difference (χ^2 = 0.003, p = 0.95, 95% CI -7.2 to 8.7) in the prevalence of moderate depressive symptoms between females and males.

Only 14 (4.2%) female students had PHQ-9 score of 15-19, which means that only the female students had moderate severe depressive symptoms.

The highest PHQ-9 score of 20-27 had 6 (1.8%) medical students (2 males and 4 females), which means that they had severe depressive symptoms.

The female students had significantly higher PHQ-9 score compared with the male students 6.37 ± 4.88 vs. 4.89 ± 4.27, (p < 0.01).

The distribution of the medical students according to the PHQ-9 score by gender and by study year is presented in Table 4.

Table 4. Distribution of medical students according to the total PHQ-9 score, by gender and the year of studying

PHQ-9 Total score	First year		Fourth year	
	Male Number	Female %	Male Number	Female %
0-4	22 (13.7)	46 (28.5)	47 (27.6)	57 (33.5)
5-9	14 (8.7)	43 (26.7)	13 (7.6)	28 (16.4)
10-14	10 (6.2)	16 (9.9)	3 (1.8)	11 (6.5)
15-19	0 (0.0)	8 (4.9)	0 (0.0)	6 (3.5)
20-27	0 (0.0)	2 (1.2)	2 (1.2)	2 (1.2)

There were significantly less first year male students compared with fourth year male students who did not have depressive symptoms (χ^2 = 6.57, p = 0.01; 95% CI 2.7 to 24.8).

It was found that the first year male students had more mild depresive symptoms than the fourth year male students (7.6% vs. 8.7%) but the observed difference was not statistically significant (χ^2 = 0.090, p = 0.8; 95% CI -6.86 to 9.1). Moderate depresive symptoms were significantly more common in the first year male students compared with the fourth year male students (χ^2 = 4.21, p = 0.04; 95% CI -0.2 to 9.5).

Male students from both the first and fourth year did not have moderate severe depressive symptoms by PHQ-score. Symptoms of severe depression were observed only in 2 (1.2%) fourth year male students.

There were more fourth year female than first year female students (33.5% vs. 28.5%) with PHQ-9 score of 0-4. The difference was not statistically significant (χ^2 = 0.96, p = 0.3; -5.3 to 15.2).The first year female students had more mild depresive symptoms than the fourth year female students (26.7% vs. 16.4%). The determined difference was statistically significant (χ^2 = 5.19, p = 0.02; 95% CI 1.0 to 19.4).

The fourth year female students had less moderate severe depresive symptoms (3.5% vs. 4.9%), and the difference was not statistically significant (χ^2 = 0.40, p = 0.5). An equal percentage of female students from the fourth and from the first year had severe depression (1.2% vs. 1.2%).

There was a statistically significant negative correlation between the PHQ-9 score and an add item, which was related to the everyday functioning (ρ = 0.610; p < 0.001).

Discussion

In our study, nearly half of the medical students had depressive symptoms of various grades based on the PHQ-9 score. The most prevalent were mild depressive symptoms and they were observed in almost every third medical student (first year 35.4% vs. fourth year 24.1%). The first year medical students had significantlly higher prevalence of depressive symptoms compared with the fourth year students (62.4% vs. 41%). Depressive symptoms were more common in the female (53% vs. 38.4%) than in the male medical students.

In our study, the prevalence of depression was 48% and it was higer than the global prevalence among medical students. According to the recent study, a global prevalence of depression among medical students of 28.0% was determined.

The prevalence of depressive symptoms in Brazilian medical students was 41.3 % (17), and depressive and anxiety symptoms were more prevalent among female medical students than in male medical students (17).

In a study conducted in Pakistan, the prevalence of depression of 35.1% was determined (18). The study conducted in Katmandu, Nepal in 2016, determined the depression prevalence of 29.9% (19). At the Malaysia Medical University, the prevalence of depression was of 41.9% (20).

A study from Lithuania found that 14% of medical students have symptoms of depression and these depressive symptoms were associated with higher vulnerability to stress (21).

According to the results of a study from South Korea, prevalence of major depression among the first year medical students was 6.5% and this was significantly higher among female students compared with males (22).

A study in India found the overall prevalence of provisionally diagnosed depressive and major depressive disorders among medical students was 39.9% (23). A study from Katmandu in 2012, found the prevalence of depression of 29.8%. Depression was more prevalent in females with significantly higher rates among female and first year medical students (24).

In the study of Quince et al. (2012), Cambridge medical students do not have a higher prevalence of depression than students in general or comparable nonstudent members of the general population. They determined that the prevalence of depression among Cambridge medical students varied from 2.2% to 14.8% (25).

In our study, the prevalence of mild and moderate depression was more often present among the first year students than among the fourth year students. Moderate severe and severe depression were significantly more often present in the female students than in the male students.

In this study, the prevalence of moderate severe depression was 14% and it was determined only in female students.

The first year students had the higher PHQ-9 score if compared with the fourth year students. The male fourth year students had two times less depression than the male first year students.

According to the presented results, the female students had significantly higher PHQ-9 score compared with the male students. The prevalence of depression among the female was 53% and in male medical students was 38.4%. Depression was significantly associated with the female gender. Similar findings had Nagasa et al. (2017) in Cameroon (26).

Each year a larger number of female than male students enroll in the Integrated Medical Studies at the Faculty of Medicine in Niš. The proportion of females in the Faculty of Medicine and in the medical profession in the city of Niš and in the whole Serbia has increased in the recent years (27). The same process exists in many countries of the world. In Texas, in the USA, this process is well investigated and it is described as feminization - the increase in the number of female workers in the medical profession (28).

In the study of Eisenber (2007), females were more likely to screen positive for major depression and the prevalence of overall positive screens for depression was identical by gender among undergraduates and slightly higher for females among graduate students (9).

Students in Oman showed no significant difference in the rate of depression between males and females (29). In the study of Niemi (2006), the gender did not turn out to be a significant factor in the stress reporting (30).

In our study, the statistically significant negative correlation between the depressive symptoms among the medical students and everyday achievement was determined.

Eisenberg (2007) suggested that students with depression may not experience severe academic impairment if they are still interested and able to engage in typical activities, but could still be experiencing elevated depressive symptomatology (9).

Results from a study among American and Canadian medical students data regarding the causes of student distress and its impact on academic performance, dropout rates, and professional development are limited (5, 6).

According to the results of a two year longitudinal study involving American students, depression was associated with the increased risk of course dropout and lower grade point average (GPA); each additional point on the depression measure by PHQ-9 was associated with 0.31% increased risk of dropout, while a 15 point increase was associated with a 0.17 point decrease in GPA scores (31).

The limitations of the study

There were several limitations of the study. Only the first and fourth year students were involved in the study, not students from all six study years. The study was conducted based on self-assessment of depression not based on clinical diagnosis. The study was done at the beginning of a new academic year, which could be a stressful academic period for medical students of both examined study years. Only medical students were examined, and because of that, the results cannot be generalized.

The strength of the study

The high response rate supports the validity of our results. This is the first study of prevalence of depression based on the PHQ-9 questionnaire in the student population in the city of Niš.

Conclusion

Simptoms of depression were prevalent in medical students. About 48% of all examened medical students had depresive symptoms of various grades. Symptoms of depression were significantly more prevalent in the first year than in the fourth year students and in female compared with male medical students. Depressive symptoms had a significantly negative impact on students' daily activities. Because medical students experience high levels of stress during their studies they should be screened for symptoms of depression.

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PREVALENCIJA SIMPTOMA DEPRESIJE KOD STUDENATA MEDICINE

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Simptomi depresije, anksioznosti i stresa često su zastupljeni kod studenata medicine i prediktori su depresije u zrelom dobu.

Rad je imao za cilj da utvrdi prevalenciju depresivnih simptoma kod studenata prve i četvrte godine medicine. Primenjena je studija prevalencije kojom su bili obuhvaćeni svi studenti prve i četvrte godine integrisanih studija medicine Medicinskog fakulteta u Nišu. Kao instrument instraživanja primenjen je anonimni upitnik the Patient Health Questionnaire-9 (PHQ-9). Izračunavani su Studentov t-test i Pirsonov koeficijent korelacije.

Od 400 distribuiranih upitnika, kompletno popunjen bio je 331 upitnik i samo su oni uvršteni u analizu (83%). Kod 48% studenata bili su prisutni depresivni simptomi. Studenti prve godine imali su statistički veći prosečni PHQ-9 skor u upitniku od studenata četvrte godine (6,75 ± 4,60 prema 5,03 ± 4,67; $p < 0,05$). Svaki treći student imao je blage depresivne simptome. Studentkinje su imale statistički veći prosečni PHQ-9 skor od studenata (6,37 ± 4,88 prema 4,89 ± 4,27; $p < 0,01$). Postoji statistički značajna negativna korelacija između depresivnih simptoma i obavljanja svakodnevnih aktivnosti ($\rho = 0,610$; $p < 0,001$).

Više od polovine studenata medicine nije imalo depresivne simptome, ali je 48% imalo depresivne simptome različite težine. Depresivni simptomi bili su zastupljeniji kod studenata prve godine u odnosu na studente četvrte godine i bili su značajno zastupljeniji kod studentkinja nego kod studenata. Depresivni simptomi imali su značajan negativan uticaj na obavljanje svakodnevnih aktivnosti studenata. Studenti medicine izloženi su velikom stresu tokom studiranja i u cilju sprečavanja pojave depresivne simptomatologije trebalo bi uvesti skrining depresije u sklopu sisitematskih pregleda studenata.

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Ključne reči: *depresivni simptomi, studenti medicine, prevalencija, PHQ-9 upitnik*

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ADVANTAGES OF UNILATERAL SPINAL ANESTHESIA VERSUS CONVENTIONAL BILATERAL SPINAL ANESTHESIA IN LOWER LIMB ORTHOPEDIC SURGERY

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Spinal anesthesia is a frequently applied technique for lower limb orthopedic surgery. Hypotension is the most frequent side effect of conventional bilateral spinal anesthesia. An exclusively unilateral block only affects the sensory, motor and sympathetic functions on one side of the body without the typical adverse side effects seen with a bilateral block.

The aim of this prospective, randomized study was to compare unilateral anesthesia versus conventional bilateral spinal anesthesia in lower limb orthopedic surgery according to the quality of sensory and motor blockade, analgesia, hemodynamic stability and side effects.

Forty ASA I – II patients scheduled for lower limb orthopedic surgery were randomly allocated into two groups. Group BS patients received bilateral spinal anesthesia with 3ml isobaric 0.5% levobupivacaine (conventional dose) and group US patients received unilateral low dose spinal anesthesia with hyperbaric spinal solution (7.5mg of 0.5% levobupivacaine and 40mg of 10% glucose) over a period of 120 seconds and the patients were kept in the lateral position for 15 minutes.

In both groups, the quality of the sensory and motor block was adequate for the surgical procedure. The time to two segment regression of sensory blockade, recovery time of motor blockade, as well as the time of complete recovery was significantly shorter in US group as compared to the BS group. Seven patients in the bilateral, and one patient in the unilateral group developed hypotension that required treatment with ephedrine (Chi-square test 7.02; $p < 0.05$).

Unilateral low dose spinal anesthesia achieves stable hemodynamics. It also results in rapid recovery compared to a bilateral conventional dose spinal anesthesia.

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Key words: *spinal anesthesia, hemodynamic, unilateral, levobupivacaine, low dose*

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Introduction

Spinal anesthesia is a frequently applied technique with its ease of performance and high success rate in lower limb orthopedic surgery. It is widely used for providing a fast and effective sensory and motor blockade. This blockage reduces the stress response to a surgical trauma, decreases intraoperative blood loss, reduces the incidence of postoperative thromboembolism and decreases morbidity and mortality in comparison with general anesthesia (1).

However, side effects such as hypotension, bradycardia, nausea and vomiting, postpuncture headache and urine retention are observed (2). Hypotension is the most frequent side effect of conventional bilateral spinal anesthesia, occurring in more than 30% of patients. Ward et al. reported a decrease in mean arterial blood pressure of 21.3% of the baseline following spinal anesthesia. He also reported that a level of spinal anesthesia to T5 resulted in an increase in heart rate by 3.7%. The cardio-accelerator fibers originate from T1-T4, so the level of spinal anesthesia affecting these dermatomes may cause bradycardia (3, 4). An exclusively unilateral block only affects the sensory, motor and sympathetic functions on one side of the body and offers the advantages of a spinal block without the typical adverse side effects seen with a bilateral block (5, 6).

The advantages of unilateral spinal anesthesia include much lower incidence of clinically relevant hypotension, lower incidence of urine retention, better patient satisfaction, better mobility during recovery time and block restriction on the operative side. Several factors are required for successful unilateral spinal anesthesia: the type of spinal needle and bevel direction, the rate of injection, volume, baricity and the concentration of local anesthetics, as well as the position of patients on the operating table (7, 8). Moreover, patient posture is thought to be fundamental in determining the level of anesthesia spread, particularly when a hyperbaric anesthetic solution is used (9, 10).

The aim of our study was to compare unilateral anesthesia versus conventional bilateral spinal in lower limb orthopedic surgery according to the quality of sensory and motor blockade, analgesia, hemodynamic stability and side effects.

Materials and methods

This prospective study included forty adult patients scheduled for unilateral lower limb surgery, except patients with degenerative hip disease or hip fracture, in routine surgical theaters at the Clinic of Orthopedic Surgery and Traumatology in Clinical Center Niš. Informed consent was obtained from all patients. Inclusion criteria were American Society of Anesthesiologists (ASA) score I–II, age 18-65 years, male and female. Exclusion criteria were contraindications for spinal anesthesia: skin infection at the site of regional anesthesia, coagulopathy, taking anticoagulant drugs, allergy to local anesthetic drugs, hypovolemia, low fixed cardiac output, neurologic and psychiatric disorder, spine deformity, body mass index (BMI) > 35kg/m^2 and chronic pain treatment.

Patients were randomly allocated into two groups of 20 patients (N = 20). The BS group patients received bilateral spinal anesthesia with 15mg isobaric 0.5% levobupivacaine (conventional doses). The US group patients received unilateral spinal anesthesia with 7.5mg hyperbaric 0.5% levobupivacaine (low doses). Hyperbaric solution was prepared by combining 7.5mg of isobaric 0.5% levobupivacaine (1.5ml) with 40mg 10% glucosae (0.4ml). All patients were given 2mg midazolam intravenously as premedication, as well as an intravenous infusion of 7mL/kg of lactated Ringer solution. Standard monitoring was used, including noninvasive blood pressure, electrocardiogram, peripheral pulse oximetry, and respiratory rate measurements. Baseline arterial blood pressure and heart rate were recorded at the end of volume expansion, before inducing spinal block.

All patients were placed in a lateral position on the operative side down, while the vertebral column was positioned as horizontally as possible. Under complete aseptic technique, dural puncture was performed using a midline approach at the L3-L4 interspace with a 27 gauge spinal pencil point needle. BS group received an intrathecal injection of 15mg plane (isobaric) levobupivacane 0.5% over a period of 10 seconds. The direction of the needle aperture was cranial during the injection. After injection of spinal solution the patients immediately were turned in supine position (conventional bilateral spinal anesthesia). US group received of 7.5mg plane levobupivacaine 0.5% with 40mg glucose (hyperbaric solution) over a period of 120 seconds (injection speed: 1ml/min) without further aspiration maneuvers. The bevel of the needle pointed down to operative site during the injection. The patients were kept in the lateral position for 15 min and then placed in the supine position for surgery (unilateral low dose spinal anesthesia).

Hemodynamic changes were recorded every 5 min after spinal anesthesia, and then until the end of surgery. Hypotension (SAP < 90 or 30% decrease from the baseline) was treated with additional intravenous bolus of 250ml crystalloid. However, if supplementation of fluids failed to reverse hypotension, intravenous ephedrine 5-10mg bolus was administrated. Bradycardia (HR < 50) was treated with 0.5 mg of atropine intravenously.

The sensory anesthesia level was evaluated by pinprick method with 22 gauge hypodermic needle along the anterior middle clavicular line of both sides. The time to onset of analgesia was defined as the time to the onset of sensory block to maximum cephalad spread. The onset and degree of motor block were evaluated using a modified Bromage scale (0 = no motor block; 1 = hip blocked; 2 = hip and knee blocked; 3 = complete motor block). Pain was assessed from the beginning of surgery using a 10cm visual analog scale (VAS). We also recorded side effects such as nausea, vomiting and headache. The urinary retention was not recorded due to a significant number of patients with preoperatively placed urinary catheter.

Statistical analysis was performed using standard data processing programs - MS EXCEL and software package R. Tests were performed with Chi-square, Fisher's exact test and t-test for independent samples. A value of $p < 0.05$ was considered as significant. Continuous variables were presented as mean ± stdev or as median (range); categorical data were presented as number (%).

Results

There were no significant differences between two groups with respect to age, gender, weight, ASA status, duration of surgery and intraoperative crystalloids (Table 1).

In both groups, anesthesia was adequate for the surgical procedure and none of the patient needed general anesthesia or intraoperative analgesics. The quality of the sensory and motor block, as well as intraoperative analgesia are shown in Table 2. T10-T12 anesthesia was achieved in both groups. The maximum level of sensory blockade was higher in the bilateral spinal group T7 (T4-T8) than in the unilateral spinal group T8 (T11-T7) thoracic dermatome, but there was no significant difference ($p > 0.05$).

Table 1. Patient's characteristics, duration of surgery and intraoperative crystalloids

	BS group (N = 20)	US group (N = 20)
Age (years)	45.4 ± 12.84	44.2 ± 12.79
Sex (Male/Female)	12/8	14/6
Weight (kg)	76.5 ± 13.2	75.2 ± 12.62
ASA classification	ASA I 4 (20%) ASA II 16 (80%)	ASA I 5 (25%) ASA II 15 (75%)
Duration of surgery (min)	64.3 ± 18.46	61.0 ± 17.22
Intraoperative crystalloids (ml)	1109 ± 522.4	1038 ± 456.86

Data are means ± sd or numbers. ASA – American Society of Anesthesiologists.
There were no significant differences between groups ($p > 0.05$).
BS group – bilateral spinal anesthesia; US group – unilateral spinal anesthesia

Table 2. Comparison of the spinal blockades

	BS group (N = 20)	US group (N = 20)
Maximum cephalad spread (dermatome)	T7 (T4 – T8)	T8 (T11 – T7)
Onset time of sensory blockade (min)	6.7 ± 0.9	8.05 ± 1.07*
Time to two segment regression (min)	91.55 ± 9.55	57.75 ± 7.32*
Intraoperative analgesia (VAS = 0 – 10)	0 (100%)	0 (100%)
Degree of motor block - operative side	Bromage III 20 (100%)	Bromage III 20 (100%)
Degree of motor block - nonoperative side	Bromage III 20 (100%)	Bromage I/II 3 (15%)*
Duration of motor block (min)	179 ± 13.74	105.25 ± 12.59*
Full recovery (min)	232 ± 17.49	167.25 ± 10.42*

Data are means±sd or numbers. VAS – visual analog scale.
*Statistical significance was set at the $p < 0.05$ level.
BS group – bilateral spinal anesthesia; US group – unilateral spinal anesthesia.

The average time to sensory onset in the unilateral group was 8.05 ± 1.07 min. In the bilateral group, this value was 6.7 ± 0.9 min (t value -4.21; $p < 0.05$). The time to two segment regression of sensory blockade was significantly shorter in the unilateral spinal group 57.75 ± 7.32 min versus 91.55 ± 9.55 min in the bilateral spinal group (t value -12.24; $p < 0.05$). Recovery time of motor blockade in unilateral spinal group (105.25 ± 12.59 min) was significantly shorter (t value 17.24; $p < 0.05$), as well as the time of complete recovery (167.25 ± 10.42 min) in the unilateral spinal group (t value -13.86; $p < 0.05$). An average Bromage score of III was achieved for the motor block in both groups. A strictly the unilateral spinal anesthesia in the US group was achieved in seventeen patients, while in three patients spinal block spread to the nonoperative side (Bromage I or II).

Table 3. Hemodynamic changes and side-effects of spinal anesthesia

	BS group (N = 20)	US group (N = 20)
Hypotension (SP<90mmHg)	7 (35%)	1 (5%)*
Bradycardia (SF < 50 / min)	4 (20%)	1(5%)
Nausea, vomiting	4 (25%)	1 (5%)
Headache	1	0

Data are numbers.
*Statistical significance was set at the $p < 0.05$ level.
BS group – bilateral spinal anesthesia; US group – unilateral spinal anesthesia.

Hemodynamic changes and side effects of spinal anesthesia in both groups are shown in Table 3. Seven patients in the bilateral, and one patient in the unilateral group developed hypotension that required treatment with ephedrine. There were significant differences in the incidence of hypotension between study groups (Chi-square test 7.02; $p < 0.05$). Bradycardia, nausea and vomiting occurred in four

patients in the bilateral group and in one patient in the unilateral group. One patient in the bilateral group and no one in the unilateral group needed treatment for headache. There were no significant differences in the incidence of bradycardia, nausea and vomiting and headache between BS versus US group ($p > 0.05$).

Discussion

The conventional bilateral spinal anesthesia is widely used in adults for lower limb orthopedic surgery. Although considered safe, it has got many complications. The most common side effects are hypotension and bradycardia due to sympathetic blockade (2, 11). Unilateral spinal anesthesia only affects the sensory, motor and sympathetic functions on one side of the body and offers the advantages of a spinal block without the typical adverse side effects seen with a bilateral block. The cardiovascular stability following unilateral spinal anesthesia is certainly one of the most important benefits. Hypotension may develop in 30% of patients with bilateral spinal anesthesia, even with intermediate doses (2, 12) compared to 0–6% with unilateral spinal anesthesia (13).

The research showed that the patient's position immediately after spinal anesthesia affects the distribution of anesthetics into the spinal cord. The baricity of local anesthetics (hypo or hyper-baricity) in relation to the specific gravity of the cerebrospinal fluid enables the achievement of a unilateral block. It is also important that the distance between the left and right nerve roots and the lumbar region is about 10-15cm, which makes it possible to achieve unilateral spinal anesthesia too (14). Kuusniemi and colleagues reported that hyperbaric bupivacaine is more effective in achieving unilateral spinal anesthesia than plain bupivacaine (15). However, determining the optimal time for lateral positioning is difficult when a high dose of hyperbaric bupivacaine (12-20mg) is used. The anesthetic drug may migrate during 30 - 60 min. Conversely, if a low dose (5-8mg) of anesthetic solution is used, putting the patient in the lateral position for 10-15 min may prevent migration of the anesthetic drug (9, 16).

In our study, we injected 7.5 mg of hyperbaric levobupivacaine slowly through pencil-point directional needles. The patient was kept in the lateral position for 15 min, which led to unilateral spinal anesthesia in 85% of cases. In three cases, the anesthetic drug spread to the other side, resulting in bilateral spinal anesthesia with Bromage scale I/II on the nonoperative side. In a study performed by Esmaoglu, the unilaterality of the block was achieved in 85.7% of patients after 10 minutes in a lateral decubitus with small doses of hyperbaric solution (17).

In both groups, the quality of the sensory and motor block was adequate for the surgical procedure. The time to two segment regression of sensory blockade, recovery time of motor blockade, as well as the time of complete recovery was significantly shorter in the unilateral spinal group as compared to the bilateral group. Unilateral spinal anesthesia is therefore suitable for outpatient surgery. This findings is also in agreement with the studies by Fanelli et al. (18) and Borghi et al. (19).

In our study, seven patients in the bilateral group had hypotension and only one patient in the unilateral group ($p < 0.05$). Chohan and Afshan administered unilateral spinal anesthesia prior to lower-limb surgery in elderly patients with ASA classification of III or IV. They used hyperbaric bupivacaine (1.1 – 1.8ml). The authors found no significant hemodynamic changes (20). The cardiovascular stability following unilateral spinal anesthesia is certainly one of the most important benefits, especially in high risk patients.

There was no significant difference in bradycardia, nausea and vomiting, as well as postdural puncture headache (PDPH). Headache after spinal anesthesia was reported in one patient in the bilateral group. We used a small gauge (G27) pencil-point (Whitacre) spinal needle. The low incidence of PDPH may be related to the type of the needle used (21).

Conclusion

We observed that both bilateral and unilateral spinal anesthesia provide adequate intraoperative conditions. Unilateral sensory and motor block, a faster recovery profile, and a stable hemodynamic state can be achieved with low doses of hyperbaric levobupivacaine (7.5ml) injected slowly through pencil-point directional needles in patients who are maintained in the lateral decubitus position for 15 min. This technique of unilateral spinal anesthesia achieves stable hemodynamics, particularly in elderly. It also results in rapid recovery compared to a bilateral conventional spinal anesthesia.

Unilateral low dose spinal anesthesia is suitable for high-risk patients, as well as for ambulatory surgery.

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PREDNOSTI UNILATERALNE SPINALNE ANESTEZIJE U ODNOSU NA KONVENCIONALNU BILATERALNU SPINALNU ANESTEZIJU U ORTOPEDSKOJ HIRURGIJI DONJEG EKSTREMITETA

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Spinalna anestezija je često primenjivana tehnika u ortopedskoj hirurgiji donjeg ekstremiteta. Hipotenzija je najčešći sporedni efekat konvencionalne bilateralne spinalne anestezije. Poseban unilateralni blok utiče na senzornu, motornu i simpatičku funkciju samo jedne strane tela bez tipičnih neželjenih sporednih efekata viđenih bilateralnim blokom.

Cilj ove prospektivne, randomizovane studije je da uporedi unilateralnu anesteziju sa konvencionalnom bilateralnom spinalnom anestezijom u ortopedskoj hirurgiji donjeg ekstremiteta, u odnosu na kvalitet senzorne i motorne blokade, analgezije, hemodinamske stabilnosti i sporednih efekata.

Četrdeset ASA I – II bolesnika, planiranih za ortopedsku hirurgiju donjeg ekstremiteta, podeljeno je randomizacijom u dve grupe. Bolesnici BS grupe dobili su bilateralnu spinalnu anesteziju sa 3 ml izobarnog 0,5% levobupivakaina (konvencionalna doza), a bolesnici US grupe dobili su unilateralnu spinalnu anesteziju malom dozom sa hiperbarnim spinalnim rastvorom (7,5 mg 0,5% levobupivakaina i 40 mg 10% glukoze) tokom 120 sekundi i bolesnici su držani u lateralnom položaju 15 minuta.

U obe grupe, kvalitet senzornog i motornog bloka bio je adekvatan za hiruršku proceduru. Vreme regresije senzornog bloka za dva segmenta, vreme oporavka od motorne blokade, kao i vreme do potpunog oporavka bilo je značajno kraće u US grupi u poređenju sa BS grupom. Sedam bolesnika u bilateralnoj i jedan bolesnik u unilateralnoj grupi razvili su hipotenziju koja je zahtevala lečenje efedrinom (chi square test 7,02; $p < 0,05$).

Unilateralna spinalna anestezija malom dozom postiže stabilnu hemodinamiku. Takođe, rezultira brzim oporavkom u poređenju sa bilateralnom spinalnom anestezijom konvencionalnom dozom.

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Ključne reči: *spinalna anestezija, hemodinamika, unilateralna, levobupivakain, mala doza*

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GENERAL, EPIDEMIOLOGICAL PARAMETERS AND IMMUNIZATION COVERAGE OF CHILDREN SUFFERING FROM MORBILLI IN CENTRAL KOSOVO AND METOHIJA

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Morbilli is a viral, highly contagious droplet infection belonging to the group of rashcausing fever. The virus enters humans via the respiratory route. The disease starts with a rise in body temperature, "facies morbillosa" cough, catarrhal changes of the mucous membrane of the upper respiratory tract followed by maculopapular rash.

The aim of the paper was to analyze epidemiological parameters and the vaccination status of affected children in central Kosovo and Metohija enclaves.

The study enrolled 91 children (57.1% boys and 42.9% girls), in the period October 2017-March 2018 in the enclaves where Serbs, Roma, Albanians, Gorani, and Turks live. The diagnosis was established according to epidemiological and clinical parameters, blood count, and findings of specific IgM antibodies. The children were grouped according to gender, ethnicity, age, the origin of the infection, and vaccination status. Numerical properties and attributes are shown. The Student's t-test was used for comparing sets of presented numerical values. The Chi squared (χ^2) test and Fisher's exact test were used to illustrate and compare the difference in the frequency of attributive characteristics.

The mean age of children was 9.74 ± 4.23 years. The greatest number of patients was in December, 34.1%. The majority of children were of Roma ethnicity. The number of affected unvaccinated Roma children (49.4%) was three times higher in comparison to Serbian children (17.6%) and five times higher in comparison to children of Albanian ethnicity (9.9%), which is a statistically significant difference (χ^2: $p < 0.05$). A great number of children (30.7%) got infected in healthcare facilities. The majority of children who received one dose of vaccine were among Serbian children (16.5%). The number of children with nosocomial infections (30.7%) was 6 times higher in comparison to children with unknown source of infection (5.5%) (χ^2: $p < 0.05$).

In the enclaves of central Kosovo and Metohija, the majority of Roma children were affected because of non-vaccination, inadequate living conditions and migrations. The incidence of nosocomial infections indicates that the morbillivirus spreads rapidly. Morbilli can be eradicated by conducting healthcare education and complete immunization, primarily of Roma children.

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Key words: *Morbilli, children, general and epidemiological parameters, vaccination*

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Introduction

Morbilli, or measles, is a viral, highly contagious disease belonging to the group of rashcausing fever. The virus enters humans via the respiratory route. The disease starts with a rise in the body temperature, as well as with catarrhal changes of the conjunctiva and mucous membrane of the upper respiratory tract. It is manifested by cough, runny nose and conjunctivitis, as well as by characteristic facial expression, *"facies morbillosa"* associated with characteristic maculopapular rash (1).

Morbilli is a cosmopolitan disease caused by RNA morbillivirus from the family *Paramyxoviridae*.

Morbillivirus has a RNA genome with two glycoprotein envelopes: surface glycoprotein–the hemagglutinin (H) and the fusion protein (F).

A membrane glycoprotein CD-150 and an adhesion molecule Nectin-4 are key cellular receptors for virus binding, thus mediating the infection. CD-150 regulates the synthesis of CD-46 regulatory molecule and protein F (1).

The presence of viral antigen in the body initiates immunological reactions in the lymphatic tissue. The virus initially inhibits the induction of type I interferon and interferon-stimulated antiviral genes. The innate immune response, regulated by the nuclear factor kappa β (NF-κβ), characterizes the expression of CD-150, nectin-4 and CD-46 molecules on host cells. They are major cell receptors which bind the virus. Binding of the virus to the host cells is mediated through protein F, CD-150 molecules, nectin-4 and CD-46 molecules, leading to cellular infection and resulting in systemic infection. CD-150 is human signaling lymphocytic activation molecule (Ly). It is primarily distributed on mononuclear cells of the lymphoid tissue and in the circulation. During morbillivirus replication in the regional lymph nodes, the fusion proteins are released causing infected cells fuse with uninfected cells. From the lymph nodes the virus is carried by mononuclear cells into the lymphatic system, and then into the circulation. Nectin-4 is distributed on the epithelial submucosa and epidermal keratinocytes. In the acute phase of the disease pathological changes primarily involve the skin, tonsils, and the mucous membrane of the throat and respiratory airways, as well as gastrointestinal tract epithelium. CD-150 is also expressed on thymocytes, macrophages, activated T Ly (CD4 and CD8), as well as on B Ly of the lymph nodes (2-5).

The initial T-cell response includes CD8-T cells and T-helper 1 CD4-T cells important for control of infectious virus. Then, the virus is released from the surface of T Ly antigen carrier into extracellular fluid where viral antigen comes in contact with B Ly. During the acute phase of the infection there is a shift of T-helper 1 to T-helper 2 CD4-T cell response that stimulates the humoral immune response. It promotes B Ly maturation, production of antibodies and mitigation of the disease (6). In case of persistent viral replication, modulation of the immune response occurs. Suppression of immune responses may suppress macrophage activation and T-helper 1 CD4-T response to new viral infections. Thus, the disease progresses and the infection is spread throughout the body (7).

The diagnosis of measles is based on medical history, epidemiological data, clinical manifestation, characteristic blood count, and findings of specific IgM antibodies in serum (ELISA test). Isolation of the virus from blood, nasopharyngeal aspirates, or liquor may be performed as well (PCR method) (1).

In the late 2017 and early 2018, mainly in the region of central Kosovo and Metohija, the cases of measles reemerged. According to the number of affected individuals in the study, it can be said that measles still represents a serious social and health problem in the regions where adequate and complete vaccination coverage has not been reached.

Aim of the paper

The aim of the paper was to analyze general and epidemiological parameters and the vaccination status of the patients suffering from morbilli in Serbian enclaves in central Kosovo and Metohija.

Methods

This retrospective study was performed on 91 children suffering from measles in Serbian enclaves of central Kosovo and Metohija. There are different ethnic groups in the enclaves, including Serbs, Albanians, the Roma population, Turks, the Ashkali, Gorani and others. The study was conducted from October 2017 to March 2018. The children were treated at the Pediatric Clinic of the Clinical Hospital Center Priština. The diagnosis of measles was established according to epidemiological and clinical characteristics, blood count, and findings of specific IgM antibodies in serum (ELISA test). In two thirds of affected children the disease was also confirmed by laboratory findings. Descriptive epidemiological and statistical methods of analysis were used for providing statistical data analysis. T-test was used for the presentation of numerical measurements. The Chi squared test was used to compare frequencies of numerical measurements. Accepted level of significance less than 5% risk level ($p< 0.05$) was the threshold for statistical significance.

The patients were divided according to age, gender, ethnicity, and the origin of the infection. They were also grouped depending on their vaccination status.

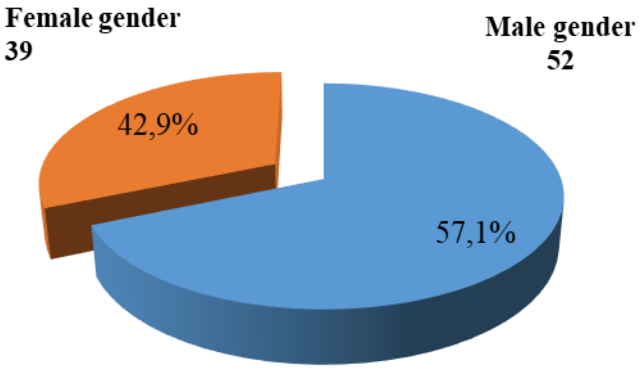
The study was conducted after Ethics Committee approval and written informed consent for each patient had been obtained.

Results

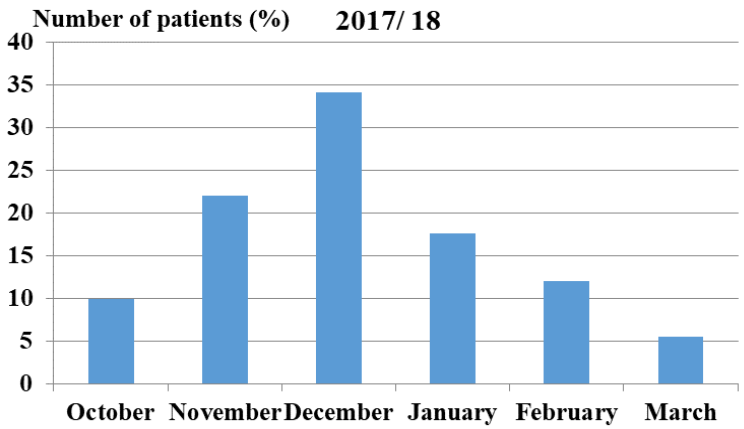
The experimental group included 91 children diagnosed with measles. There were 52 (57.1%) male and 39 (42.9 %) female patients (Graph 1). They were between the ages of 8 months to 18 years. The mean age of the children was 9.74 ± 4.23 years. The male children were older than female children for 2.15 years. The study also included children predisposed to malnutrition and obesity. Records of affected children were performed in the period of six months (Graph 2).

The first cases of the disease were registered in October 2017, 9 cases (9.9%). The greatest number of patients was in November, 20 patients (22%), and December 2017, 31 patients (34.1). A reduction in the incidence has been observed in 2018, 16 patients in January (17.6%), 11 patients in February (12.1%) and 5 patients in March (5.5%). There was a statistically significant difference in the number of patients in December compared to the number of patients in October, February and March (χ^2: $p< 0.05$).

The number of affected children was analyzed in relation to gender, ethnicity and vaccination status (Table 1). The majority of infected children were among Roma population, 45 (49.4) followed by Serbian children, 35 (38.5%). Children of other ethnicities were significantly less infected, 11 children (12.08%). There was a statistically significant difference between the number of Roma and Serbian affected children in comparison to children of other ethnicities (χ^2: p< 0.05).



Graph 1. Number of patients by gender



Graph 2. Number of affected children distributed by months

Table 1. Distribution of affected children according to ethnicity

Ethnicity	Male gender	Female gender	Number of patients (%)		Vaccination status
Roma	24 0 0	21 0 0	45 0 0	49.4 0 0	0 1 2
Serbian	10 7 5	6 4 3	16 11 8	17.6 12.1 8.8	0 1 2
Others	4 2 0	5 0 0	9 2 0	9.9 2.2 0	0 1 2

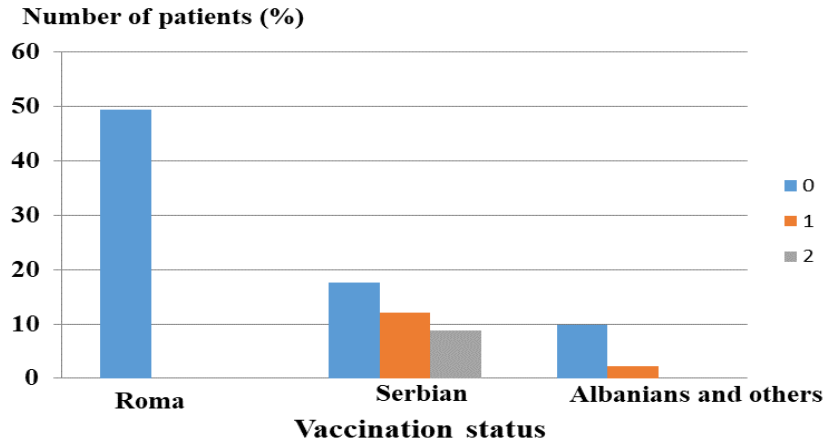
Vaccination status 0 – unvaccinated, 1 – incompletely vaccinated, 2 - fully vaccinated

The number of affected children in relation to vaccination status has been analyzed. (Graph 3)

The majority of unvaccinated children and those with unknown vaccination status were in the Roma population, 49.4% of them. They are followed by children of Serbian ethnicity, 17.6%. There were 12.1% incompletely vaccinated children. There was a statistically significant difference in the number of unvaccinated children of Roma and Serbian ethnicity (χ^2: $p < 0.05$)

Statistically significant difference was registered in the incidence of incompletely vaccinated children of Serbian population in comparison to children of other ethnicities (χ^2: $p < 0.05$). There were 8.8% fully vaccinated, but still affected Serbian children (χ^2: $p < 0.05$). Statistically significant difference was noted between the incidence of fully vaccinated Serbian children and children of other ethnicities (χ^2: $p < 0.05$). Statistically significant difference was registered in the incidence of affected children of Albanian ethnicity (11.1% of them) in comparison to children of Roma ethnicity (49.4% of them) and Serbian ethnicity (38.5% of them), (χ^2: $p < 0.05$).

Affected children were divided into six age-related, gender-related, and vaccination status related groups (Table 2).



Vaccination status 0 – unvaccinated, 1 – incompletely vaccinated, 2 - fully vaccinated

Graph 3. The number of affected children according to ethnicity and in relation to vaccination status

Table 2. Age-related and vaccination-related status of affected children

Age groups	Age	Male gender	Female gender	Number of patients (%)		Vaccination status
First	< 1	3 0 0	2 0 0	5 0 0	5.5 0 0	0 1 2
Second	< 2	6 0 0	2 1 0	8 1 0	8.8 1.1 0	0 1 2
Third	2- 4	2 0 0	1 0 0	3 0 0	3.3 0 0	0 1 2
Fourth	5- 9	10 2 2	7 2 2	17 5 4	18.8 5.5 4.4	0 1 2
Fifth	10- 14	16 4 1	8 3 0	24 7 4	26.4 7.7 4.4	0 1 2
Sixth	15- 18	7 2 1	4 1 0	11 3 1	12.1 3.3 1.1	0 1 2

Vaccination status 0 – unvaccinated, 1 – incompletely vaccinated, 2 - fully vaccinated

The majority of affected children, 76 of them (83.7%), was in the fourth, fifth and sixth groups (between 5 and 18 years of age). Statistically significant difference was recorded in the incidence of affected children in the fourth, fifth and sixth group in comparison to the number of patients in the first three groups (χ^2: p< 0.05).

The greatest number of unvaccinated affected children was in the fourth and fifth group, (between 5 and 14 years of age), 52 children (57.3%). Statistically significant difference was noted in the incidence of affected unvaccinated children in the fourth and fifth group in comparison to incidence in the first three groups. Statistically significant difference was also registered in the incidence of affected incompletely vaccinated children in the fourth, fifth and sixth group in comparison to the first three groups (χ^2: p< 0.05).

The number of affected children according to source of infection and gender was analyzed in relation to vaccination status (Table 3).

Table 3. Distribution according to source of infection and in relation to vaccination status

Source of infection	Male gender	Female gender	Number of patients (%)		Vaccination status
Healthcare facility	15	7	22	24.2	0
	1	2	3	3.3	1
	2	1	3	3.3	2
Preschool institution	5	4	9	9.9	0
	1	1	2	2.2	1
	0	0	0	0	2
School institution	9	7	16	17.6	0
	1	1	2	2.2	1
	2	2	4	4.4	2
Household contacts	8	10	18	19.8	0
	1	1	2	2.2	1
	0	0	0	0	2
Catering facilities	0	0	0	0	0
	3	0	3	3.3	1
	0	2	2	2.2	2
Unknown origin	4	0	4	4.4	0
	0	1	1	1.1	1
	0	0	0	0	2

Vaccination status 0 – unvaccinated, 1 – incompletely vaccinated, 2 - fully vaccinated

The majority of children got infected within healthcare facilities, 28 of them (30.7%), followed by those affected at schools, 22 (24.2%), and by household epidemics, 20 (21.9%). Catering facilities and unknown sources accounted for the minority of affected children, 5 children respectively (5.5%). A statistically significant difference was observed between the number of patients affected within school institutions in comparison to pre-school institutions (χ^2: p < 0.05).

There was a statistically significant difference between the incidence of affected children within healthcare facilities and due to household epidemics in comparison to affected ones within catering facilities and unknown sources (χ^2: p < 0.05).

The greatest number of affected unvaccinated children was registered within healthcare facilities, 22 (24.2%) and family epidemics, 18 (19.8%). Statistically significant difference was found between the incidence of unvaccinated children within healthcare facilities and family epidemics in comparison to affected ones within catering facilities and unknown sources (χ^2: p < 0.05).

Discussion

According to the World Health Organization (WHO) data, infectious diseases are still a leading cause of death worldwide. Morbillivirus is transmitted by aerosol droplets and is one of the most contagious pathogens of infectious diseases. Before the introduction of measles vaccine there were between 2 and 3 million deaths caused by measles (1). In the period between 1990 and 2010 vaccination reduced the number of deaths by measles from 0.63 to 0.13 million (9). Also, the WHO data suggest that in the period between 2000 and 2012 there was a 78% drop in measles deaths worldwide, and a 95% drop in 2015. However, according to the data of a study by Muscat M et al., there has been a growing tendency of measles cases in Europe and worldwide in the second decade of the 21st century. The World Health Organization set the Global Measles Strategic Plan 2012-2020 for achieving measles elimination (10, 11).

The epidemic of measles in Kosovo emerged in early October 2017 in Priština. The majority of affected individuals was identified within the Roma

population, followed by children of Albanian ethnicity. There were 98 registered cases of measles infection at that time. Most of affected persons were not vaccinated (12).

Our study showed the results of morbilli epidemics in the regions of Serbian enclaves in central Kosovo and Metohija in the period of six months, when 91 affected children were registered. The affected individuals were dominantly males. Similar to our results, Giefing-Kröll C. et al. also showed in their study the predominance of younger male patients. One of the reasons is poor immune response toward infectious diseases in males in comparison to females (13). The immune and endocrine systems show age-related interactions in patients with infectious diseases. Out of the total number of patients in Africa and Asia in the period 2013-2107, two-thirds were male patients (14). Contrary to these results, Garenne et al. showed in their study that in big regions of the world, such as Europe, North and South America, the morbidity of females was higher than morbidity in males (15).

The first cases of the disease in the regions of Serbian enclaves in October 2017 were registered in Kosovo Polje. In this Serbian enclave, there were over 70% cases of unvaccinated children, representing 'a large critical mass for an outbreak'. In November and December 2017, a two-fold and a four-fold increase in the number of affected children was observed. The greatest number of affected children was registered in December, 34.1%. In the last four years there has been a significant increase in measles incidence in Europe. Over 20.000 cases of measles, dominantly in children, were registered in Europe in 2017 (16). The Centers for Disease Control and Prevention reported in 2017 over 20 million of measles cases worldwide in 2017 (8). Our study revealed a significant decrease in the incidence of measles. In January 2018 there was a two-fold decrease in the number of affected children, and in February a three-fold reduction. A significant reduction in affected children was registered in March 2018. The main reason for decreasing measles incidence is intensive supplementary immunization of unvaccinated and incompletely vaccinated children. The vaccination included children up to the age of 14 as well (12).

The area of Serbian enclaves in central Kosovo and Metohija is a multiethnic community. The structure of the population in Serbian enclaves is different in comparison to other regions. There is a variety of ethnicities living in Serbian enclaves, such as Serbs, Roma, Albanians, Ashkali, Gorani, Turks and others populations. Ethnic structure of the enclaves is different from other regions in Serbia. The main reason of measles epidemics is a rise in unvaccinated children. Parental fear and skepticism of possible side effects of the MMR vaccination contributed to inadequate immunization. Concerns were related to the vaccine preservative thimerosal being involved in this multivalent live attenuated vaccine, weakening the immunological system and causing autism (17). Due to inadequate vaccination among the populations in Kosovo, it has always been an endemic region for measles outbursts. Epidemic waves up to 2000 were registered in epidemic cycles every 3-4 years (18). Factors also leading to the emergence and development of the disease in some areas of Kosovo include: malnutrition, vitamin A and zinc deficiency, resulting in reduced function and modulation of T- and B-lymphocytes and immune response deficits (19). Similar to our results, Gignoux E et al. demonstrated that in DR of Congo vulnerable rural families, poverty and micronutrient deficiency during measles epidemics significantly affected the development of complications and fatal outcome (20). Prolonged modulation and suppression of cellular and humoral immune response enhance exposure to pathogens, viruses and bacteria. The contagious pathogen-related morbidity is increased, causing development and spreading of the infection through the population (21).

Constant migration and immigration of all pulations from/in Kosovo is an additional cause for the disease spreading, regarding the fact that different morbillivirus genotypes are characteristic for specific geographic regions. Rima BK et al. Demonstrated in their study that the movements of different ethnic groups due to migrations, trade and wars throughout centuries have played a critical role in transmission of infectious diseases (22). Demographic characteristic of specific populations, such as genetics, culture, ecology and epidemiology may have an impact on the spread of the disease in different populations (23). As for environmental factors, air pollution has the potential to affect mutations, sustainability and virulence. Morbillivirus is highly resistant in an immunosuppressive organism, it survives several rounds of autophagy and may contribute to a new infection (24). According to epidemiological data it has been observed that the Roma population was a primary source of infection among affected children. The study results show that the majority of affected Roma children, 49.4% of them, were not vaccinated or they were with unknown vaccination status. One of the factors of a great number of affected, unvaccinated Roma children is economic instability-induced early-age migration to countries with economic stability (25). Factors including poverty, overpopulation, poor hygiene, and the absence of vaccination against infectious diseases contributed to rapid spread of the disease among them (26). European Roma Society pointed out a poor socioeconomic and nutritional status, as well as inadequate immunization among the Roma population. The lack of healthcare education of the Roma population is a reason for high rates of contagious and chronic diseases (27). Mobility of the Roma population groups contributed to transmission of the virus among other populations in Kosovo that were unvaccinated or incompletely vaccinated. According to the results of our study, the next most affected children were of Serbian population, 38.5%, and there was a significantly lower number of children from other ethnicities, 12.1% of them. "Dr Milan Jovanović Batut", Institute of Public Health in Serbia , according to the data of the Center for Disease Control and Prevention, Institute of Public health Kosovska Mitrovica, recorded first dose coverage of 84.6% and second-dose coverage of 14.3% in the region of Serbian enclaves in the central region of Kosovo and Metohija (28).

One of the factors that contributes to morbilli disease and its evolution is the age of subjects (29). The results of our study showed that in 83.7% of cases affected children were between 7 and 18 years of age. The youngest patient was 8 months old and the oldest one was 18 years old. During the first six months of life infants are protected by maternal passive measles immunity. Protective antibodies (immunoglobulin γ) are transplacentally transferred during the pregnancy or through breast milk if the mother had morbilli or if she is fully vaccinated (30). In 67.2% of cases affected children were between 8 and 18 years of age. They comprised a sensitive group for two reasons. The first one is that they were more socially active, and the other one is inadequate vaccination in the post-war period. Children aged 0-4 years were significantly less affected (five-fold). The lowest number of affected children was between 2 and 4 years of age, 3.3% of them. The greatest number of patients, 67.4% of them, was between 10 and 14 years of age. A study by Garenne M. et al. also confirmed that children of the same age were more affected in the Philippines and Thailand (15). Similar to our results, in certain regions of the USA, the majority of diseased children were between 5 and 14 years of age (31). Lee KY et al. showed in their study no difference in age distribution in Korea. Children under the age of 5, as well as those over 10 years of age were equally affected (29). Contrary to our results, it has been observed that children aged up to 2 and 4 years get affected more commonly in some regions of Europe, North and South America and Australia (15, 32). Within our region in different geographical environments, age distribution of the disease is different. In Foča, in the period between October 2014 and February 2015, age distribution of affected individuals was different than in our study. The majority of patients were between 18 and 22 years of age, the youngest patient was 13 years old (33). In Tuzla, in the period 2014–2015, 57.2% of affected children were up to 6 years of age due to absence of immunization. They are followed by 27.1% of children aged between 11 and 18 years and 15.6% children aged between 6 and 10 years. These children were inadequately vaccinated in the war and postwar period due to parental hesitation and the anti-vaccination movement (34). Waaijenborg showed that children who have lower concentrations of maternal antibodies are at greater risk of infection (35).

In the 1990s measles was exclusively a disease of early-school and pre-school children (18). Our study showed that the majority of children acquired the infection within healthcare facilities, 30.8%. This indicates that healthcare workers, even without clinical manifestations of the disease, may be a source of infection transmission (36). Then, there are children who got infected in school institutions, 24.2% of them, followed by children who acquired infection within household epidemics, 21.9%. It has been observed that the number of affected children within healthcare facilities and household epidemics is significantly higher (five- and four-fold) in comparison to infected children within catering facilities or by unknown sources.

The number of affected Roma children was three times higher in comparison to affected Serbian children. The number of affected Albanian children was five times and two times lower in comparison to affected Roma and Serbian children, respectively. Non-vaccination and inadequate living conditions (inadequate hygiene, overcrowding) are responsible for high rate of affected Roma children (half of the total number of affected children). The main reason of non-vaccination among them is insufficient healthcare knowledge on the importance of immunization in the prevention of infectious diseases. The lowest incidence of affected Albanian children and children of other ethnicities (a 10-fold less than the total number of affected children) was because they were treated at the Priština Pediatric Hospital. The affected children were also grouped according to vaccination status. There were 74.7% unvaccinated children or two thirds of affected children, 14.3% were incompletely vaccinated, 8.8% were fully vaccinated, while 3.2% had unknown vaccination status (37, 38). Similar to our results, in the USA there were 85% cases of affected unvaccinated children, 7.8% vaccinated affected children, and 4.7% of affected children with unknown vaccination status (31). In China in 2014, the vaccination coverage rate was also only 84 % (39). In our study the greatest number of unvaccinated children of the Roma population was between 10 and 14 years of age, followed by unvaccinated children of Serbian population, aged between 10 and 18 years. The majority of incompletely vaccinated children belonged to Serbian population, aged between 10 and 18 years. The reason for one-dose vaccination of Serbian children is related to vaccination failure during the war and postwar period after 1999. Parental hesitancy and concerns about adverse effects of the vaccines were also related to outbreaks of measles in the enclaves (38).

Among children of the Roma population there were no incompletely or fully vaccinated children. Roma children pose a risk for the spread of the infection among children of other populations in Kosovo. Morbilli can be eradicated in the region of Kosovo by employing epidemiological and healthcare surveillance of Roma children, as well as by their isolation, immunization and treatment (40). Among the children affected by measles there were 8.8% atypical cases, meaning they were fully vaccinated, but still affected. However, atypical cases of affected children presented milder clinical manifestation. Le Baron et al. showed in their study that a titer less than 120 mIU/mL weakens immune response. Such a low antibody titer in the circulation indicates potential sensitivity to infection (41). The affected unvaccinated and incompletely vaccinated children of other ethnicities comprised a total of 11.1%. Infiltration of sporadic cases of patients with measles from the neighbouring countries of the region also facilitated the outbreak in Kosovo.

The Center for Disease Control and Prevention in Europe has observed in the last 5 years that measles vaccination coverage rate was 90%. The WHO reported in 2016 that vaccination coverage in 8 European countries was in the range between 74% and 98% (42). In the countries of the region,

insufficient vaccination coverage results in sustainable transmission of the virus among the populations. Romania was affected with several thousand cases of infected children in the period 2016-2017 with 17 deaths (43). Such a vaccination status in the countries of the Region indicated the possibility of epidemics in our country. The results of Moss et al. demonstrated that morbillivirus is spread rapidly and easily and they suggested increasing the level of immunization globally for eradication of measles (44).

Conclusion

According to the study research it can be concluded that there are several factors responsible for measles outbreaks in Serbian enclaves in central Kosovo and Metohija. The main factor is a high number of unvaccinated Roma children and incompletely vaccinated Serbian children. A lot of factors facilitated the eruption of the diseases among Roma children. They include inadequate healthcare education on the importance of immunization among the Roma population, inappropriate plan of vaccination in marginalized groups of the Roma population, poor living conditions, and their migration to socioeconomically stable countries. A high incidence rate of nosocomial infections suggests that the morbillivirus spreads rapidly. It may be concluded that eradication of measles requires necessary realization of adequate prophylactic, epidemiological measures, and immunization. In this way measles outbreaks may be prevented in Kosovo, as well as regionally and globally.

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OPŠTI I EPIDEMIOLOŠKI PARAMETRI I IMUNIZACIJA DECE OBOLELE OD MORBILA NA CENTRALNOM KOSOVU I METOHIJI

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Morbili su virusna, veoma kontagiozna kapljična infekcija iz grupe osipnih groznica. Virus u organizam dospeva respiratornim putem. Bolest počinje povišenom telesnom temperaturom, plačnom maskom, kašljem, kataralnim promenama sluzokože gornjih delova respiratornih puteva i pojavom makulopapulozne ospe.

Cilj rada bilo je ispitivanje epidemioloških parametara i vakcinalnog statusa obolele dece u enklavama centralnog Kosova i Metohije.

Istraživanje je obuhvatalo 91 dete (57,1% dečaka i 42,9% devojčica), u periodu oktobar 2017. - mart 2018. godine, u enklavama u kojima žive Srbi, Romi, Albanci, Goranci i Turci. Dijagnoza je postavljena epidemiološkim i kliničkim parametrima, krvnom slikom i dokazom IgM antitela. Grupisanje je izvršeno po polu, etničkoj pripadnosti, starosti, poreklu infekcije i vakcinalnom statusu. Prikazana su numerička i atributivna obeležja. Za prikaz numeričkih obeležja rađen je Student t-test. Za prikaz i poređenje učestalosti atributivnih obeležja kori-šćeni su Hi^2 i Fišerov test.

Prosečna starost dece je 9,74 godine ± 4,23 godine. Najviše obolelih bilo je u decembru (34,1%). Najviše je bilo romske dece. Broj obolele nevakcinisane romske dece (49,4%) bio je tri puta veći od broja srpske dece (17,6%), kao što je broj romske dece bio 5 puta veći od broja albanske dece (9,9%), što je statistički značajna razlika (χ^2: $p < 0,05$). Veliki broj dece (30,7%) oboleo je u zdravstvenim ustanovama - najviše je srpske dece vakcinisane jednom dozom (16,5%). Broj dece oboleo u zdravstvenim ustanovama (30,7%) bio je 6 puta veći od broja dece obolele na nepoznat način – 5,5% (χ^2: $p < 0,05$).

U enklavama centralnog Kosova i Metohije, najviše obolele romske dece bilo je zbog nevakcinacije, neadekvatnih uslova života i migracije. Najviše obolele dece u zdravstvenim ustanovama ukazuje na brzo širenje virusa morbila. Morbili se mogu eradikovati zdravstvenom edukacijom i potpunom imunizacijom, pre svega romske dece.

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Ključne reči: *morbili, deca, opšti i epidemiološki parametri, vakcinacija*

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CHARACTERISTICS OF FAMILIES WITH ADOLESCENTS WHO HAVE ENGAGED IN NON-SUICIDAL SELF-INJURY

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Non-suicidal self-injury (NSSI) in adolescents is a complex phenomenon determined by numerous individual, family and sociocultural factors. The aim of the study was to determine whether families with adolescents who have engaged in NSSI differ in functionality from families with no NNSI adolescents. The study involved 99 adolescents, of both sexes, aged 14-18, divided into two groups: the clinical and the control one. The clinical group included adolescents who had engaged at least once in deliberate self-injury, confirmed by an objective physical examination and anamnestic interview with the respondents. The control group consisted of adolescents with no history of NSSI or another psychiatric disorder. A questionnaire designed for the purpose of this study and FACES III (Family Adaptability and Cohesion Evaluation Scale) were used. Disengaged and separated families (51% and 24.5%, respectively) were dominant in the clinical group, while the dominant ones in the control group were separated (53.1%) and connected families (26.5%). The clinical group was dominated by rigid (51.0%) and chaotic (22.4 %) families, while in the control group they were flexible (42.9%) and structured (36.7%). The results showed a markedly significant difference in the categories of cohesiveness and adaptability between the examined groups. The clinical group had predominantly disengaged/rigid families (36.7%), while the presence of all other levels was less than 10.0%. The control group was dominated by flexible/separated (30.0%) and structured/separated families (20.0%). The study revealed that families with adolecsents who had engaged in NSSI differed from the ones with no NNSI adolescents in terms of functionality on FACES III. These results confirmed the previously obtained results on the connection between family dysfunctionality and engaging in NSSI behavior in adolescents and can have clinical implications in working with the vulnerable group of adolescents and their families.

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Key words: *non-suicidal self-injury, family functionality, FACES III*

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Introduction

Nonsuicidal self-injury (NSSI) refers to the deliberate, self-inflicted destruction of body tissue without suicidal intent, and for purposes not socially sanctioned. It includes behaviour such as cutting, burning, biting and skin scratching (1). NSSI has been shown to be a common phenomenon in adolescents both in clinical and community samples. The incidence and prevalence of self-injury is mainly unreliable due to the fact that self-injury is inflicted in secret or it is not clearly recognizable. In general population, only 10%-15% of adolescents who engage in self-injury seek help in hospitals, which indicates that there is a large number of unrecorded cases of adolescents with mental disorders, including serious psychiatric disorders (2-4). In the clinical population of adolescents, self-injury is more common in comparison with the general population, and is often in comorbidity with borderline personality organization, depressive or anxiety disorders (PTSD), eating disorders, and psychoactive substance abuse (4). Nonsuicidal self-injury may also be present without any psychiatric comorbidities (5).

Motivation for NSSI as well as its function is extremely individual. Adolescents who engage in self-injury can also be classified according to the function of NSSI. These functions can change and

overlap over time, and serve to express different aspects of the same events. Klonsky (6) carried out a comprehensive review of theoretical views on the functions of NSSI and research to date in the field. Seven main categories of NSSI functions were derived from this review: affect regulation, self-punishment, anti-dissociation, interpersonal influence, interpersonal boundaries, sensation-seeking, and anti-suicide (6). The emotional cascade model asserts that NSSI serves as a form of distraction which temporarily reduces negative emotion and increases a perception of relief or even wellbeing. In this way, NSSI represents a negative reinforcer in the emotion-behavior interaction (7).

There are various interpretations as well as numerous prejudices about the causes of non-suicidal self-injury in young people. Its genesis involves a large number of individual, family and sociocultural factors. With regard to family factors, authors differ in stressing the role of families in genesis and maintenance of NSSI in young people. It is stated that dysfunctional families (8), lack of parental support (9, 10), parental criticism (11) and family conflicts (12) increase the risk of non-suicidal self-injury in children.

Within the framework of systemic family therapy, Olson's Circumplex Model of marital and family relationships, reviews family functionality through two basic dimensions: family cohesion and adaptability (13). Cohesion refers to the emotional connections existing among the family elements and describes the way the family understands the balance between union and individuation.There are four levels of cohesion: disengaged, separated, connected, and enmeshed. It is assumed that the central levels of cohesion (separation and connection) are the most desirable for optimal family functioning, since they allow family members to freely experience separation and connection, while being separated and connected to their family at the same time. Extreme leves of cohesiveness (disengaged and enmeshed) are generally seen as a problem in family functioning (13). Adaptability refers to the balance between stability and change. A familial system's adaptability describes its flexibility in changing its structure, roles and relational rules in response to different situations and developmental stress. There are also four levels of adaptability: rigid, structured, flexible, and chaotic. It is believed that the central levels are better for the successful functioning, while the extremes are problematic. According to Olson's theory, in order for a family to be functional, it must be flexible in terms of adaptability and separated in terms of cohesiveness (13).

To the authors' knowledge, there have been no studies using Olson's Circumplex Model for the assessment of the functionality in families with adolescents who have engaged in NSSI in our surroundings. Hence the idea for precisely examining these dimensions of family functionality, which would contribute to a better understanding of this complex phenomenon from the aspect of family functioning.

Aims

The aim of the study was to determine whether families with adolescents who have engaged in NSSI differ in functionality from families with no NNSI adolescents on FACES III. The hypothesis was that families with NSSI adolescents are generally less functional than families with no NSSI adolescents on FACES III.

Methodology

The study was conducted in the period from December 2017 to December 2018 at the Department of Child and Adolescent Psychiatry, Center for Mental Health Protection, Clinical Center Niš, Serbia.

Sample description

The study included a total of 99 adolescents divided into two groups: the clinical and control one. The clinical group included adolescents who had engaged at least once in deliberate self-injury, confirmed by an objective physical examination and anamnestic interview with the respondents. The control group consisted of adolescents with no history of NSSI or another confirmed psychiatric disorder. The respondents in the groups were of both sexes, aged 14-18, chosen by convenience sampling. The clinical group consisted of respondents who were treated at the Department of Child and Adolescent Psychiatry at the Department of Mental Health Protection at the outpatient clinic or hospital, and were willing to participate in the study, which they confirmed by signing an informed consent. The control group consisted of adolescents from the general population, without the diagnosis of psychiatric disorders.

Instruments

The study used FACES III (Family Adaptability and Cohesion Evaluation Scale), i.e. a questionnaire for assessing family adaptability and cohesion (14). Family cohesion represents the degree of separation or connection among family members and differentiates among four levels of cohesion: disengaged, separated, connected, and enmeshed. There are also four levels of adaptability: rigid, structured, flexible, and chaotic. FACES III consists of 10 cohesion items and 10 adaptability items. The instrument asks the respondents to indicate how frequently the described behavior occurred in his or her family on a Likert scale from 1 (almost never) to 5 (almost always). The total scores of cohesion and adaptability respectively ranged from 10 points to 50 points. Internal consistency was also tested in the sample of adolescents and was deemed acceptable (Cronbach α = 0.76 for family adaptability; α = 0.81 for family cohesion). A general questionnaire designed for the purpose of this study included information regarding the respondents' gender and age, as well as family structure and socio-economic status.

Data processing

The data are presented in the form of an arithmetic mean and a standard deviation, i.e. in the form of absolute and relative numbers. Continuous variables were compared using the t test, while Chi-square test was used to compare the observed and expected frequencies. The hypothesis was tested with a significance threshold of $p < 0.05$. Statistical data processing was performed using the SPSS 16.0 software package.

Results

The study included 49 respondents within the clinical group and 50 respondents in the control group. The groups were age-balanced ($p = 1.000$). There were significantly more female respondents in the clinical group than in the control group (69.4% vs 40.0%, $p = 0.006$) (Table 1). Families with two children were dominant in both groups (63.3%, and 72.0%, $p = 0.416$). There were more families with divorced parents in the clinical group (30.6%), compared to the control group (14.0%), but no statistically significant difference was found ($p = 0.081$). In both groups, the socio-economic status was predominantly average (63.3%, or 70.0%, $p = 0.605$) (Table 2).

Disengaged and separated families (51% and 24.5%, respectively) were dominant in the clinical group, while the dominant ones in the control group were separated (53.1%) and connected families (26.5%). There was a statistically significant difference in cohesion between the two examined groups ($p < 0.001$) (Table 3).

Disengaged and separated families (51% and 24.5%, respectively) were dominant in the clinical group, while the dominant ones in the control group were separated (53.1%) and connected families (26.5%). There was a statistically significant difference in cohesion between the two examined groups ($p < 0.001$) (Table 3).The clinical group was dominated by rigid (51.0%) and chaotic (22.4 %) families, while in the control group they were flexible (42.9%) and structured (36.7%). The results showed a statistically significant difference in adaptability between the examined groups ($p < 0.001$) (Table 3).

The clinical group had predominantly disengaged/rigid families (36.7%), while the presence of all other levels was less than 10.0%. The control group was dominated by flexible/separated (30.0%) and structured/separated families (20.0%). All other family types were present in a significantly lower percentage (Table 4).

Table 1. Demographic characteristics in the clinical and control group

Parameter	Clinical group		Control group		p
Gender	Number	%	Number	%	
Male	15	30.6	30	60.0	0.006[1]
Female	34	69.4	20	40.0	
Age†	15.00 ± 1.22		15.00 ± 1.21		1.000[2]

[1]Chi-square test, [2]t test, †Arithmetic mean±standard deviation

Table 2. Family structure and economic status in the clinical and control group

Parameter	Clinical group		Control group		p[1]
	Number	%	Number	%	
№ of children in the family					
One	6	12.2	7	14.0	0.416
Two	31	63.3	36	72.0	
More	12	24.5	7	14.0	
Divorce					
Yes	15	30.6	7	14.0	0.081
No	34	69.4	43	86.0	
Socio-economic status					
Below average	16	32.7	12	24.0	0.605
Average	31	63.3	35	70.0	
Above average	2	4.1	3	6.0	

[1]Chi-square test

Table 3. Cohesion and adaptability in the examined groups

Parameter	Clinical group		Control group		p[1]
Cohesion	Number	%	Number	%	
Disengaged	25	51.0	5	10.2	< 0.001
Separated	12	24.5	27	53.1	
Connected	5	10.2	13	26.5	
Enmeshed	7	14.3	5	10.2	
Adaptability					
Rigid	25	51.0	5	10.2	< 0.001
Structured	8	16.3	19	36.7	
Flexible	5	10.2	21	42.9	
Chaotic	11	22.4	5	10.2	

[1]Chi-square test

Table 4. Distribution of families according to the dimensions of cohesion and adaptability in the examined sample

Clinical group								
Cohesion	**Disengaged**		**Separated**		**Connected**		**Enmeshed**	
Adaptability	Number	%	Number	%	Number	%	Number	%
Rigid	18	36.7	4	8.2	3	6.1	0	0.0
Structured	2	4.1	3	6.1	1	2.0	1	4.1
Flexible	1	2.0	2	4.1	0	0.0	2	4.1
Chaotic	4	8.2	3	6.1	1	2.0	3	6.1
Control group								
Cohesion	**Disengaged**		**Separated**		**Connected**		**Enmeshed**	
Adaptability	Number	%	Number	%	Number	%	Number	%
Rigid	1	2.0	2	4.0	2	4.0	0	0.0
Structured	2	4.0	10	20.0	5	10.0	2	4.0
Flexible	1	2.0	15	30.0	5	10.0	0	0.0
Chaotic	1	2.0	0	0.0	1	2.0	3	6.0

Discussion

The study found that adolescents who had engaged in NSSI were more likely to have divorced parents in comparison with the control group. While some studies do not find an increased incidence of NSSI in children with divorced parents (15, 16), others confirm this connection (17). One study found a significant increase in the incidence of NSSI in children whose parents remarried (18). It is believed that increased demands in performing parental functions, lack of support from the biological partner, and often low socio-economic family status can lead to an insufficient emotional and physical presence of the parent with whom the child lives. Regardless of age, research shows that 25% of children from incomplete families (compared to 10% of children with both parents) have difficulties in school, behavioral problems (delinquent behavior, emotional outbreaks), mood disorders, low self-esteem and unsuccessful intimate relationships (19). In terms of the socio-economic status, there was no significant difference among the families in the examined groups, which is in accordance with literature data (20).

It is a unified view that families with adolescents are in a very specific life cycle phase that sets new tasks and goals before both the adolescent and their family. The family dynamics significantly changes when a child goes through the period of adolescence. Due to the essential importance for the development of young people, adolescence is a period in which all weaknesses and failures in the family system are revealed. In case a family fails to meet the need for security and love, when there is emotional disengagement of family members or excessively rigid boundaries and the inability to establish new ones and reorganize family rules, the adolescent will show their dissent openly, turbulently or

specifically - through various emotional problems or specific psychopathological symptoms.

One of the most common findings mentioned in the literature on the non-suicidal self-injury in adolescents is that individuals who engage in NSSI come from families which are dysfunctional at multiple levels (21). Dysfunctional families are characterized by disturbed structures, boundaries, roles, leadership, unnatural alliances, and failure to solve problems. Dysfunctional families are inflexible and poorly adaptable, their interactions do not change according to the child's developmental needs and events in the surrounding. The literature seems to confirm associations between family functioning and various forms of dysfunction, especially depression and anxiety symptoms (22) as well as suicidal behavior (23) in adolescents.

The study found that the functionality of families with NSSI adolescents is significantly different from the family functionality in the control group in both examined dimensions on FACES III – cohesiveness and adaptability. The largest number of adolescents in the study group came from disengaged families, i.e.families with extreme low cohesion. Members of such family systems very rarely interact with other family members, and significantly promote separation and independence, at the expense of closeness and togetherness. In disengaged family systems, everyone usually performs their own tasks and prefers to have their own time, interests and space. Family members cannot rely on each other when it comes to support or solving problems (13).

Emotional attachment and family support facilitate psychological development in adolescence. Some authors (24) showed that perception of family cohesion and adaptability were associated with adolescents' ability to express emotions and to manage stressful situations through positive coping skills. In contrast, low cohesion and poor satisfaction with family relationships represent a serious risk for the psychological adjustment of adolescents. Those adolescents who have negative perceptions of family relationships have more psychopathological symptoms when dealing with stressful situations than adolescents with harmonious family relationships (25). Studies confirm that low family cohesion indirectly increases the risk of NSSI in children (through emotional regulation), especially in females (12).

While some studies show no differences in NSSI risk associated with family adaptability (26), the other results point out that elevated risk for NSSI is associated with greater family rigidity (27). The respondents from the clinical group in our study significantly more often come from families with low adaptability compared to the respondents from the control group, i.e. they come from families with rigid family functioning. Rigid families exhibit excessive rigidity and control, no negotiation, and most decisions are made by the leader. The rules of conduct are therefore strict and limited and there is limited communication among family members (13).

The results of our study on the functioning of families with NSSI adolescents indicate that the examined families are, in most cases, disengaged in terms of cohesion and rigid in terms of adaptability. According to Olson's Circumplex Model, this type of family is considered to be extreme in terms of both dimensions of family functioning (13). The limitation of this study is in its methodology and refers to a small sample of respondents and a method of assessing family functioning. Having in mind that a self-assessment tool was used (which always carries the risk of subjectivity), it is recommended that more family members be included in the following studies on this topic to verify the conformity of their assessment, which would give a more realistic picture of family functionality.

With regard to the implications of the obtained results on the relationship between family functionality measured by FACES III and NSSI in adolescents, we can point out that this simple family self-assessment can be useful in dealing with high risk families. It is also possible to effectively single out the cases of young people where work with the family would be of significant importance, while functional scores for some of the FACES III dimensions could be of benefit to family therapists.

Conclusion

The study revealed that families with adolescents who had engaged in NSSI differed from the ones with no NNSI adolescents in terms of functionality on FACES III. It was shown that families with adolecsents who had engaged in NSSI are more commonly grouped within the zones of low family cohesion and adaptability in comparison to the control group. These results confirmed the previously obtained results on the connection between family dysfunctionality and engaging in NSSI behavior in adolescents and can have clinical implications in working with the vulnerable group of adolescents and their families.

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KARAKTERISTIKE PORODICA ADOLESCENATA SA NESUICIDALNIM SAMOPOVREĐIVANJEM

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Nesuicidalno samopovređivanje (engl. NSSI) među adolescentima je kompleksni fenomen determinisan mnogobrojnim individualnim, porodičnim i sociokulturalnim faktorima.

Cilj istraživanja bio je da se utvrdi da li se porodice adolescenata koji se samopovređuju razlikuju po stepenu funkcionalnosti u odnosu na porodice u kojima nema samopovređivanja adolescenata.

U istraživanju je učestvovalo 99 adolescenata, oba pola, uzrasta od 14 do 18 godina, koji su podeljeni u dve grupe: kliničku i kontrolnu. Kliničku grupu činili su adolescenti koji su načinili najmanje jednu namernu samopovredu, što je potvrđeno objektivnim kliničkim pregledom i anamnestičkim intervjuom ispitanika. Kontrolnu grupu činili su adolescenti koji nemaju istoriju samopovređivanja, niti drugi psihijatrijski poremećaj. U istraživanju je korišćen upitnik sačinjen za potrebe istraživanja i FACES III skala (Family Adaptability and Cohesion Evaluation Scale).

U kliničkoj grupi dominiraju razjedinjene (51,0%) i udaljene (24,5%) porodice, a u kontrolnoj grupi dominiraju udaljene (53,1%) i povezane (26,5%) porodice. U kliničkoj grupi dominiraju rigidne (51,0%) i haotične (22,4%) porodice, a u kontrolnoj grupi fleksibilne (42,9%) i strukturirane (36,7%). Utvrđeno je da postoji statistički značajna razlika u kategorijama kohezivnosti i adaptabilnosti među ispitivanim grupama. U kliničkoj grupi najviše je razjedinjenih/rigidnih porodica (36,7%), a učestalost svih ostalih porodica manja je od 10,0%. U kontrolnoj grupi dominiraju fleksibilne/odvojene porodice (30,0%) i strukturirane/odvojene porodice (20,0%).

Ovim istraživanjem utvrdili smo da se porodice adolescenata, koji se samopovređuju razlikuju po stepenu funkcionalnosti u odnosu na porodice u kojima nema samopovređivanja adolescenata, mereno skalom FACES III. Rezultati su potvrda, na uzorku naše populacije, ranije dobijenih rezultata o vezi između porodične disfunkcionalnosti i samopovređujućeg ponašanja adolescenata i mogu imati kliničke implikacije u radu sa vulnerabilnom grupom adolescenata i njihovim porodicama.

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Ključne reči: *nesuicidalno samopovređivanje, porodična funkcionalnost, FACES III*

Case report **UDC: 613.83:[616.127+616.345**
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MORPHOMETRIC ANALYSIS OF MYOCARDIAL AND INTERSTITIAL CONNECTIVE TISSUE IN THE HEROIN ADDICTS: A CASE-CONTROL STUDY

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Sudden deaths are mostly caused by substance abuse and overdose, whether they were used separately or combined with other substances having depressor effect on the central nervous system. Apart from the pathology associated with the central nervous system and the lungs, a large portion in sudden death occurrence in opiate abusers lies also in the pathology of cardiovascular system, especially the heart. A group of 42 long-term heroin addicts was observed (35 men and 7 women), aged 18-48 years, whose sudden death was related to heroin abuse, whether heroin taken intravenously (38 cases) or by sniffing (4 case). Myocardial tissue samples were processed with the modified Movat's staining procedure and analysed statistically. In the current study, standard histological examinations of the heart muscle in heroin addicts found cardiomyocyte hypertrophy and interstitial and/or perivascular fibrosis with statistical significance ($p < 0.001$) in comparison with the control group. In 24% (10 cases) of all examined cases, histological picture in the heart muscle matching the picture of the acquired cardiomyopathy was determined. We believe that in cases of repeated intoxication, even in the doses of heroin that are not inherently lethal, sudden changes in hemodynamics and disturbances in the rhythm of operation of such altered and more vulnerable heart muscle can affect the occurrence of sudden cause of death.

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Key words: *drug abuse, heroin, myocardial fibrosis, cardiomyocyte hypertrophy*

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Introduction

Substance abuse is a major social and health issue predominantly affecting younger population (1-4). Sudden deaths are mostly caused by substance abuse and overdose (5-7), whether they were used separately or combined with other substances having depressor effect on the central nervous system.

According to numerous studies performed on autopsy material, it is clear that besides the pathology associated with the central nervous system and the lungs, a large portion in sudden death occurrence in opiate abusers lies also in the pathology of cardiovascular system, especially the heart.

Numerous studies are dealing with this issue, and especially those related to heart and heart muscle alterations caused by cocaine and amphetamines abuse (8-14). These substances, acting as cardiovascular system stimulants, lead to the increase in heart rate and vasoconstriction of the coronary blood vessels, which in susceptible individuals may lead to disturbances in cardiac rhythm and/or myocardial infarction (4). In addition, it was noticed in the autopsy material that in such cases there is a slight chronic inflammatory heart muscle infiltration, multiplication of connective tissue (6), as well as cardiomyocyte hypertrophy (15-17).

The issue of cardiac pathology in relation to heroin abuse has been insufficiently considered, despite the fact that at the present time heroin addiction is very important and growing problem, especially among young people. Myocardial ischemia and

infarction as well as a poorly characterized cardiomyopathy have been reported (18, 19).

The aim of this paper was to present the morphological changes in the heart muscle and their relationship with the immediate cause of death in heroin addicts.

Materials and methods

A group of 42 long-term heroin addicts was observed (35 men; 7 women), aged 18-48 years, whose sudden death was related to heroin abuse, whether heroin taken intravenously (38 cases) or by sniffing (4 cases). For 11 cases there are no data about the length of the heroin usage, 13 cases used heroin < 5 years, 11 cases used heroin for 5-10 years, and 7 cases used heroin > 10 years.

Autopsy results of 10 heroin addicts (24%) showed hypertension or hypertrophy of left cardiac ventricular wall, and these cases were not further examined.

The control group included 10 persons, aged 22–35 years, for whom the data on antemortem opiate abuse and existence of cardiac disease was not obtained and the cause of their death was the traffic trauma (severe traumatic shock, haemorrhage or polytrauma).

All autopsies were performed within 48 hours of the death.

Histological examination

Cardiac muscle samples were systematically sampled from standard positions (front, rear and side wall of the left ventricle, cranial part of the interventricular septum, anterior and posterior wall of the right ventricle). From each standard position, three cardiac muscle tissue samples were taken. The tissue samples were stored in a neutral, buffered 4% formalin solution, for 18-24 hours, dehydrated in ethanol of progressive concentration and embedded in paraffin wax. From the paraffin blocks, the tissue was cut into 4-5μm thin samples, routinely stained with the hematoxylin and eosin method, while the cardiac muscle fragments were processed with the modified Movat's staining procedure also. The significance of the modified Movat's staining usage is the easier and shorter procedure lasting (about 70 min); and shows minimal overstainig and maximal efficiency in differential histochemical staining (20). Microscopic analysis was performed by Leica DM1000 (Wetzlar, Germany), with digital microscopic camera Leica EC3 (Wetzlar, Germany) with Leica LAS EZ imaging software V1.8.0.

Morphometric and image analysis

The thickness of cardiomyocytes was considered, as well as the ratio between the areal fractions of the cardiomyocytes and fibrous connective tissue. The morphometric analysis was performed in ImageJ program ver 1.50

http://rsb.info.nih.gov/ij/index.html).

Before analysis, system was calibrated first. Digital cross-sectional views of modified Movat's pentatachrome stain were observed and photographed under magnification x200 and in a further procedure used to measure the stereological parameters of connective tissue in myocardium. By random selection method, 30 fields of vision were selected per one case. Across digital images of the aforementioned field of view is superposed is a digital line network system generated by the software using the options Plugins-Analyse-Grid (Figure 1).

The volume density of the connective tissue (VVT) was calculated as the number of points affecting connective tissue (PVT) in the appropriated field of vision and the total number of points of the test system (PT) (VVT = PVT / PT). From the obtained values of the volume density of all analyzed fields of vision, the average value of the volume density of each case was expressed in percentages, and mean values per case were shown.

Morphometric analysis of the cardiomyocytes was performed under magnification x400. The thickness of 10 cardiomyocytes randomly selected per field was measured and mean values per case were shown (Figure 2).

Statistical analysis

Differences among groups were examined by F–Test for testing statistical significance of the variance, and by Student's t–test for two independent samples. Values are expressed as the mean with standard error (SE). Values of $p \leq 0.05$ were considered statistically significant. All statistical analyses were performed with software Jandel SigmaStat 2.0.

Results

Myocardial Hypertrophy

F–Test for testing statistical significance of the variance of two independent samples revealed the existence of statistically significant difference in the study groups ($p < 0.05$). Consequently, testing the difference between the average values of cardiomyocytes thickness in the group of addicts and the control group was performed using the Student's t–test for two independent samples with unequal variances. The results of this test showed that the average thickness of cardiomyocytes in the group of heroin addicts was statistically and significantly higher compared to the control group ($p = 0.001$; Table 1) which represents the moderate intensity difference (Cohen's $d = 0.23$).

Ratio between the areal fraction of the interstitial connective tissue and areal fraction of the myocardium

F–test for testing statistical significance of the variance of two independent samples also indicated the existence of statistically significant differences in the groups studied ($p < 0.001$). Student's t–test for two independent samples with unequal variances showed that the average areal fraction of interstitial connective tissue in relation to the areal fraction of

cardiomyocyte, in the group of heroin addicts was statistically significant higher than in the control group ($p < 0.001$; Table 1) which represents the moderate intensity difference (Cohen's $d = 0.4$).

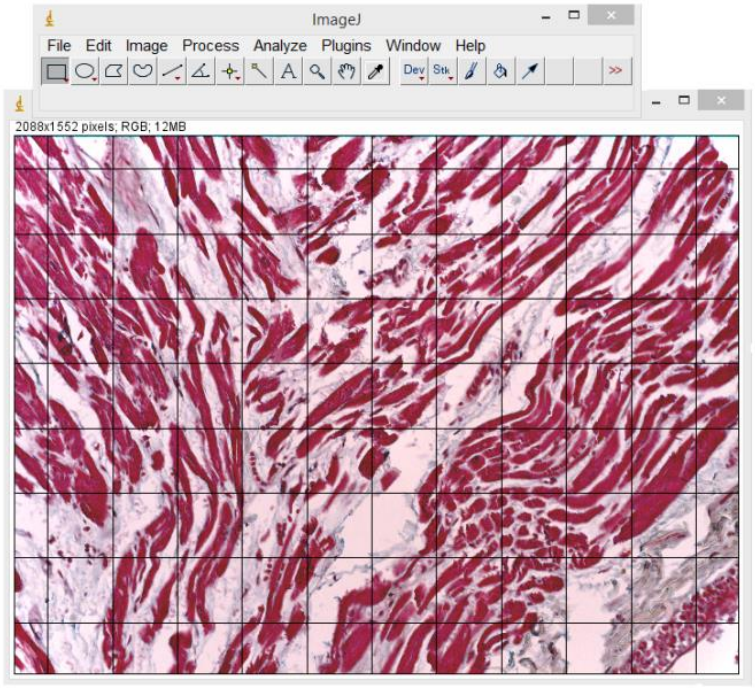

Figure 1. Measuring of the stereological parameters of connective tissue in myocardium (modified Movat's; original magnification x400) in ImageJ program

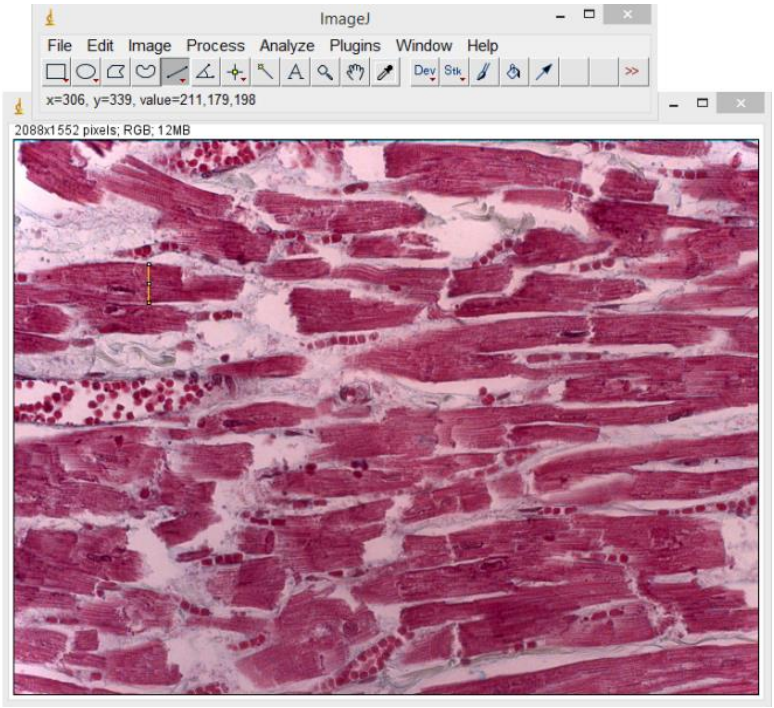

Figure 2. Morphometric analysis of the cardiomyocytes (modified Movat's; original magnification x200) in ImageJ program

Table 1. Myocardial thickness and ratio between the areal fraction of the interstitial connective tissue and areal fraction of the myocardium of the evaluated cases

№	Parameter			
	Myocardial thickness (μm)		Interstitial connective tissue/ myocardium ratio (%)	
	Heroin addicts	Control group	Heroin addicts	Control group
1.	14.414	13.686	41.45	33.88
2.	14.814	12.018	33.63	32.34
3.	14.265	12.180	35.38	34.79
4.	15.789	11.234	40.40	30.42
5.	16.994	12.142	39.03	30.85
6.	16.298	10.828	38.18	31.73
7.	14.245	11.985	44.29	29.79
8.	15.452	10.816	43.63	30.01
9.	15.958	11.169	40.30	29.15
10.	15.819	10.340	36.18	29.00
11.	12.644		40.00	
12.	8.022		38.16	
13.	7.588		29.52	
14.	10.774		31.66	
15.	14.376		49.48	
16.	12.592		40.53	
17.	7.936		40.09	
18.	9.209		44.60	
19.	7.298		45.06	
20.	11.006		40.68	
21.	15.144		45.81	
22.	16.488		44.44	
23.	15.06		30.63	
24.	15.725		32.82	
25.	15.421		33.53	
26.	14.439		35.67	
27.	15.652		31.75	
28.	13.547		34.06	
29.	15.323		31.07	
30.	14.731		35.43	
31.	14.389		33.13	
32.	17.06		31.59	
Mean	13.7*	11.6	34.9*	33.4
SE	0.51	0.31	0.7	0.6

SE – Standard error
* – heroin addicts vs. control group p = 0.001

Histological examination of other organs, in most cases, among other findings, found acute blood stasis in the internal organs and brain swelling. Hemorrhagic pulmonary edema was found in 40%

of the tested cases, while in others cases, pulmonary edema was found in low to medium degree of severity, without hemorrhagic component. In all cases in the group of heroin addicts the interstitial fibrosis was found (Figure 3), while in 22 cases (70%), in addition to the interstitial, the perivascular fibrosis was also found (Figure 4).

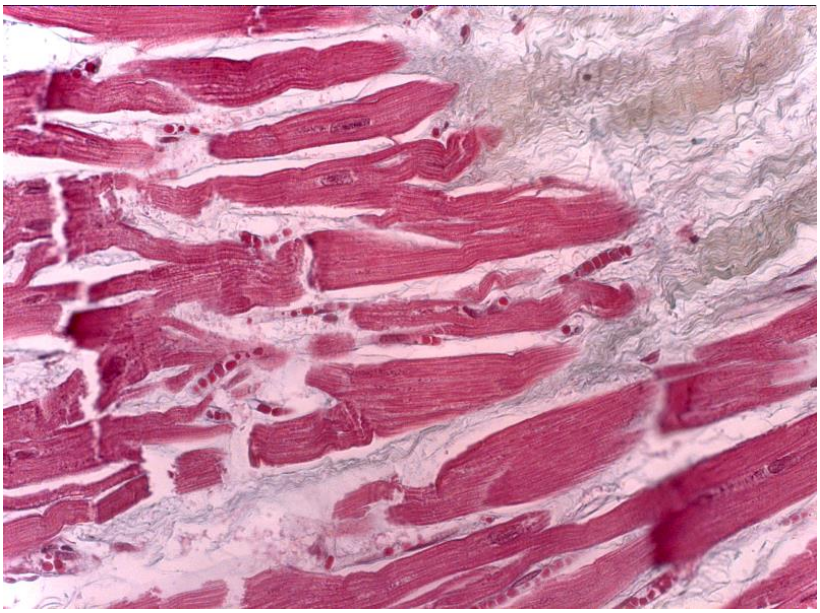

Figure 3. Fibrosis in the interstitium (modified Movat's; original magnification x400)

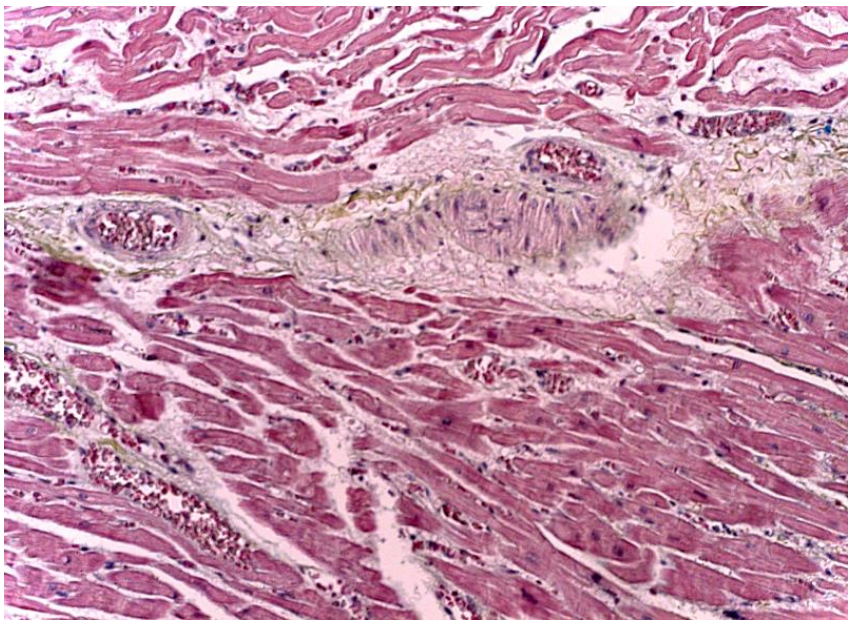

Figure 4. Concentric fibrosis around small artery (modified Movat's; original magnification x200)

Six cases (19%) from the group of heroin addicts also had lipomatosis, which was predominantly expressed in the region of the front wall muscle of the right ventricle. There was also an increase in the number of mast cells in the interstitium of the heart muscle particularly in the level of the

multiiplied connective tissue surrounding the small blood vessels in heroin addicts compared to the control group.

In the examined group of heroin addicts, the concentration of morphine measured in femoral blood sample ranged from 0.067 to 2.03mg/L, and 6–monoacetylmorphine (6–MAM) concentration ranged from 0.06 to 0.87 mg/L. In 10 cases (31.25%), ethanol was detected in blood sample in the range from 0.09‰ to 3.05‰, while diazepam in therapeutic or lower concentration together with heroin metabolites were detected in 9 cases (28.13%).

Discussion

Looking at data from the literature, it can be observed that in explaining the occurrence of death among heroin addicts the focus is on the malignant hemorrhagic pulmonary edema as a consequence of acute heroin intoxication. Although it is unknown precisely why it arises, the possible reasons for occurrence of the malignant hemorrhagic pulmonary edema are considered to be hypoxia caused by the increased capillary permeability, cardiomyocyte contractility depression, centrally induced respiratory depression, primary toxic heroin effects on the alveolar-capillary membrane and acute anaphylactic shock (21). The toxic effects of heroin and its direct metabolites directly to the heart muscle with an aim to explain cardiac failures as the possible cause of death in heroin addicts were considered sporadically (1-5). The tests conducted so far were mainly based on existing chronic inflammatory changes in the heart muscle, while morphological changes resulting from the inflammatory processes, in explaining the immediate cause of death in heroin addicts, were generally perceived as supportive rather than dominant (15, 22).

In the current study, standard histological examinations of the heart muscle in all cases so far found cardiomyocyte hypertrophy and interstitial and/or perivascular fibrosis, while in 24% of the examined cases, histological picture in the heart muscle matching the picture of the acquired cardiomyopathy was determined (18-19). Significance of the difference between the areal fractions of the connective tissue in the tested group of heroin addicts and the control group was established, as well as statistically and significantly thicker cardiomyocytes in the group of heroin addicts ($p < 0.001$), which is in agreement with the literature data and the pathohistological mechanism of the cardiac muscle response to toxic effects of heroin. Namely, the morphological changes in the cardiac muscle that occur in response to the effects of heroin are probably of hypoxic type and are based on sporadic death of certain cardiomyocytes along with the hypertrophy of the preserved cardiomyocytes and reduction in their total number. In the process of remodeling the damaged myocardium after the death of cardiomyocytes, it is in these places that fibrosis occurs in the process of excessive accumulation of extracellular matrix (ECM), predominantly composed of collagen.

Research conducted on experimental animals determined that heroin and morphine reduce blood pressure and heart rate, explained by histamine release, especially from mast cells (15, 23). In 60% of studied sudden death cases among heroin addicts, the routine processing of the tissue did not establish the existence of malignant hemorrhagic pulmonary edema, the occurrence of which require a certain period of time. Also, the fact that the finding of the increased number of mast cells in the cardiac muscle, where the impact of histamine from their depos in the case of the repeated intoxication can further contribute to the stated cardiovascular abnormalities (15).

Conclusion

Test results clearly indicate that the effect of chronic heroin abuse on the cardiac muscle causes cardiomyocyte hypertrophy and multiplication of fibrous tissue. In cases of repeated intoxication, especially in patients in the methadone therapy, even in the doses of heroin that are not inherently lethal, sudden changes in hemodynamics and disturbances in the rhythm of operation of such altered and more vulnerable heart muscle, can affect the occurrence of sudden cause of death. The disruption in morphologically altered cardiac muscle functioning in repeated heroin intoxication cannot be a priori taken only as a supportive factor for the occurrence of death, but as a competitive cause of death.

Namely, in most cases, and especially in cases where heroin was taken together with alcohol and/or benzodiazepines, morphological changes in the heart muscle can be considered as a significant factor in the occurrence of death.

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MORFOMETRIJSKA ANALIZA MIOKARDIJALNOG I INTERSTICIJALNOG VEZIVNOG TKIVA HEROINSKIH ZAVISNIKA: STUDIJA SLUČAJEVA

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Naprasne smrti najčešće su izazvane zloupotrebom raznih opioidnih supstanci i predoziranjem, bez obzira da li su korišćene ponaosob ili u kombinaciji sa drugim supstancama, izazivajući depresorni efekat na centralni nervni sistem. Pored patologije centralnog nervnog sistema i pluća, veliki broj slučajeva naprasnih smrti opioidnih zavisnika leži u patologiji kardiovaskularnog sistema, naročito u patologiji srca. Analizirana je grupa od 42 dugogodišnja heroinska zavisnika (35 muškaraca i 7 žena), starosti od 18 godina do 48 godina, čije su iznenadne smrti bile u vezi sa zloupotrebom heroina, bez obzira da li je heroin uzet intravenski (38 slučajeva) ili ušmrkavanjem (4 slučaja). Isečci tkiva miokarda obrađivani su modifikovanom Movatovom procedurom bojenja i statistički analizirani. U aktuelnoj studiji, standardna histološka analiza srčanog mišića heroinskih zavisnika pokazala je zadebljanje kardiomiocita i umnožavanje intersticijale i/ili perivaskularne fibroze sa statistički signifikantnom razlikom ($p < 0,001$) u poređenju sa kontrolnom grupom. U 24% (10 slučajeva) svih ispitivanih slučajeva histološka slika srčanog mišića odgovara stečenoj kardiomiopatiji. Smatramo da u slučajevima ponavljanih intoksikacija, čak i u slučajevima gde aplikovane doze heroina nisu bezuslovno letalne, nagle promene u hemodinamici i distribuciji u ritmu rada izmenjenog i vulnerabilnog srčanog mišića mogu uticati na nastanak naprasne smrti.

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Ključne reči: *zloupotreba droge, heroin, miokardijalna fibroza, hipertrofija miokardiocita*

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VASCULOGENIC POTENTIAL OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS *IN VITRO* INDUCED INTO OSTEOBLASTS APPLIED WITH PLATELET-RICH PLASMA IN AN ECTOPIC OSTEOGENIC MODEL

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Bone tissue deficiencies can be caused by fractures, bone loss or tumors. Insufficient vascularization is the main problem in successful bone tissue regeneration. In order to improve vascularization during bone tissue regeneration, a promising methods have been developed in the field of bone tissue engineering (BTE) by using adipose-derived stem cells (ADSCs). The aim of this study was to examine vasculogenic potential of ADSCs *in vitro* induced into osteoblasts (OBs) combined with platelet-rich plasma (PRP) and bone mineral matrix (BMM) in ectopic osteogenic implants, and compare it with implants consisting of uninduced ADSCs, PRP and BMM. ADSCs isolated from mice epididymal adipose tissue cultivated up to the third passage were divided into two groups: ADSCs *in vitro* induced into OBs and ADSCs expanded without osteoinduction. Based on biological triad principle, two types of implants were composed: implants containing BMM, PRP and ADSCs *in vitro* induced into OBs (BPO implants), and implants containing BMM, PRP and uninduced ADSCs (BPU implants). The BPO implants had higher expression of endothelial-related genes compared to the BPU implants. Additionally, VCAM-1 immunoexpression increases during *in vivo* experimental period in the BPO implants, while in the BPU implants VCAM-1 immunoexpression decreases during *in vivo* experimental period. Therefore, vasculogenic potential of ADSCs *in vitro* induced into OBs and combined with PRP and BMM in ectopic osteogenic implants is higher compared to the implants composed of uninduced ADSCs, PRP and BMM, which makes implants enriched with ADSCs induced into OBs good candidates for improving vascularization in bone tissue-engineered constructs.

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Introduction

Bone tissue deficiencies can be caused by fractures, bone loss or tumors (1). Insufficient vascularization is still the main problem in successful bone tissue regeneration. In order to improve vascularization during bone tissue regeneration, as an alternative to osteosynthetic stabilizing techniques and autologous bone transplantations (2), promising methods have been developed in the field of bone tissue engineering (BTE) (1). For the construction of successful bone grafts, it is of vital importance to improve angiogenesis in the early stage as well as in the long-term process of ossification (3). To accomplish this goal, different approaches have been applied (4-8).

A special place in BTE belongs to cell-based therapies that include the application of adipose-derived mesenchymal stem cells (ADSCs) (9-11). ADSCs secrete numerous growth factors (12) with a significant impact on tissue regeneration (13) so they can be applied *in vitro*-expanded, uninduced (14) for bone implant construction. On the other hand, ADSCs posses the ability for *in vitro* differentiation into various cell types including osteoblasts (OBs) (15, 16) which represents one more possibility for their application in BTE. ADSCs, whether induced into OBs or not, can be applied in BTE in combination with natural source of growth factors

and a biomaterial that represents bone mineral matrix (BMM) carrier for growth factors and cells. In this manner, the principle of biological triad is respected (16-20).

Data related to the impact of uninduced ADSCs as well as osteoinduced ADSCs on vascularization during bone tissue regeneration vary depending on the types of chosen experimental model, species of chosen experimental animals, types of biomaterial, source of growth factors, localization of fat taken for the isolation of ADSCs. Motif of this study was to see whether osteogenic induction of ADSCs is preferable over application of uninduced ADSCs in improving vascularization of ectopic osteogenic implants that were composed based on biological triad principle. Vascularization was observed via endothelial-related gene expression and immunoexpression of vascular cell adhesion protein-1 (VCAM-1) because endothelial cells and their progenitors are necessary for vascular network formation (21, 22), while VCAM-1 is a protein involved in molecular mechanism of blood vessel maturation (23).

Aim

The aim of this study was to examine vasculogenic potential of ADSCs *in vitro* induced into osteoblasts (OBs) combined with platelet-rich plasma (PRP) and BMM in ectopic osteogenic implants, and compare it with the implants consisting of cultivated, uninduced ADSCs, PRP and BMM.

Materials and methods

Experimental animals

The present research was done on syngeneic, male, BALB/c mice (Military Medical Academy, Belgrade, Serbia), each at the age of eight weeks and weighting between 22 g and 24 g. Local Ethical Committee approved all procedures that were done on the animals (approval number 01-2857-8). Whole research was conducted according to the Animal Welfare Act (Republic of Serbia). The mice were treated in accordance with the regulation of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS no. 123 Appendix A)".

Isolation and expansion of ADSCs from stromal vascular fraction

ADSCs were obtained out of the adipose tissue that surrounds epididymis of BALB/c mice conforming to our earlier described protocols (16-19). In brief, after extraction from the animals, maceration and washing in the sterile conditions, adipose tissue was subjected to digestion in the water bath, at 37 °C, by using collagenase type I (Sigma-Aldrich, Hamburg, Germany) solution at the concentration of 2000 i.j. in low glucose Dulbecco's Modified Eagles Minimal Essential Medium (DMEM, PAA Laboratories, Pasching, Austria). When 45 minutes of digestion had passed, the process was stopped by using pre-warmed DMEM supplemented with 10% fetal bovine serum (FBS), 2 mmol/L of L-glutamine and 1% antibiotic-antimycotic solution (all from PAA Laboratories GmbH, Pasching, Austria)−complete DMEM (cDMEM). Isolated cells contained within stromal vascular fraction (SVF) were passed through 180 μm mesh, centrifuged (1500 rpm, 10 min, 4 °C) and after discarding white, "lipid" ring from the top of the tube, the cells were counted and seeded at the density of 10^6 per 25 cm^2 growth area of cell culture flask. Stromal vascular fraction cells (SVFs) were cultivated in cDMEM. In order to expand ADSCs from SVF and to eliminate other types of cells, nonadherent and terminally differentiated, SVFs were expanded up to the third passage (P03).

Osteogenic differentiation of ADSCs

At P03, the cells were counted and divided into two 24 well plates. Cell density per well was 1×10^4. In the first plate, ADSCs were subjected to osteogenic differentiation. Osteogenic media was prepared by adding the following supplements into cDMEM – 10^{-8} M dexamethasone, 50 μg/mL ascorbic acid and 10 mM β-glycerophosphate (24). The cells were *in vitro* induced into OBs for 15 days, since we have previously shown that osteoblast-related genes expression and osteocalcin immunoexpression are the highest at this time point during osteogenic differentiation (16). In the second plate, ADSCs were expanded in cDMEM for twelve days. These uninduced ADSCs were the control group. Both types of cell cultures were monitored on in--verted light microscope Axio Observer. Z1 that is equipped with AxioCam HR camera (Carl Zeiss, Oberkochen, Germany). After cultivation time ended, these two cell cultures were passaged, counted and used for the implant construction.

Preparation of implants and implantation procedure

Based on biological triad principle, implants were composed out of three components – biomaterial, source of growth factors and cells.

Deproteinized, sterilized bovine bone Bio-Oss®, size S (Geistlich-Pharma, Wolhusen, Switzerland) that represents a BMM was used as a carrier for growth factors and cells.

PRP was applied as a natural source of growth factors and prepared in two steps (25) in order to get 4-6 times higher platelets concentration in comparison with the one in physiological conditions. Platelets were counted manually in the Malassez counting chamber (Paul Marienfeld GmbH & Co. KG, Lauda-Konigshofen, Germany) and established number of these blood components was $1.89 \pm 0.5 \times 10^6/\mu l$. We used 10% v/v of PRP in the liquid implant component since this concentration has shown to be the optimal for combining with ADSCs (14, 26, 27).

Two types of cell cultures were used in this experiment: uninduced ADSCs cultivated in cDMEM for 12 days after P03 and ADSCs *in vitro* induced into OBs for 15 days after P03. Regardless of the

used cell type, each implant contained 10 mg (~0.02 cm^3) of BMM and 20 µl of liquid component. Based on the used cell type, two types of implants were constructed:

1) BPU type of implants contained 10 mg of BMM, 2 µl of PRP (finally 10% v/v) and 1 × 10^4 of uninduced ADSCs in 18 µl of DMEM.

2) BPO type of implants contained 1 0mg of BMM, 2 µl of PRP (finally 10% v/v) and 1 × 10^4 of ADSCs *in vitro* induced into OBs.

The implants were prepared in the sterile, flat, glass plates. The cells were allowed to attach to BMM surface and fibrin fibers were allowed to form within implants for around 10-15 min before the implantation procedure (28). After that, each implant was shaped in a lump and implanted using sterile biopsy needle into the interscapular subcutaneous tissue of anaesthetized mice. In both groups, each mouse had four implants of the same type. Both experimental groups consisted of twenty animals (each). The extraction of implants was done one, two, four and eight weeks after implantations so that five animals per group were sacrificed per each single experimental period. The implants from each experi-mental period were placed in RNAlater® solution (RNA Stabilization Solution, Ambion, Life Technolo-gies, USA), at -80 °C, in order to preserve RNA until gene expression analysis. The implants extracted two and eight weeks after implantations were fixed by using 10% neutral buffered saline (NBF) for im-munohistochemical analysis.

Relative gene expression analysis

Concentration of total RNA isolated from the implants was determined by using RNeasy Mini Kit® (Qiagen, Hilden, Germany) and measured by using Qubit® RNA assay Kit and a Qubit® 2.0 fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The procedures were done in accordance with the manufacturers' recommendations. DNase I Rnase-free set (Qiagen, Hilden, Germany) was used for digestion of the residual DNA, after which isolated RNA was subjected to reverse transcription into cDNA by using High-capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) and in a thermal cycler SureCycler 8800 (Agilent Technologies, Santa Clara, CA, USA). Relative gene expression analysis was performed in a Stratagene MxPro-Mx3005P Real-Time thermal cycler (Agilent Technologies, Santa Clara, CA, USA). The reactions for endothelial-related genes (Table 1) expression were prepared by using Quanti-Tect primer assays (Qiagen, Hilden, Germany) and KapaSybr® Fast Universal 2 × qPCR Master Mix (Kapa Biosystems,Wilmington, MA, USA), in accordance with the manufacturers' instructions. The results were presented as relative to these genes' expression in PRP (calibrator sample). A housekeeping gene beta-actin was used as a gene normalizator (Table 1).

Table 1. List of primers used for qPCR

Genes	Gene symbol	QuantiTect Primer assay
beta-actin	*Actb*	Mm_Actb_2_SG, QT01136772
von Willebrand factor	*Vwf*	Mm_Vwf_1_SG, QT00116795
early growth response 1	*Egr1*	Mm_Egr1_1_SG, QT00265846
vascular endothelial growth factor receptor 1	*Flt1*	Mm_Flt1_1_SG, QT00096292
vascular cell adhesion molecule 1	*Vcam1*	Mm_Vcam1_1_SG, QT00128793

Immunohistochemistry

The samples of implants previously fixed with 10% NBF were decalcified in ethylenediaminetetraacetic acid solution (pH 7.4). Decalcified tissue was processed, embedded in paraffin and sliced at 4 µm on a Leica RM2235 microtome (Leica Microsystems, Solms, Germany). Prior to incubation with the primary antibody, heat-induced antigen-retrieval procedure was performed on sliced tissue sections by using 10 mmol/L of sodium citrate buffer (pH 6.0), in the microwave oven pre-warmed at 96 °C, for 30 min. Primary antibody used for the experiment was anti-VCAM-1 (1:1000, ab106777, Abcam, Cambridge, USA) and it was omitted in the negative controls. For visualization, rabbit-specific horseradish peroxidase/diaminobenzidine (HRP/DAB) detection Kit (ab64261, Abcam, Cambridge, USA) was used, in accordance with the manufacturer's instructions. Tissue sections were counterstained with Mayer's Haematoxylin (5 min, room temperature), mounted with VectaMount® (Vector Laboratories, Burlin-game, CA, USA) and analyzed on LEICA DMR light microscope (Leica Microsystems, Solms, Germany). Immunoreactivity in the sections was visualized as brown

colour and indicates immunopositivity for the applied antibody.

Statistical analysis

Statistical analysis was done in Microsoft Office Excel. The results are shown as mean value ± standard deviation. For the comparison of mean values, Student's T-test was applied.

The differences were considered significant for $p < 0.05$.

Results

Expression patterns and dynamics of endothelial-related genes *Vwf, Egr1, Flt1* and *Vcam1* in BPU and BPO implants extracted one, two, four and eight weeks after the implantations are presented in Figure 1.

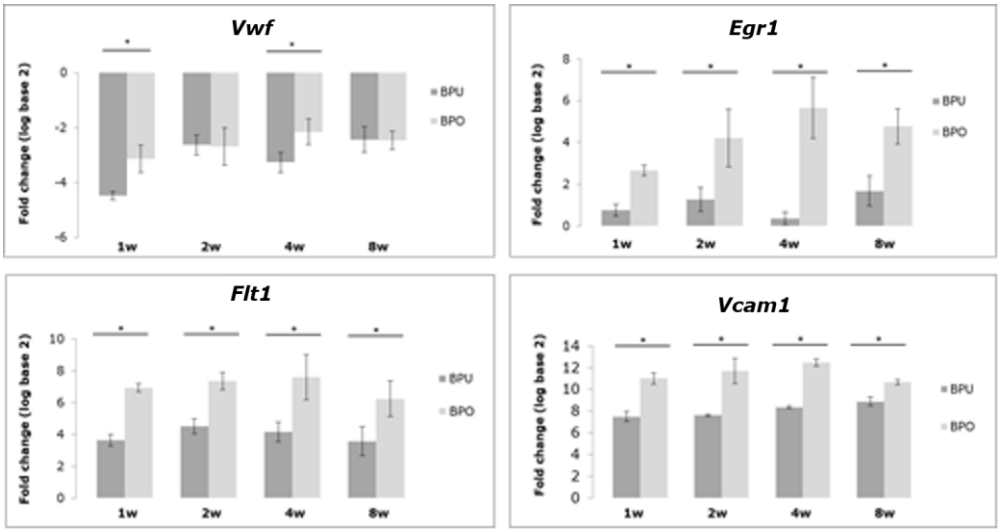

Figure 1. Patterns and dynamics of relative expression levels of endothelial-related genes in examined implants[1]

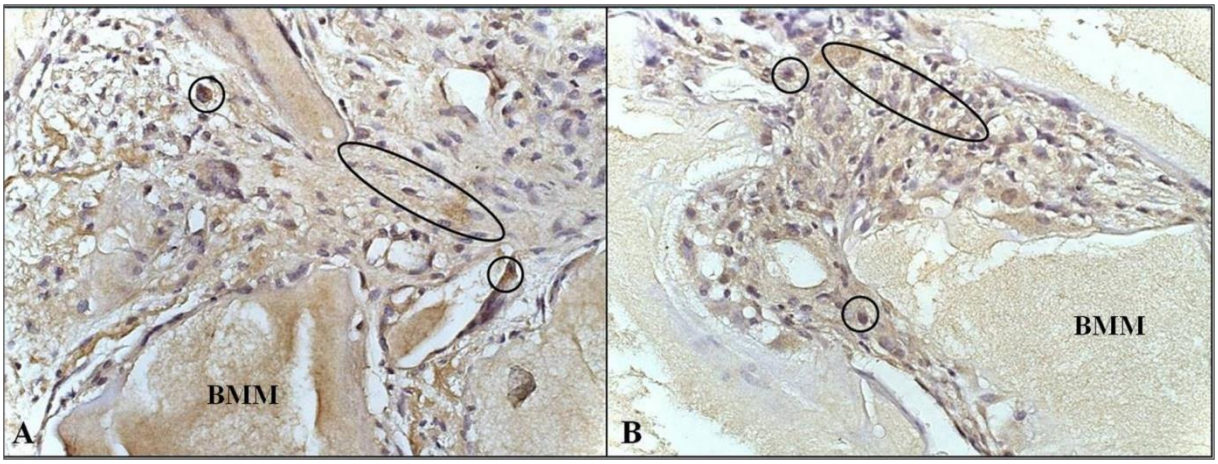

Figure 2. VCAM-1 immunoexpression at two weeks in BPU (A) and in BPO implants (B)[2]

[1] Patterns and dynamics of relative expression levels of endothelial-related genes: *Vwf, Egr1, Flt1* and *Vcam1* in BPU and BPO implants extracted at one, two, four and eight weeks of the *in vivo* experimental period. Significant differences between BPU and BPO groups of implants within the same experimental period: *$p < 0.05$.

[2] Ellipse presents VCAM-1 immunoexpression in the tissue between BMM granules. Circle presents VCAM-1 immunoexpression in the single cells. BMM - bone mineral matrix granules. Magnification: 400 ×. From: Jelena G. Najdanović. Uticaj mezenhimskih ćelija belog masnog tkiva miša, indukovanih *in vitro* ka endotelskim i osteogenim ćelijama, na vaskularizovanost ektopičnih osteogenih implanata, doktorska disertacija, Biološki fakultet, Univerzitet u Beogradu, 2016.

At one and four weeks, the expression of *Vwf* gene was significantly higher in BPO compared to the BPU implants ($p < 0.05$). At two and eight weeks, significant difference of *Vwf* gene expression between BPU and BPO groups was not detected and the level of *Vwf* expression was nearly the same in compared groups.

The *Egr1* gene expression was significantly higher ($p < 0.05$) in BPO compared to BPU implants at each single observation point.

At each observation point, the expression of *Flt1* was significantly higher ($p < 0.05$) in BPO than in the BPU group.

The expression of *Vcam1* gene was significantly elevated ($p < 0.05$) at each observation point in the BPO than in the BPU implants.

At two weeks, VCAM-1 immunoexpression in the BPU implants was intensive in the tissue between BMM granules (Figure 2A). At the same observation point, the tissue between BMM granules in the BPO type of implants had also strong VCAM-1 immunoexpression (Figure 2B).

In the BPU implants extracted eight weeks after implantations, VCAM-1 immunoexpression decreased (Figure 3A) compared to the earlier observation point (Figure 2A). However, VCAM-1 immunoexpression in the BPU implants was still present at eight weeks but mostly near BMM granules rather than between them (Figure 3A). Unlike in the BPU implants, VCAM-1 immunoexpression in the BPO type of implants increases at eight weeks (Figure 3B) in comparison with the earlier observation point within the same group (Figure 2B). VCAM-1 immunoexpression is noticeable in the tissue between BMM granules, in the single cells within the tissue as well as in the blood vessel wall cells (Figure 3B) and it is stronger compared to the BPU implants (Figure 3A) at the same observation point.

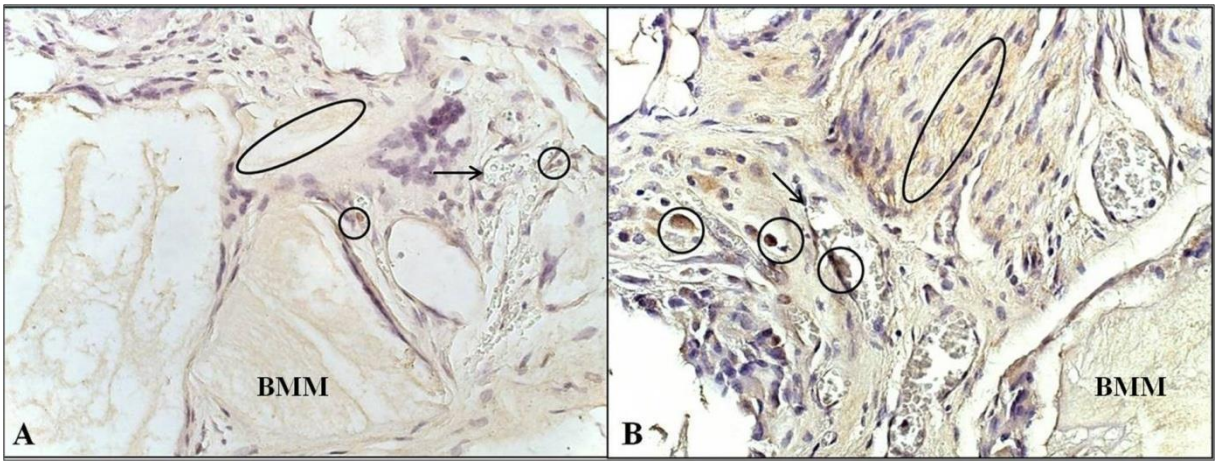

Figure 3. VCAM-1 immunoexpression at eight weeks in BPU (A) and in BPO implants (B)[3]

Discussion

The expression of *Vwf* was negatively regulated in both examined type of implants, at each observation point. *Vwf* is a gene that has a role in later stages of angiogenesis (29). This can be explained by the fact that, besides endothelial cells, *Vwf* gene is also expressed in platelets (30) and we evaluated gene expression in BPU and BPO implants relative to the gene expression in freshly isolated PRP. There was almost no difference in *Vwf* gene expression at two and eight weeks between BPO and BPU implants, but *Vwf* gene expression was significantly higher ($p < 0.05$) in BPO compared to the BPU implants at one and four weeks. This difference is a sign of better vasculogenic potential of ADSCs *in vitro* induced into OBs in comparison with ADSCs cultivated without osteoinductive factors.

The expression of *Egr1, Flt1* and *Vcam1* genes was positively regulated in both examined type of implants, at each single observation point. Positively regulated relative expression of endothelial related genes in BPU implants is probably influenced by biological factors released out of activated PRP. Biological factors released from activated platelets can accelerate *in vitro* and *in vivo* differentiation of ADSCs that were not previously cultivated in cell culture media inductive for differentiation towards certain cell line (14, 26, 31). However, the expression of all examined genes was lower in BPU in comparison with the BPO group.

[3] Ellipse presents VCAM-1 immunoexpression in the tissue between BMM granules. Circle presents VCAM-1 immunoexpression in the single cells. Blood vessels are presented with arrows. BMM - bone mineral matrix granules. Magnification: 400 ×. From: Jelena G. Najdanović. Uticaj mezenhimskih ćelija belog masnog tkiva miša, indukovanih in vitro ka endotelskim i osteogenim ćelijama, na vaskularizovanost ektopičnih osteogenih implanata, doktorska disertacija, Biološki fakultet, Univerzitet u Beogradu, 2016.

The *Egr1* gene can be positively regulated in response to hypoxia, cytokines and growth factors (32). It could be assumed that activation of *Egr1* gene in the examined implants was stimulated by growth factors released from cells that are their components.

The *Flt1* gene is specific receptor for vascular endothelial growth factor (VEGF), a "trigger" protein that controls differentiation of precursor cells towards endothelial and not hematopoietic cell line (33). Significantly higher ($p < 0.05$) expression of *Flt1* in BPO implants in comparison with BPU implants at each observation point can be related to more numerous endothelial cells and their progenitors (34) in BPO than in BPU implants.

The expression of *Vcam1*, a gene that encodes inducible endothelial cell adhesion molecule VCAM-1 which recruits leukocytes to the site of inflammation (35), was significantly elevated ($p < 0.05$) at each observation point in the BPO compared to the BPU implants. VCAM-1 is expressed on small and large blood vessels after stimulation of endothelial cells by cytokines (36) where it binds to its corresponding receptor on the leukocytes very late activation antigen-4 (VLA-4). However, it should be kept in mind that VCAM-1 and its receptor VLA-4 cannot be expressed on quiescent cells which indicates that, once the blood vessel formation is finished, these molecules are negatively regulated and are not necessary for the normal functioning of blood vessels in the quiescent phase (23).

VCAM-1 immunoexpression was strong at two weeks in BPU as well as in BPO implants which means that blood vessel formation had early onset in both examined types of implants. However, VCAM-1 immunoexpression decreases in the BPU implants during *in vivo* experimental period and, at eight weeks, it is weaker compared to the one observed in BPO implants. Numerous giant, multinucleated osteoclast-like cells were observed in both types of implants, at both observation points, but their presence was the most prominent in BPU implants at two weeks. Close to these cells, moderate VCAM-1 immunoexpression can be observed, which could be correlated not only with blood vessels formation but also with osteoclastogenesis process (37). The existence of osteoclast-like, giant multinucleated cells in BPU implants is probably the consequence of synergistic effect of PRP combined with uninduced ADSCs (9). On the model of bisphosphonate-related osteonecrosis of the rat jaw, combination of cultivated, uninduced ADSCs taken from the third passage and PRP led to higher osteoclasts number in the jaws of treated rats in comparison with the rats that haven't received ADSCs/PRP treatment for the prevention of bisphosphonate-related osteonecrosis (9).

Man and associates (14) used also an ectopic model and showed that blood vessel network is well developed after the implantation of uninduced ADSCs isolated from rabbit inguinal fat pads, PRP and alginate-based biomaterial. This effect could be attributed to the cytokines and chemokines secreted by ADSCs since these molecules are signals for the attraction of resident MSCs and precursor cells to the site of injury (1). The difference in the effect of this and our study is that ADSCs were obtained from adipose tissue of different localization−inguinal fat pads. Also, the implants were prepared in the different manner−the components of our implants were mixed just before the implantation procedure while the preparation of implants in the study of Man and associates included *in vitro* encapsulation of ADSCs and PRP with alginate biomaterial during three weeks.

Conejero and his team (38) compared the effects of uninduced ADSCs with those of osteoinduced ADSCs on the repair of rat palatal bone. They seeded uninduced ADSCs on poly-L-lactic acid scaffolds and, simultaneously, osteoinduced ADSCs on the same type of scaffolds. ADSCs induced into OBs triggered more significant bone tissue regeneration compared to the uninduced ADSCs. Higher bone mineral density, bone regeneration and vascular density was estimated in critical-size calvarial defects of male Lewis rats when the defects were filled with hydroxyapatite/poly(lactide-co-glycolide) [HA-PLG] seeded with ADSCs induced into OBs compared to the defects filled with uninduced ADSCs (39). Better vasculogenic potential of ADSCs induced into OBs than the one seen in uninduced ADSCs which we have shown in the present study, could explain more improved osteogenic process in two above mentioned and similar studies.

Conclusion

Vasculogenic potential of ADSCs *in vitro* induced into OBs and combined with PRP and BMM in ectopic osteogenic implants is higher compared to the implants composed of uninduced ADSCs, PRP and BMM, which makes implants enriched with ADSCs induced into OBs good candidates for improving vascularization in bone tissue-engineered constructs.

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VASKULOGENI POTENCIJAL MEZENHIMSKIH MATIČNIH ĆELIJA MASNOG TKIVA INDUKOVANIH *IN VITRO* U OSTEOBLASTE, PRIMENJENIH SA PLAZMOM OBOGAĆENOM TROMBOCITIMA U EKTOPIČNOM OSTEOGENOM MODELU

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Nedostaci koštanog tkiva mogu biti posledica povreda, gubitka kostiju ili tumora. Nedovoljna vaskularizacija glavni je problem za uspešnu regeneraciju koštanog tkiva. Kako bi vaskularizacija tokom regeneracije koštanog tkiva bila poboljšana, obećavajuće metode su razvijene u okviru tkivnog inženjerstva kosti (TIK), uz pomoć primene mezenhimskih matičnih ćelija (MMĆ). Cilj ovog istraživanja bio je da se ispita vaskulogeni potencijal MMĆ *in vitro* indukovanih u osteoblaste u kombinaciji sa plazmom obogaćenom trombocitima (PRP) i mineralnim matriksom kosti (MMK) u ektopičnom osteogenom modelu i uporedi sa implantima sastavljenim od neindukovanih MMĆ, PRP i MMK. MMĆ izolovane iz epididimalnog masnog tkiva miša kultivisane su do trećeg pasaža i potom podeljene u dve grupe: MMĆ *in vitro* indukovane u osteoblaste i ADSC ekspandirane bez osteogene indukcije. Na osnovu principa biološke trijade, sastavljena su dva tipa implanata: implanti koji su sadržali MMK, PRP i MMĆ *in vitro* indukovane u osteoblaste (BPO implanti) i implanti koji su sadržali MMK, PRP i neindukovane MMĆ (BPU implanti). BPO implanti imali su višu ekspresiju gena endotelnih ćelija u poređenju sa BPU implantima. Uz to, imunoekspresija VCAM 1 raste tokom *in vivo* eksperimentalnog perioda u BPO implantima, a opada u BPU implantima. Prema tome, vaskulogeni potencijal MMĆ indukovanih *in vitro* u osteoblaste i kombinovanih sa PRP i MMK u ektopičnom osteogenom modelu viši je u poređenju sa neindukovanim MMĆ, PRP i MMK na ovom modelu. To čini implante obogaćene MMĆ *in vitro* indukovanim u osteoblaste dobrim kandidatima za poboljšavanje vaskularizovanosti u tkivno inženjerisanim konstruktima kosti.

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Ključne reči: *ektopična osteogeneza, mezenhimske matične ćelije masnog tkiva, osteogena diferencijacija, ekspresija gena endotelnih ćelija, plazma obogaćena trombocitima*

Case report

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JEJUNO-JEJUNAL INTUSSUSCEPTION CAUSED BY SKIN MELANOMA METASTASES: A CASE REPORT

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Skin melanoma is a relatively rare malignant tumor with a raising incidence in last decades. Biological course is characterized by lymphatic and hematogenous spread, but metastases in intestine and mesenteric lymph nodes are frequent. These metastases can lead to acute intestinal occlusion as a sign of acute abdomen requiring surgical emergency.

Patient 68 year old, admitted with clinical and radiology signs of acute intestinal occlusion, underwent emergency surgery. The jejuno-jejunal intussusception was found and the lead point of intussusception was intramural melanoma metastasis 4cm in diameter. The small bowel resection length was 40cm. Postoperative course was without complications. Five years before, patient underwent surgery for melanoma of the skin of left scapular region.

Acute intestinal occlusion in patients operated from skin melanoma could seldom be caused by hematogenous intra-abdominal metastases of skin melanoma.

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Key words: *intestinal occlusion, invagination, melanoma*

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Introduction

Only in 15% of intussusceptions, lead points in the small bowel are malignant lesions. They are frequently metastatic in nature and commonly caused by a melanoma (1).

According to recent reviews, the small intestine is a frequent site of melanoma metastases and this is the main cause of secondary intestinal tumors. Superficial spreading melanoma is the most common type of melanoma (70% to 80%) and therefore responsible for most gastrointestinal metastases, which can develop even more than 10 years after resection of the primary cutaneous lesion. Around 60% of the patients who suffer from melanoma have small bowel metastases at the moment of death, but in only 1% to 4% of the cases they are detected as complications occur (2).

The intussusception caused by metastatic melanoma is more often as primary melanoma (3).

Clinical findings are indolent with intermittent crampy abdominal pain leading to acute obstruction with abdominal distension, pain and vomiting. Less than 20% have blood in stool. The diagnosis in plain radiography is highly demanding showing the absence of lower liver edge sign (absence of the subhepatic angle) in upper right abdomen. Other signs are: target sign, crescent sign and a bowel obstruction. The target sign is a mass in the right upper quadrant. It sometimes does not have a target appearance and just resembles a solid mass. It is sometimes called a pseudokidney sign because it may have the shape of an oval mass in the right upper quadrant. The crescent sign is caused by the intussuscepting lead point (intussusceptum) protruding into a gas filled pocket, which often results in a crescent shaped gas pocket. But if the pocket is large, it may not be crescent shaped.

On ultrasound, it could be seen as concentric alternating echogenic and anechogenic bands and defined as target sign, looking as doughnut or bull`s eye (signs are synonyms) The echogenic bands are formed by mucosa and muscularis whereas the submucosa is responsible for the hypoechoic bands. Other sign could be defined as pseudokidney sign, where other part mimics cortical part of kidney but inner part mimics medulla.

Case report

Male Caucasian, 68-year old, underwent excision of nodular skin melanoma (Breslow IV -6 mm in depth) in left scapular region 5 years ago. Patient refers to surgeon complaining of diffuse abdominal

pain and acute abdominal distension from last night. The pain is continuous, sharp and diffuse in the abdomen. He vomits nonbilious and the body temperature was normal. The symptoms had progressively worsened over the last month with colicky intermittent pain mostly in periumbilical region and no changes in bowel habits. Blood samples confirmed raised lactate-dehydrogenase 545U/L and hypochromic microcytic anemia (Hb 9.4g/dl; hematocrit 29.0%).

On physical examination, his vital signs were normal. The hallmark physical findings were a sausage-shaped mass palpable in periumbilical region and moderate abdominal distension. There were no clinical signs of peritonitis. Murphy's sign was negative.

Diagnosis of intestinal occlusion was proved by erected plain abdominal radiography with huge dilated bowel loop with air-fluid levels, distention of the whole small intestine and a solid pseudo-mass in the right upper quadrant (arrow head in Figure 1).

The ultrasound examination reveals pathognomonic bull-eye sign (arrow head in Figure 2).

Therefore, an emergency laparotomy was performed through median incision and jejuno-jejunal intussusception was found (Figure 3).

The manual reposition of intussusception was not possible and a segmental jejunal resection (40 cm in length) was performed. The lead point for intussusception was intramural metastasis of melanoma. There were a lot of black colored, round shaped nodular metastases in the mesentery and two melanotic lesions were present in the jejunal wall, approximately 75cm distal to the duodeno-jejunal junction (Treiz ligament) (Figure 4). The bowel continuity was obtained by end-to-end anastomosis and a drain was placed in abdominal cavity before closing.

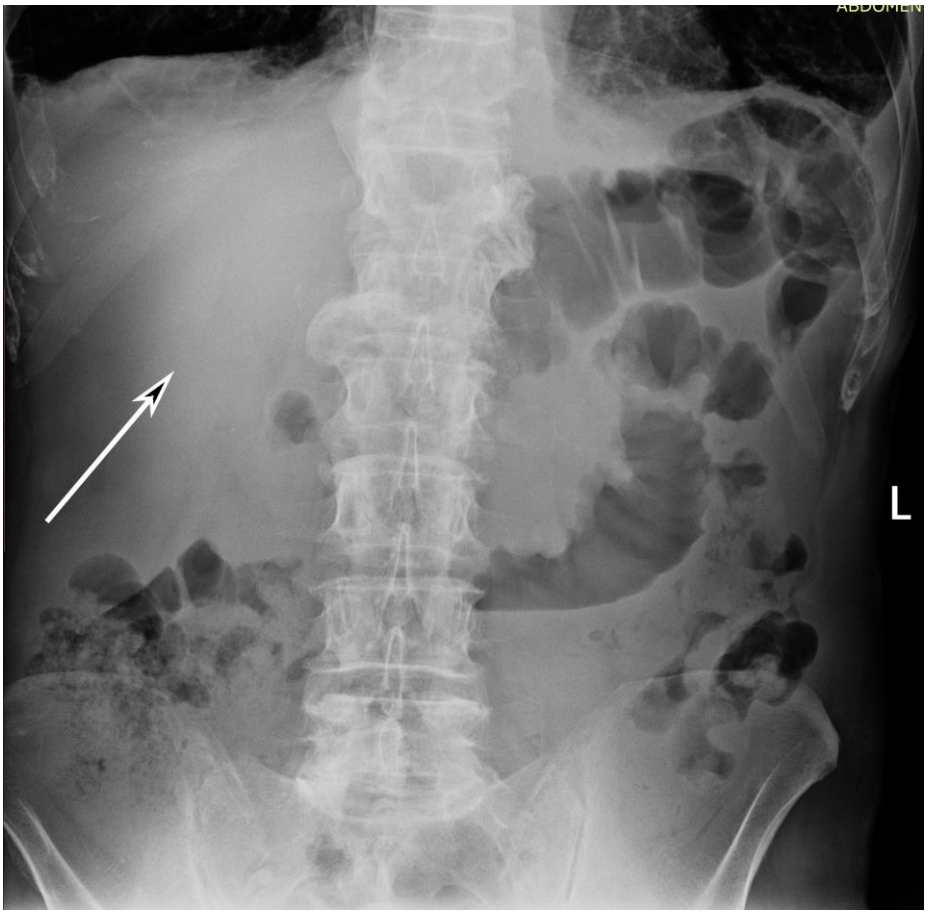

Figure 1. Abdominal radiograph illustrating a dilated small bowel loop and solid mass in right upper quadrant (arrow head) with scanty bowel gas elsewhere

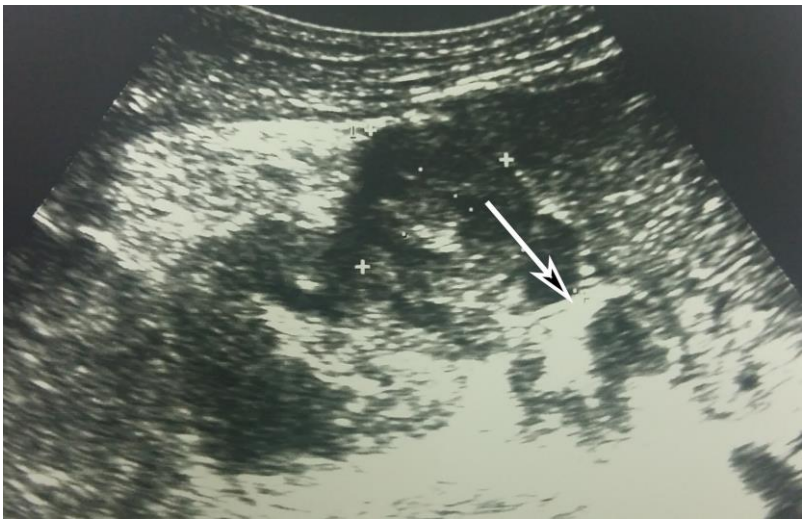

Figure 2. Ultrasound depicts target sign (arrow head)

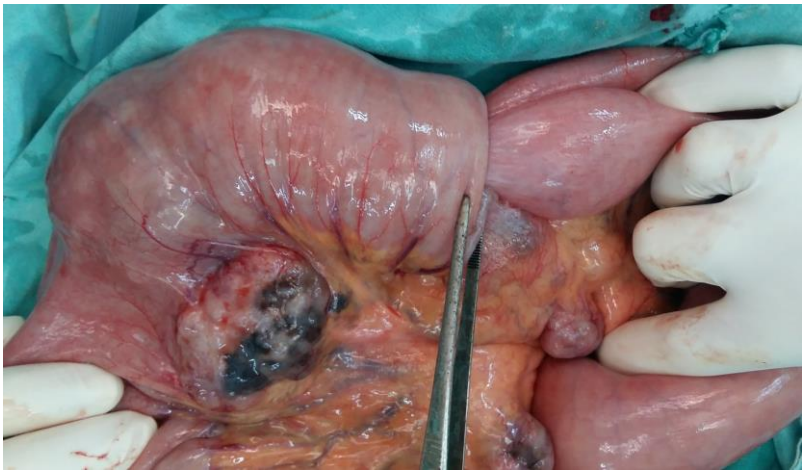

Figure 3. Intraoperative photograph indicating jejuno-jejunal intussusception

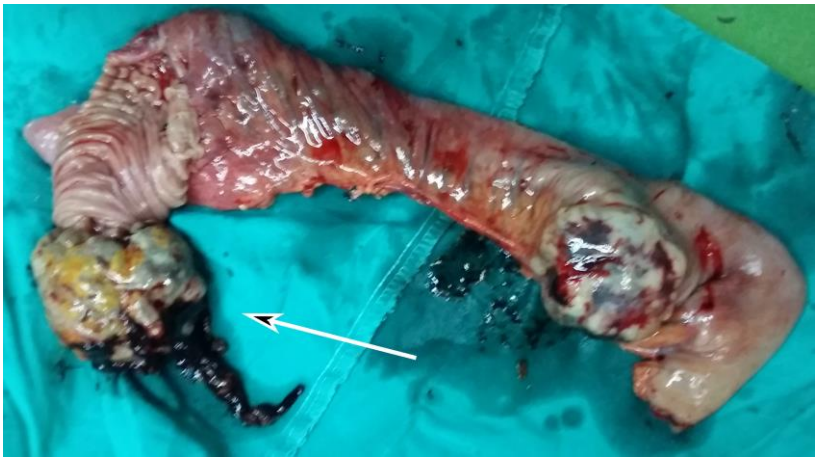

Figure 4. Open surgical specimen showing invaginated bowel segment.
The metastatic melanoma as an ulcerated polypoid mass is shown as the lead point

Postoperative course was uneventful. Postoperatively, the patient was treated with intravenous antibiotics and monitored closely for postoperative ileus. Supportive management in the form of analgesia, nasogastric tube and intravenous fluids was required for 72 h until normal dietary intake was reestablished. The patient was discharged on the 10th postoperative day.

The histopathologic examination revealed intestinal malignant melanoma lesions with a maximum diameter of 3.5cm.

Discussion

Incidence of clinically evident small bowel metastatic deposits after skin melanoma surgery is 2% to 5% of patients and is most commonly expressed as an acute intestinal obstruction (4).

Some authors refer the propensity of melanoma to metastasize to the GI tract, and the small bowel is a common site of involvement (35% to 70% of GI metastasis from skin melanoma). In the gastrointestinal tract, the small bowel is the most frequent site of metastasis of melanoma, mainly because of its rich blood supply (5).

In 58% of the patients with malignant melanoma, intestinal metastases were found at autopsy.

That means less than 5% of patients with metastases to the gastrointestinal tract are diagnosed antemortem. Though malignant melanoma is the most common cause of the extra-abdominal source of intestinal metastasis, it is rare to find them presenting as jejuno-jejunal intussusception (6). The magnitude of silent metastases coupled with the fact that these metastases can present with almost any GI symptom highlights the need for high clinical suspicion in patients with a previous malignant melanoma presenting with GI symptoms. The rarity of jejuno-jejunal intussusception is the prime reason for reporting our case.

The clinically evident bowel metastasis in our case appeared 5 years after primary excision what is in accordance with literature review that the average time from excision of the primary cutaneous melanoma to the occurrence of an intestinal metastasis is 3-6 years. Due to the difficulty in exploring the whole length of the small bowel using common diagnostic procedures, a preoperative diagnosis is often challenging to establish. Routine examinations are plain radiography and ultrasound. When the patient presented with abdominal pain, vomiting and distension, the diagnosis of intestinal obstruction in the emergency setting must be confirmed by erect abdominal X-ray. Ultrasonography showed small intestine intussusception. In cases with small bowel intussusception, plain radiography shows signs of bowel obstruction such as dilated loops of bowel or air-fluid level in the bowel lumen, and rarely a mass lesion or intraluminal air trapped between the walls of the intussusceptum and intussuscipiens (air crescent sign). These findings nevertheless lack the specificity and sensitivity to diagnose intussusception (7). Plain abdominal radiographs are of limited value in the diagnosis of intussusception due to their reduced sensitivity and specificity. However, they are often performed as part of the initial investigations for patients presenting with an acute abdomen. One of the radiographic features consistent with intussusceptions is signs of intestinal obstruction proximal to the lead point. In our case, we referred the air fluid level and solid mass in upper right abdomen.

The next examination should be abdominal ultrasonography as a useful technique in the diagnosis of intussusception in adults and children. The features described include a target and doughnut signs on the transverse view and a pseudokidney sign on a longitudinal view. Ultrasonography carries no radiation risks. The limitations include obesity and bowel gas which may obscure the typical findings (7). We defined target sign in ultrasound in our case.

Although different imaging techniques such as barium examinations and CT, they both may be able to depict larger intestinal lesions. In developed countries, CT of the abdomen seems to be the radiological investigation of choice, with a sensitivity of 71.4% to 87.5% and a specificity of 100% in the prospect of diagnosis of intussusception in adults (7). In adult patients with long-term nonspecific abdominal pain, barium study is contraindicated because it brings the risk of intestinal perforation. Despite of various imaging modalities have been used to help in establishing the diagnosis, it is frequently confirmed only during surgical intervention. Surgery is currently the treatment of choice without a precise surgical strategy. There is not a clear consensus about the optimal surgical approach and there is still controversy about reduction before resection (8). The current controversy remains on the extent of surgical resection vs. reduction of the intussusception. The initial favor to resect enbloc the intussuscepted segment of bowel was based on the theoretical risks of venous embolization of the tumor cells on bowel manipulation and also the risks of perforating the ischemic bowel with contamination of the peritoneal cavity (9-11). Resection without reduction was the standard of care for intussusception caused by tumor and advocated by most surgeons (12, 13). In our case, we also performed resection without attempts to reduction. Some authors advise that simple reduction is acceptable in post-traumatic or idiopathic intussusceptions, where no pathological cause could be identified, obviously after the exclusion of bowel ischemia or perforation (14). Some authors suggest that reduction prior to resection can be safely performed in selected patients with suspected benign disease, especially when small bowel intussusception is presented without ischemia or there is a risk of short gut syndrome after wide en block resections (2). Diagnostic laparoscopy and resection has been used successfully in selected patients. In patients with chronic and subacute presentation with partial small bowel obstruction, laparoscopy offers the benefit of a conservative approach with possible reduction of the bowel but laparoscopy in acutely obstructed patients with bowel distension where visualization may be poor, and bowel manipulation may further risk perforation and increase the morbidity of an operation (15). However, surgery is not curative and it should be considered as a good means

of palliation with a chance of improving prognosis (5 year survival up to 40% and a disease-free interval up to 10 years) when free surgical margins can be achieved (16).

Conclusion

Metastatic melanoma of the gastrointestinal tract, especially bowel intussusception caused by metastatic melanoma should be suspected in patients with history of melanoma of the skin and acute gastrointestinal symptoms. Emergency surgery is the mainstay of treatment and bowel resection is appropriate treatment. A high index of clinical suspicion combined with the appropriate imaging might help in establishing an early diagnosis, emergency surgery and avoiding serious complications like perforation and peritonitis. In the presence of a lead point lesion but no preoperative tissue diagnosis, surgical intervention in the form of bowel resection without reduction is advisable.

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Prikaz bolesnika

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JEJUNO-JEJUNALNA INVAGINACIJA UZROKOVANA METASTAZAMA MELANOMA KOŽE: PRIKAZ SLUČAJA

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Melanom kože je relativno redak tumor, ali je incidencija ovog maligniteta u stalnom porastu. U biološkom toku opisane su limfogene i hematogene metastaze, a često mogu nastati metastaze u creva i limfne čvorove mezenterijuma. Ove metastaze mogu dovesti do crevne okluzije, koje mogu uzrokovati akutni abdomen kao urgentno hirurško oboljenje.

Bolesnik star 68 godina primljen je zbog kliničkih i radioloških znakova i akutne intestinalne okluzije i hitno je operisan. Nađena je invaginacija jejunuma, a vodeći deo invaginata činila je intramuralna metastaza melanoma prečnika 4cm. Učinjena je resekcija tankog creva dužine 40cm i post-operativni tok je protekao uredno. Pet godina pre prijema, bolesnik je operisan zbog melanoma na koži leđa.

Akutne crevne okluzije kod bolesnika koji su operisani od melanoma kože mogu retko biti uzrokovane hematogenim intraabdominalnim metastazama melanoma.

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Ključne reči: *crevna okluzija, invaginacija, melanom*

Case report

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INTRAOPERATIVE RUPTURE OF THE RECONSTRUCTED AORTIC VALVE LEAFLET: A CASE REPORT

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The autologous pericardium is often used for the reconstruction of aortic valve, due to its easy accessibility and adequate strength. Reconstruction of insufficient bicuspid aortic valve was performed by resecting the leaflet and reconstructing it by using autologous pericardium. Initial perioperative transesophageal echo registered a sudden increase in regurgitation up to 2+. The rupture of the autologous pericardium used for leaflet reconstruction was noted. Although accompanied with a small number of early and late failures, the reconstruction of aortic valve with autologous pericardium still presents a good choice of treatment of aortic valve insufficiency. The development of new materials is needed for achieving better results.

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Key words: *bicuspid aortic valve, insufficiency, pericardium, reconstruction*

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Introduction

A bicuspid aortic valve (BAV) is present in 1-2% of general population (1). The most frequent causes leading to aortic insufficiency are: aortic root dilatation, incomplete closure of a congenitally bicuspid aortic valve, and infective endocarditis, postinflammatory disease, etc. (2). The most frequent cause of aortic insufficiency in people between 20 and 50 years of age lies in the existence of a BAV (3). The two major ways of treating valve insufficiency are either by a valve replacement with a mechanical or biological prosthesis or by the repair of the native valve (4). Aortic valve replacement increases the risk of adverse effects of anticoagulant therapy or endocarditis (5). When we are talking about valve repair we need to emphasize that the presence of calcifications on the valve leaflets requires decalcification, while any large defects need to be replaced. Autologous pericardium represents easily accessible material that can be used for the repair of valve leaflets (6). The literature contains many descriptions of cases of aortic regurgitation caused by a late rupture of the suture line of the pericardial patch (7). In our case, rupture of the free edge of the pericardium used for leaflet reconstruction took place immediately after surgery resulting in significant aortic regurgitation which led to valve replacement.

Case description

A 48 year old patient was admitted for elective surgical procedure on the aortic valve. For about a year, the patient had been aware of a heart murmur accompanied by fatigue, palpitations and choking during exercise. Ultrasound examination revealed BAV with a decreased separation of leaflets, pressure gradient of 36/14 mmHg and severe aortic regurgitation of up to 3+. The aortic root diameters were 31 mm, while the ascendant aorta was slightly dilated up to 39 mm. During the surgery, and after aortotomy was performed, BAV with a fusion of the right and non-coronary cusp was noticed. An excision of the calcification was performed. The resulting defect was reconstructed with a triangular 2% glutaraldehyde - fixed autologous pericardial patch. A subsequent plication of the central part of the pericardial patch was performed with 6-0 polypropylene sutures in order to ensure adequate leaflet coaptation. A water test confirmed good leaflet symmetry. Following aortic closure and cardiac dearing, the aortic clamp was removed, and the heart spontaneously returned to sinus rhythm. After performing a control transesophageal echocardiogram (TEE), central AR up to 1+ was noticed. Once the heart lung machine

was stopped and full heart activity resumed, the TEE registered sudden increase in the regurgitation up to 2+. Due to the less than optimal reconstruction result, it was decided to substitute the reconstructed native valve with a mechanical prosthesis. Following a repeated aortotomy, a new defect on the free edge of the pericardial patch was visualized (Figure 1). It was a small tear far from suture line, on the free edge of pericardium. That phenomenon was interpreted as the cause of the newly created leaflet insufficiency. The cusps were completely excised. Interrupted mattress suture technique was used to implant the mechanical aortic valve prosthesis (St. Jude medical, USA) No 25. Aortic closure was performed, deaeration completed and aortic clamp removed. After period of reperfusion the patient was weaned from the Heart Lung machine without inotropic support. The further postoperative period was uneventful.

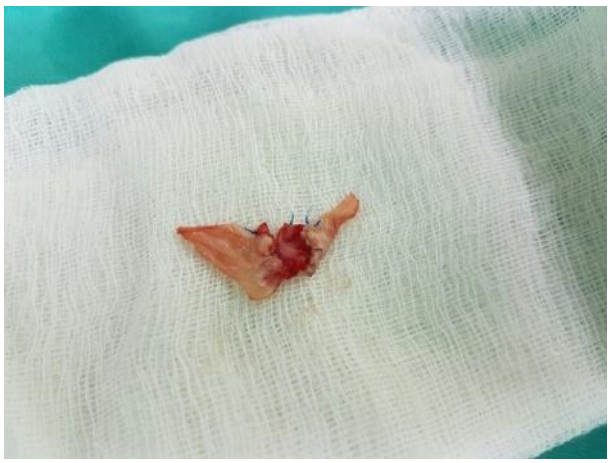

Figure 1. Excided aortic valve leaflet with the visible rupture of free edge of the pericardium used for reconstruction

Discussion

BAV is one of the most frequent congenital heart anomalies with a prevalence of 1.3% (8). It is accompanied by an increased number of complications like aortic stenosis, insufficiency and infectious endocarditis. Many patients do not demonstrate significant hemodynamic alterations prior to 70 years of age (9). Incidence of isolated aortic regurgitation of the bicuspid valve is less frequent than incidence of stenosis, but tends to appear at an earlier age (10). A bicuspid valve substituted with a mechanical prosthesis requires a lifelong use of anticoagulant therapy. Biological valves, on the other hand, are prone to degeneration and require substitution after a while. An implanted valve is accompanied by complications including endocarditis, thromboembolism and repeated surgeries. A reconstruction of the bicuspid valve in younger patients has become a significant alternative to its substitution (11). Leaflet defects occurring during the reconstruction can be repaired with the use of various materials. The most frequently used are the dura mater, fascia lata or bovine pericardium, but autologous pericardium has particular traits. This material is easily accessible and durable. Lausberg et al. (2006) (6) confirmed good results in using autologous pericardium in the reconstruction of aortic valve leaflets. Al Halees et al. (2005) (12) have demonstrated no significant deterioration of tissue structure between bovine and autologous pericardium after or during 16 years of research, but have proven that bovine pericardium is more prone to calcifications in comparison to the autologous. Price et al. (2013) (13) claim that in their 10-year follow-up after aortic leaflets reconstruction with autologous pericardium, the results were comparable to the results of valve substitution with a biological valve, which confirms good results of the reconstruction procedure. The leading reasons for a recurrence of valve insufficiency after reconstruction are the progression of the rheumatic disease, while the second most common cause is the dehiscence of the suture in late postoperative period. Other less common causes are endocarditis and progressive calcification of the pericardial patch (7, 14). In our case, description the acute insufficiency of the reconstructed leaflet was caused by a rupture of the pericardial patch outside the suture line, or the intact pericardium, to be more precise. To the best of our knowledge this is the first case of the kind ever published in a scientific journal.

Conclusion

Aortic valve insufficiency caused by degenerative disease of bicuspid valve can be successfully surgically treated by various reconstruction techniques, thus avoiding potential complications after the implantation of the mechanical prosthesis. Complications like a recurrence of AR, acute or chronic dehiscence of the suture or the bursting of the used pericardium have been described. The published mortality and morbidity data show good results of reconstruction in carefully selected patients, especially younger ones. Leaflet reconstruction is an established modality in treating aortic valve disease. In order to achieve better results in the use of this procedure, constant improvements in surgical techniques and usage of new materials are essential.

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Prikaz bolesnika

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INTRAOPERATIVNA RUPTURA REKONSTRUISANOG LISTIĆA AORTNOG ZALISTKA – PRIKAZ SLUČAJA

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Autologni perikard često se koristi za rekonstrukciju aortne valvule, zbog lake dostupnosti i odgovarajuće čvrstine. Rekonstrukcija insuficijentne bikuspidne aortne valvule učinjena je resekcijom listića i rekonstrukcijom uz upotrebu autolognog perikarda. Tokom inicijalnog perioperativnog transezofagealnog pregleda, registrovan je iznenadni nastanak aortne regurgitacije (do 2+). Iako praćena izvesnim brojem akutnih i hroničnih komplikacija, rekonstrukcija aortne valvule autolognim perikardom predstavlja dobar izbor za lečenje insuficijencije aortne valvule. Razvoj novih materijala je neophodan da bi se poboljšali rezultati lečenja.

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Ključne reči: *bikuspidna aortna valvula, insuficijencija, perikard, rekonstrukcija*

Case report

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A 64 YEAR OLD PSYCHIATRIC PATIENT SUFFERING FROM DEPRESSION, VERTIGO AND SUICIDE THOUGHTS WITH NYSTAGMUS AND DIPLOPIA: A CASE REPORT

Horst J. Koch

A 64 year old patient was referred to the psychiatric ward due to a depressive mood and suicide thoughts. An antidepressant treatment was started. Intermittently, he showed a rightward horizontal gaze-evoked nystagmus. We arranged a cerebral magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) analysis, which revealed an autoimmune disorder - dipeptidyl-peptidase-like protein 6 (DPPX) antibody - which was the probable underlying cause. The patient was treated with steroids and azathioprine which improved his condition substantially. The case emphasizes the relevance of putative autoimmune etiology of acute psychiatric diseases.

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Key words: *psychosis, nystagmus, autoimmune encephalitis, dipeptidyl-peptidase-like protein 6 antibody, immune suppression*

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Introduction

Antibody-associated neuropsychiatric syndromes were first identified in the 1980ies and initially allocated to different forms of cancer as paraneoplastic syndromes (1-4). Meanwhile, a plenty of antibodies directed to neuronal structures have been detected, which have not necessarily to be related to tumors (5-8). In 2007, the anti-NMDA (N-methyl-D-aspartate)-receptor encephalitis was discovered, which is related to ovarial carcinoma in some 50%. Apart from schizophrenia-like symptoms, the patients of this quite frequent disorder – more than 1000 documented cases - may suffer from dyskinesia, dystonia or epileptic seizures. Likewise, potassium channel antibody associated diseases play an important role (voltage gated potassium channel complex, VGKC), which bind to proteins such as LGI1 (leucine-rich, glioma inactived 1) or Caspr2 (contactin associated protein) (6). LGI1-encephalitis often shows psychosis-like findings including epilepsy in older men, whereas Caspr2 is often related to the so-called Morvan syndrome, which is characterized by neuromyotonia and insomnia or confusion. Some practically important differential diagnoses of autoimmune encephalopathies with their core syndromes are summarized in Table 1.

DPPX (dipeptidyl-peptidase-like protein 6)-associated encephalopathies are comparably rare and in most cases not associated with tumors. This IgG subclass antibody regulates the Kv4.2 (potassium channel, voltage dependent) channel, which is abundant in brain and myenteric plexus. Often paranoid symptoms or mutism are observed in addition to weight loss and diarrhea (5). The channel is possibly involved in soma-dendritic signal and decreases neural back propagation (9). The latter may have importance in neural plasticity or long-term potentiations, i.e. in memory function (9). Neuropsychiatric symptoms include hyperekplexia (startle reaction), myoclonus, tremor or seizures (6, 8). The DPPX-associated encephalitis may sometimes coincide with systemic lymphoma or may resemble the PERM syndrome (progressive encephalomyelitis with rigidity and myoclonus) (7, 8). The clinical findings are most important. The MRI often yields unspecific findings as do EEGs. The decisive method is the confirmation of the antibody in the CSF.

Case report

A 64 year old patient was referred to the psychiatric ward due to suicide thoughts, depression and increasing rotatory vertigo for a few weeks by his

general practitioner. He had a history of alcohol and nicotine abuse but was completely abstinent for at least 4 years. A chronic pancreatitis hyperuricemia were preexisting. An obsessive-compulsive disorder - thoughts - was diagnosed in the run-up of the admission. He had no autonomic complaints and slept well. He worked as truck driver and was divorced 15 years ago having contact neither with his daughter nor with his earlier friends.

On admission, his psychopathology showed a cooperative man, well orientated with only minimal brain impairment without psychotic symptoms. The leading symptoms were recurrent suicide impulses. Auscultation revealed a 2/6 systolic murmur (Punctum maximum at Erb). Neurological findings including reflex status of the upper and lower limb were initially normal apart from a slight ataxia. Routine CCT revealed a minimal bifrontal and cerebellar atrophy and an old white matter lesion in the left lobus parietalis inferior. The MRI of the brain confirmed the lacunar stroke and a II microangiopathic leucoencephalopathy. The patient took aspirin (100mg sid), simvastatin (20mg hs), pantoprazol (40mg hs), mirtazapine (45mg hs) on a regular base.

During a word round the patient´s eye movements were conspicuous. He complained of an increasing vertigo and showed a horizontal gaze-evoked nystagmus with a vertical component, pronounced when looking to the right side. Additionally, the ataxia was somewhat more emphasized. He did not complain of nausea. Ear nose throat specialist examination did not reveal a peripheral disorder. The ophthalmologist described an incomplete paresis of the right oculomotorius nerve. The diagnostic was extended to reveal the origin of the encephalopathy (Time of Flight MRI (TOF)), echocardiography, long-term ECG, neurosonography, abdominal sonography, CSF). The TOF revealed a microaneurysm of the anterior communicans, neurosonography a slight atherosclerosis, echocardiography showed a minimal left cardiac hypertrophy, and abdominal sonography, a hepatopathy in conjunction with a chronic pancreatic atrophy.

The cerebral MRI did not show inflammatory changes or acute ischemia but only a slight degree 2 subcortical glioses with global cerebral atrophy. The EEG only showed a normal basic rhythm with a few interjacent single sharp waves. The ABEP (auditory brainstem evoked potentials) indicated a brain stem lesion, which could not be localized. VEP (visual evoked potentials) could not be interpreted due to the nystagmus. The CSF revealed positive oligoclonal bands as a hint for an immunological process, otherwise no critical pathological results (clear, 1 cells/µl, no erythrocytes, protein, albumin, glucose and lactate within normal range) were found. Finally, we could confirm Anti-DPPX-antibodies (di-peptidyl-peptidase-like protein 6 – subunit protein of the potassium channel) which could explain the exacerbated encephalopathy including psychiatric symptoms.

Having a normal TMTP (thiopurine-methyltransferase) activity treatment with azathioprine was initiated (50 sid or bid according to WBC) and prednisolone (50mg mane, including a reduction scheme) was started. Within 4 to 5 weeks time the patient improved with normal cognitive test results (DemTect, Mini-Mental-Status-Test). He could be discharged in sufficient state of health and was referred to the outpatient clinic. He was again seen a month after discharge from the ward showing again a deterioration, which well responded to an intravenous pulse therapy with prednisolone (500mg per day) and an increased dose of azathioprine (150mg per day). CSF analysis was repeated and oligoclonal bands were no longer present. Since the discharge from the ward, no relapses occurred for 9 months and no further admission to the hospital was necessary. The author thanks the patient for his written informed consent to publish the case.

Discussion

We saw a 64 year old patient with psychiatric symptoms who developed a nystagmus during his treatment on the psychiatric ward. The neurosomatic diagnostics revealed an autoimmune disease which could be treated with success and which improved his well being in addition to his symptomatic psychiatric medication. The case report emphasizes the relevance of differential diagnosis of autoimmune disorders in patients with acute psychotic symptoms.

The DPPX antibody syndrome is a rare disorder which - like a chameleon - can vary in its clinical presentation. Therefore, it is an advantage to consider both neurological and psychiatric disorders in a broader sense, especially autoimmune diseases (Table 1). The combination of affective disorder and a neurological deficit gave the initial hint to look for an organic source of the disease. The major benefit for patient was the specific immune suppressive treatment option which improved both symptoms and life quality. Moreover, oncological treatment may help in paraneoplastic disease if available. Psychiatric treatment is guided by the syndrome of psychopathology. No standard causal treatment exists and, as a rule, the therapy has off-label character. In general, steroids, intravenous immune globulins, azathioprine or cyclophosphamide are mentioned in the literature (6-8). For sure, controlled studies will be necessary to improve the safety and efficacy of strategies and approaches.

Table 1: Summary of selected autoimmune encephalitic processes associated with psychopathological symptoms

Antibody directed to	Core symptoms and findings
NMDA-receptor	Psychosis, perioral dyskinesia, epileptic fits, dystonia, coma - frequently in children, 3/4 women, often associated with ovarial tumors
LGI1	Dystonia of face and arm, fits, amnesia, psychosis, hyponatriemia
Caspr2	Neuromyotonia, Morvan Syndrome*
AMPA receptor	Epileptic fits, Amnesia, psychosis
DPPX	Limbic encephalitis, mutism, paranoia
$GABA_B$ receptor	Epileptic fits, amnesia
nGluR5	Personality change, affective instability, Orphelia syndrome**, associated with Hodgkin lymphoma
Glycine receptor	Cognitive impairment, hyperexcitability, PERM, stiff person syndrome
Hu, Ma1, Ma 2, CV-2, ANNA3	Limbic encephalitis (paraneoplastic): fits, mnestic deficit, confusion, psychosis, depression
Hu (70%) and	PERM syndrome (paraneoplastic), variable: limbic, brain stem, cerebellar, motor, sensory, myenteric symptoms

* limbic encephalitis with neuromyotonia
** Hodgkin associated with autoimmune limbic encephalitis due to mGLuR5.
Abbreviations:
NMDA: N-Methyl-D-Aspartate
Caspr: contactin associated protein
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
LGI1: Leucine-rich, glioma inactived 1
mGluR5: metatropic glutamate receptor 5
GABA: gamma amino butyric acid
PERM: progressive encephalomyelitis with rigidity and myoclonus
Hu, Ma: initials of the first patient
ANNA: antineuronal antibody
CV-2 (CRPM5): anticollapsin response-mediator protein

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PSIHIJATRIJSKI BOLESNIK STAR 64 GODINE KOJI PATI OD DEPRESIJE, VERTIGA I SUICIDALNIH IDEJA SA NISTAGMUSOM I DIPLOPIJOM: PRIKAZ BOLESNIKA

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Bolesnik star 64 godine upućen je na odeljenje psihijatrije zbog depresivnog raspoloženja i suicidalnih ideja. Određena je terapija antidepresivima. Bolesnik je intermitentno imao horizontalni nistagmus udesno. Urađena je magnetna rezonanca mozga, kao i analiza cerebrospinalne tečnosti, gde je utvrđeno postojanje autoimunog poremećaja - *dipeptidyl-peptidase-like protein 6 (DPPX) antibody* – što je verovatno bio osnovni uzrok oboljenja. Bolesnik je potom lečen steroidima i azatioprinom, što je značajno poboljšalo njegovo stanje. Ovaj slučaj naglašava značaj putativne autoimune etiologije akutnog psihijatrijskog oboljenja.

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Ključne reči: *psihoza, nistagmus, autoimuni encefalitis, dipeptidyl-peptidase-like protein 6 antibody, imuna supresija*

Review article

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THE IMPORTANCE OF OLD ANTIBIOTICS IN OVERCOMING RESISTANCE TO ANTIBIOTICS

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Antibiotics are medications used to prevent or cure infections caused by bacteria. Discovery and introduction of antibiotics into medical practice brought about revolutionary changes in therapy and eradication of infectious diseases. There is a rise of interest for usage of old antibiotics. These drugs could be invaluable in the treatment of certain infections and, in order for them to remain effective, it is necessary to conduct certain measures which would prove their worth. The aim of antibiotic therapy is to deliver the antibiotic to the place of infection and to retain it in the place of infection for a long period of time. Most of all, it is of utmost importance to educate about this all medical personnel in health care system, especially physicians. The fact that the availability of these drugs remains low in comparison to the efforts made in attempt of discovering new antibiotics, imposes the need for national regulatory agencies to get involved in regulating the usage of these drugs.

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Key words: *antibiotics, resistance, infections*

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Introduction

Antibiotics are medications used to prevent or cure infections caused by bacteria. Discovery and introduction of antibiotics into medical practice brought about revolutionary changes in therapy and eradication of infectious diseases. It is believed that the "antibiotic era" has begun at the beginning of the twentieth century, but the evidence suggests that antibiotics have been used since ancient times. It is clear that antibiotics had been used in forms completely different from contemporary industrially produced ones. Nevertheless, this does not diminish the fact that certain herbal medicines which have antibiotic properties were known to ancient Greeks and Romans and that they constituted an important part of their medical practices (1).

Uncontrolled and excessive antibiotic use quickly led to the emergence of antibiotic resistant strains of bacteria. Results of many studies have shown that it is of utmost urgency that the use of antibiotics must be put under control. Unless certain changes are made, it is estimated that by the year 2050, 50.000.000 deaths will be caused by bacterial infections annually, a number surpassing the number of deaths caused by cancer (2).

Data suggests that 80% of antibiotics are used out of hospitals, while 20% fall into the category of intrahospital treatment. 20%-50% of prescribed antibiotics are not used rationally. All of these circumstances led to the emergence of multiresistant bacterial strains, and some strains of bacteria, such as *Enteroccocus faecium*, are resistant to all known types of antibiotics (3). The research has also shown that resistant strains of bacteria frequently occur in intensive care units, proving that even rational antibiotic use leads to emergence of resistant bacteria.

Due to the global problem of bacterial resistance, data is being collected in Europe and certain strains of bacteria of great epidemiological importance, such as methicillin resistant Staphiloccocus aureus (MRSA), vancomycin resistant Enterococcus (VRE), III generation cephalosporin and carbapenem resistant Enterobacteriae (Escherichia Coli, Klebsiela

pneumoniae), carbapenem resistant Pseudomonas aeruginosa and Acinetobacter baumanii, and penicillin resistant Streptococcus pneumoniae, are being monitored (4).

Acknowledgment that the Earth's population might become powerless even when it comes to the most banal infections, raised alarm among member countries of the UN at the annual gathering of General Assembly in New York in 2016 and it was decided that certain measures are to be introduced in order to reduce the risk of infections caused by multiresistant strains of bacteria (5):

1. Acceleration of development of new antimicrobial drugs
2. Acceleration of development of new vaccines
3. Acceleration of development of reliable diagnostic tests which would enable the reduction of antibiotic use
4. Monitor and collect data about antibiotic use, as well as about antibiotic resistant bacteria
5. Impose closer monitoring of antibiotic use
6. Establish national strategies which would allow for a more rational prescription of antibiotics
7. Attempt to re-introduce old ("forgotten") antibiotics

Since the introduction of new antibiotics is fairly slow and expensive, and the occurrence of resistant bacteria increased, the idea about making the old antibiotics available and used again is becoming an important topic. Unfortunately, many of these antibiotics are unavailable in most countries (the EU, the US, Australia), reasons being varied and complex.

It is not easy to explain the definition "old" and "forgotten". These are antibiotics once used, but, for various reasons (in connection to their properties or unprofitability) removed from the pharmacies or were never used in clinical practice. However, there are findings pointing that these antibiotics could be administered at the present moment or in the future and that their usefulness is beyond doubt.

Old antibiotics with narrow spectrum of action which were withdrawn from the pharmacies or never used in praxis could potentially prove to be useful in treatment of certain infections which would reduce the usage of broad spectrum antibiotics whose frequent prescription results in emergence of multiresistant bacteria (6). Moreover, old antibiotics could be used in treatment of infections caused by multiresistant bacteria, such as sepsis, endocarditis and meningitis.

Usefulness and effectiveness of old antibiotics could be summed up as following (7):

1. Old antibiotics could be useful because of their special microbiological criteria (antimicrobial spectrum; special mechanisms of action)
2. According to special pharmacological criteria
3. According to clinical criteria
4. Based on the fact that they are the only efficient antibiotics in relation to the cause of infection
5. Based on the fact that a certain antibiotic has no replacement
6. Based on the fact the certain antibiotic is the only efficient antibiotic in a certain group of antibiotics.

Overview of selected antibiotics

Penicillin G is used parenterally in the form of sodium and potassium salts. In addition to penicillin G, procaine benzylpenicillin and bezanthine benzylpenicillin are used. Despite the fact that many bacteria have become penicillin G resistant, it is important to stress that Streptococcus pyogenes remains susceptible to this drug. Therefore, this old antibiotic is important when it comes to the treatment of infections caused by this bacterium, such as sepsis, endocarditis and meningitis (8). This is the drug of choice in treatment of syphilis. Due to these facts, penicillin G should be available for treatment of streptococcal infections and syphilis. The main reason behind the deficit of this drug is of economic nature (a cheap antibiotic). This drug is available in the Serbian pharmacies.

Temocillin is a derivative of ticarcillin. Despite its unique properties it was never widely used. It is a narrow spectrum of action drug and it is stable in relation to beta lactases. What makes this antibiotic unique is that resistant strains of bacteria have never emerged. It is active against Burkholderia cepaciae and the specific and unique indication for this antibiotic is an infection caused by this bacterium. This type of infection occurs frequently and can be se-rious in patients with cystic fibrosis (9).

The main causes of infrequent use of this antibiotic are low prices and the lack of knowledge among physicians about the usefulness of this narrow spectrum of action antibiotic. This drug is not available in the Serbian pharmacies.

Izoxazole penicillins are halfsynthetic penicillins resistant to beta lactamase which is synthetised by Staphylococcus. The basic and extremely important indications for the use of these antibiotics (oxacillin, cloxacillin, dicloxacillin and flucloxacillin) are infections caused by Staphylococcus aureus susceptible to methicillin, such as dermal infections, cellulitis, infected burns, postoperative abscesses. They could be used in treatment of serious cases of pneumonia, meningitis, endocarditis and septic arthritis. If Staphylococcus aureus is proven to be the cause of osteomyelitis, these antibiotics are the drugs of choice (10). The reason behind the deficiency of these antibiotics in the pharmacies is their low price.

Cefoxitin is a cephalosporin of II generation which is administered intravenously and which penetrates through the surgical patient’s peritoneum very well. Streptococcus and Staphylococcus (apart from MRSA) are very susceptible to this drug. A special characteristic of cefoxitin is its excellent action against Mycobacterium abscessus, fast growing nontubercular bacteria which cause infections of soft tissues, medial nervous system, eye and bacteriemia. Fifty percent of Mycobacterium fortiutum are susceptible to cefoxitin. Therefore, this drug is used to treat infections caused by this bacterium (dermal infections, osteomyelitis, and joint and cornea infections).

These infections are common in immunocom-promised patients what makes this antibiotic necessary and valued in the treatment of immunocompromised patients and infections caused by atypical mycobacteria (11). This drug is available in the Serbian pharmacies.

Chloramphenicol was the first broad spectrum antibiotic to have been discovered. The advantage of this antibiotic lies exactly in its broad spectrum of action and the possibility of treating the infections caused by multiresistant bacteria (Enterococcus, Rickettsia, Strenotrophomonas maltophilia). The great importance and advantage of this drug is its ability to penetrate into the brain and eye tissues (12).

This antibiotic was withdrawn because it caused the depression of bone marrow. However, its low price means it is still being used in developing countries. If introduced back into the markets of developed countries, it could be used to treat infections in elderly patients, as well as in brain infections when treatment with other types of antibiotics fails. Chloramphenicol is especially important because of its ability to penetrate the brain and eye tissues. This drug is not registered in Serbia.

Quinupristin/dalfopristin is an antibiotic from the group of streptogramins. This antibiotic has excellent action against gram-positive bacteria. Enterococcus faecium, which is resistant to vancomycin, is especially susceptible to this drug. This constitutes the main reason behind its use in treatment against infections caused by this bacterium. Its use is also indicated in treatment of infections caused by Enterococcus faecium which are resistant to ampicillin and which are connected to the implantation of intravascular catheter (13). Despite its usefulness, this antibiotic is not widely used and it's rarely available in many countries.

Spectinomycin is structurally similar to streptomycin, but it is not an aminoglycoside. Today, it is used solely in the treatment of gonorrhea in the case of anorectal disease or gonococcus infections in patients allergic to beta-lactam antibiotics. Never the less, it is important to have this antibiotic available in the market due to the rise of gonococcus bacteria resistant to most antibiotics (14).

Teicoplanin is a glycopeptide antibiotic similar to vancomycin. It is effective exclusively against gram-positive bacteria, including MRSA resistant staphylococci. Teicoplanin is the antibiotic of choice in the treatment of infections caused by Enterococcus gallinarum and Enterococcus casseliflavus which are vancomycin resistant and which are not affected by any other antibiotic.

The advantage of teicoplanin in comparison to vancomycin lies in the possibility of one-day parenteral application, as well as in nonhospitalized patients, in addition to having less unwanted effects than vancomycin (15).

Tobramycin is an aminoglycoside antibiotic. The main advantage of this drug in comparison to other aminoglycosides is its effectiveness against Pseudomonas aeruginosa. Moreover, significant benefit of tobramycin is the possibility of administration by inhalation in patients with cystic fibrosis (16). It could also be applied intrarectally when the indications are appropriate. The use of tobramycin could reduce the prescription of wide spectrum antibiotics such as carbapenems and colistin. This drug is available in the Serbian pharmacies.

Colistin is a polypeptide antibiotic which is used in the form of colistin sulfonate and colistin methanesulfonate (prodrug). This drug is active against gram negative multiresistant bacteria such as Pseudomonas aeruginosa, Acinetobacter baumani and Klebsiella pneumoniae.

Colistin was withdrawn from the market due to dosage-dependent nephrotoxicity which passed with when the treatment with this antibiotic ceased. It was reintroduced to the market and it represents irreplaceable and valued antibiotic for treatment of infections caused by multiresistant gram negative bacteria (17). This drug is available in the Serbian pharmacies.

Colistin and quinupristin/dalfopristin are the antibiotics without alternatives. Colistin is the last drug of choice in the treatment of infections caused by gram negative bacteria, and quinupristin/dalfopristin is used to treat infections caused by Enterococcus faecium which are resistant to vancomycin, daptomycin and amoxicillin.

Fosfomycin is an antibiotic with a specific mechanism of action. This drug is effective against gram positive (Staphylococcus aureus, including MRSA strains and Enterococci) and gram negative (Salmonela, Shigellas, Proteus mirabilis, Enterobacteriacae, Serratia strains and Citrobacter) bacteria. Fosfomycin was abandoned, then reintroduced, and today, it represents a valued antibiotic in the treatment of acute cystitis in women without complications. Intravenously applied fosfomycin is used in the treatment of sepsis, serious cases of pneumonia, osetomyelitis, infections of medial nervous system, but only in cases when proven-efficacy antibiotics have no effect (18).

The main pharmacokinetic advantage of fosfomycin is the possibility of one time oral application of this drug in patients with acute cystitis since long lasting high concentrations of this drug are achieved. In addition, intravenously applied fosfomycin achieves high concentrations in brain tissues, but it is rarely used in the treatment of meningitis.

Fusidic acid is chemically categorized as an antibiotic similar to steroids. It is active against gram positive bacteria such as Staphylococcus aureus, including MRSA strains. It is used in the treatment of staphylococcal infections, but it is not the antibiotic of choice (19). This drug is available in the Serbian pharmacies.

Nitrofurantoin is a drug from the group of nitrofurans, used in the treatment of cystitis and in the prophylaxis of urinary infections. It is effective against many gram positive and gram negative bacteria (Enterococcus, Staphylococcus aureus, epidermidis and saprophyticus) (20).

Certain old antibiotics are gaining importance because of their special mechanism of actions which enable both the application of the antibiotic itself and the possibility of combining antibiotics with purpose of achieving synergistic action.

Conclusion

There is a rise of interest for usage of old antibiotics. These drugs could be invaluable in the treatment of certain infections and, in order for them to remain effective, it is necessary to conduct certain measures which would prove their worth. The aim of antibiotic therapy is to deliver the antibiotic to the place of infection and to retain it in the place of infection for a long period of time.

Most of all, it is of utmost importance to educate all medical personnel in health care system, especially physicians. The fact that the availability of these drugs remains low in comparison to the efforts made in attempt of discovering new antibiotics, imposes the need for national regulatory agencies to get involved in regulating the usage of these drugs.

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ULOGA STARIH ANTIBIOTIKA U PREVAZILAŽENJU REZISTENCIJE NA ANTIBIOTIKE

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Antibiotici su lekovi koji se koriste za sprečavanje ili lečenje infekcija uzrokovanih bakterijama. Otkrivanje i uvođenje antibiotika u medicinsku praksu dovelo je do revolucionarnih promena u terapiji i iskorenjivanju infektivnih bolesti. Trenutno postoji povećanje interesa za korišćenje starih antibiotika. Ovi lekovi mogu biti neprocenjivi u lečenju određenih infekcija i, kako bi oni ostali efikasni, neophodno je sprovesti određene mere koje bi mogle dokazati njihovu vrednost. Cilj antibiotske terapije je da se isporuči antibiotik do mesta infekcije i da se zadrži na mestu infekcije u dužem vremenskom periodu. Pre svega, izuzetno je važno edukovati, na ovu temu, sve zdravstveno osoblje u sistemu zdravstvene zaštite, posebno lekare. Činjenica da je dostupnost ovih lekova i dalje niska, u odnosu na napore u pokušaju otkrivanja novih antibiotika, nameće potrebu da se nacionalne regulatorne agencije uključe u regulisanje korištenja ovih lekova.

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Ključne reči: *antibiotici, rezistencija, infekcije*

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SURGICAL SITE INFECTION AFTER ELECTIVE COLORECTAL SURGERY: A REVIEW OF PREVENTION

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Colon cancer is the third leading cause of the disease in the world. In the world, about 1,200,000 people suffer from it every year. The leading cause of morbidity and mortality with about 500,000 deaths per year, SSI (surgical site infections) are most often complications in surgical practice. It is estimated that about 2-5% of patients receive an infection of the operating site after "pure" non-abdominal surgery, and even 20% after interventions in the abdomen. Infections of the operating site are the most common types of hospital infections in the countries of the European Union (19.6%). The reported incidence of these infections in the field of colorectal surgery ranges from 5% to 26%. Knowing the risk factors for the occurrence of surgical infections is a prerequisite for their prevention. Prevention of SSI in the field of colorectal surgery requires the implementation of a variety of preoperative, intraoperative and postoperative measures. More and more performed laparoscopic surgery in elective surgery on the colon against laparotomy with large incisions represents a selection technique that results in a smaller number of SSIs. Studies suggest that delaying resection in urgent conditions by stoma or stent with subsequent resection improves results in terms of a lower rate of complications including SSI, while overall survival time is considerably prolonged.

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Introduction

Annually, around a million people suffer from colon cancer, and half a million die. In 20 to 25% of newly detected patients, already in the diagnosis, there are distant metastases. With the use of multimodal therapy, the average survival of patients with metastases is about 2 years; only 10% of such patients live for five years. In the case of non-metastatic disease, surgery is the main form of treatment for colorectal cancer.

SSIs are the most common complications in surgical practice. It is estimated that about 2-5% of patients acquire an infection of the operative area after "pure" non-abdominal surgery, and even 20% after the procedure in the abdomen. SSIs are the most common and most complicated infections in surgical patients, which contribute to perioperative morbidity, prolonged postoperative hospital stay and increased treatment costs (1, 2, 3). Colorectal surgery is associated with the highest risk of SSI, mainly due to severe bacterial accumulation of the colon (1). Reported incidence of SSI after colorectal surgery ranges from 5% to 26% (4-8).

Surgical site infections and risk factors for their development

Hospital (infantile, intrahospital) infection is an infection that has occurred in patients and staff at the hospital or some other healthcare institution. It occurs as a local or systemic condition (state), which is the result of a reaction of the organism to the presence of an infectious agent (one or more) or its toxins, which was not present in the patient, nor was it incubated in the patient on admission to a hospital or other health institution. This internationally recognized definition was established by experts from the Center for Disease Control (CDC) in Atlanta, 1988. The name "nosocomial" comes from the Greek word nosus meaning "disease" and komeion meaning "to take care of", or Latin words nosocomium meaning

"hospital". Infections of the operating site can be divided into superficial infections, deep infections and organ infections (9).

Superficial infection

The infection occurs within 30 days of surgery and affects only the skin and the subcutaneous tissue of the incision (cut) and the patient has at least one of the following findings:

a) leakage of pus from surface incision;

b) microorganisms isolated from the culture of the secretion or tissue of surface incision (samples taken under aseptic conditions);

c) at least one of the following signs or symptoms of the infection: pain or sensitivity to the palpation, localized swelling, redness, or feeling of heat and deliberately opened wound by a surgeon, unless the culture of incision is negative;

d) the diagnosis of an infection by a surgeon or a treating physician.

Deep infection

Infection occurs within 30 days of surgery if no implant is implanted (a foreign body that is implanted during the operation and remains permanently in the patient's organism, e.g., artificial heart valves, heterologous vascular graft, mechanical heart, or joint prosthesis) or for a year if the implant is implanted and is associated with surgery and involves the deep subcutaneous tissue of the incision, such as facial and muscular lodges, and the patient has at least one of the following findings:

a) leakage pus from deep-tissue incisions;

b) a spontaneous development of wound dehiscence or the wound was intentionally opened by a surgeon because patient has had at least one of the following signs or symptoms: fever (38 °C), localized pain or palpation sensitivity, unless the culture of incision is negative;

c) an abscess or other evidence of an infection determined by a direct insight of the surgeon during a reoperation or a histopathological or radiological examination;

d) diagnosis of a deep infection of the operating site by a surgeon or a treating physician.

Infection of the organ/space of the operative site

Infection of the organs/space of the operative site includes any part of the body, except for incisions of the skin, fasciae or muscle boxes, which have been opened or manipulated with during the operation.

As far as the time of its occurrence is concerned, it must meet the previously mentioned criteria:

a) leakage of pus from the drain placed in the body/space of the operating site;

b) microorganisms isolated from the culture of the secretion or tissue of the operating site taken under aseptic conditions;

c) an abscess or other evidence of an infection of the organs/space of the operating site determined by the direct insight of the surgeon during a reoperation or histopathological or radiological examination;

d) the diagnosis of an organ/space infection by a surgeon or a treating physician.

The incidence of hospital infections varies in developed and developing countries, but it is also different in individual hospitals and in individual departments. Their incidence is 5-10%, prevalence in developed countries is on average 7.6% (between 3.5% and 12%), while in developing countries it is on average 10.2% (from 5.7% to 19.1%). Infections of the operating site are the most common types of hospital infections in the countries of the European Union (19.6%) (10-13).

Knowing the risk factors for the development of surgical infections is a prerequisite for their prevention. Risk factors for the development of surgical infections include factors related to the patient (age, sex, obesity, diabetes, compromised immune system, comorbidity, etc.), factors related to therapeutic approach (invasive procedures that damage normal host defense mechanisms such as urinary and vascular catheters, mechanical ventilation, irrational antibiotic therapy, etc.) and factors related to the work methods of health workers (application of measures to control infection) (14-17). Risk factors for the development of surgical infections vary depending on the type of hospital and the department where the patient is hospitalized. Patients in intensive care units are particularly exposed to many risk factors. Although we cannot influence most of the risk factors, which concern the patient itself, their knowledge is necessary, as health professionals will treat patients with risk with special care. Studies conducted in countries with high economic standards have shown that the most common risk factors for the development of SSI: age over 65 years, admission as an emergency in the intensive care unit, hospitalization longer than seven days, use of the central venous catheter, urinary catheter or endotracheal tube, surgical intervention, trauma-induced immunosuppression, neutropenia, rapid or extreme fatal disease (according to McCabe-Jackson classification) and reduced functional status or coma (7, 16). In medium-developed and underdeveloped countries, other risk factors have been identified for the development of surgical infections such as malnutrition, parenteral nutrition, and the existence of two or more comorbidities. The significant risk factors in these countries are the lack of financial support, the insufficient number of trained staff involved in controlling the infection, the lack of health workers in hospital departments and the insufficient capacity of equipment and tools (17).

Prevention of SSI in colorectal surgery

Preventing SSI in colorectal surgery requires the implementation of a variety of preoperative, intraoperative and postoperative measures to control risk factors. Patients undergoing colon and rectum surgery have potentially numerous risk factors for infection, apropos, the infection can develop as a result of many specific events during the surgical intervention itself. Choosing surgical technique,

strategy and preparation of patients as well as postoperative monitoring can lead to an improvement in the outcome of surgical treatment in these patients (17). For didactic reasons, preventive measures will be divided into preoperative, intraoperative and postoperative.

Preoperative preventive measures

Pre-hospital cleaning of the surgical field or site. Significant roles in preoperative preparation for planned interventions are played by preoperative bathing, showering and/or cleaning of the proposed surgical site with antiseptic soap and/or antiseptic. Despite a series of clinical studies, meta-analysis did not demonstrate a decrease in SSI rates in pure surgery or in any group of operations (18). One recent study indicates the need for repetition of showering or peeling of the antiseptic area, in order to achieve adequate efficacy in preventing SSI (19). The main source of microbiological contamination in colon surgery is actually the lumen itself, and not the skin, and it is unlikely that SSI will be prevented by aggressive prehospital cleansing.

Prolonged preoperative treatment. Classic of Cruse and Foord's study (18) and recent Vogel's et al. studies (19) showed that prolonged preopera-tive hospitalization, 3-4 days before surgery, incre-ases SSI rates and the incidence of other hospital infections. Prolonged preoperative hospitalization is likely to be related in connection with the case itself, namely other factors requiring a more rigorous pretreatment. Prolonged hospitalization also represents a permanent exposure to pathogens of the hospital environment that negatively affects the skin's resistance, and even the microflora of the colon.

Depilation. Follicules at the site of surgery are always considered to be at risk of accumulating bacteria. However, there is no evidence to support hair removal and with a reduced rate of SSI (20). Study of Alexandra et al. (21) has shown that any removal of the hair, the night before surgery increases the risk of SSI. Mechanical hair removal with a shaver results in cutting and damaging the skin. These injuries on the surface of the skin on the night before the surgery are likely to become sites for microbial growth of the skin microflora (e.g. Staphylococcus aureus) and increase the probability of developing infection at the site of the incision. These studies also identified that the removal of hair in the patient's room was also associated with an increased rate of SSI. If hair removal is considered necessary, it should be done immediately before surgery, in the patient preparation room, immediately prior to the application of the antiseptic.

Preparing the site of the incision. The three main antiseptic solutions used to prepare the site of the incision are chlorhexidine, povidone iodine and isopropyl alcohol. Isopropyl alcohol has the best antibacterial efficacy but is highly inflammable and there is a risk of fire in the operating room when used in combination with an electrocautery. Fires in the operating rooms occur more than 500 times a year in the United States and consistently identified were flammable antiseptics, oxygen and flammable foils (23) as the main causative agents. Chlorhexidine is associated with a better antiseptic effect than povidone iodine and it is more effective in the prevention of infections (22-24). One review and one meta-analysis conclude that better preparation of the field in the prevention of SSI is with using chlorhexidine (22, 25, 26).

Plastic foils/wound dressings. Plastic foils are placed on the skin at the site of the incision and used for a certain period of time to prevent colonization of the microbes on the skin. Initial cases reported unexpectedly higher rates of infection with these plastic films, which was probably due to the effect of "greenhouse", sweat and microbiological proliferation under plastic (27). Recent versions of these plastic films now use an antiseptic (e.g. povidone iodine) on the surface of the adhesive and have better adhesion to the surface of the skin. However, recent meta-analysis has not shown any reduction in SSI rates with the use of newer generation foils (28). It is proposed to cleanse the surgical site with antiseptic, completely dry the antiseptic and press the plastic film before the incision. Another variation on the topic of plastic film is a ring structure that is inserted into the abdomen, which with a simple twist, completely separates the wound from the site of surgical work. This makes sense for temporary protection when the contamination that occurs in the colorectal operations is concerned. Meta-analysis has identified benefits for this type of film (29), but additional clinical trials are necessary.

Preventive use of antibiotics. Preventive use of antibiotics in elective colon surgery is generally seen as an important method that has positive effects in the prevention of SSI. With the introduction of antibiotics in clinical practice during the Second World War, a wide use of antibiotics in operative procedures begins, especially in the digestive tract. A positive effect should be no especially in colorectal surgery where the rate of contamination is high. The initial enthusiasm, especially in patients treated with colorectal disease, has very quickly whittled away due to the low rate of reduction of surgical wound infections. The question was: when is the right time to give antibiotics? At that time, antibiotics were given after surgery, and in cases with high rates of infection (e.g., colon surgery), as well as in cases of low-infection surgery (e.g. inguinal hernia reparation). The impact of the period of administration of antibiotic prophylaxis in surgery was identified in experimental studies by Miles et al. (30), and in clinically relevant experimental models, Burke (31). The key characteristics of the preventive use of antibiotics in these experimental studies were that a tissue antibiotic was needed at the time of bacterial contamination of soft tissues and that in this way the applied antibiotic prevents the spread of infection in the tissues. The antibiotic that was given more than two hours after contamination had no effect on the onset of infection. Polk and Lopez made the first clinical study on the importance of prophylactic administration of antibiotics before surgery, which showed a statistically significant reduction in the rate of SSI using antibiotic (cephaloridine) before the surgical incision (32). Patients received the second and third dose 5 and 12 hours after the initial dose, and then all antibiotics were

abolished. Subsequent studies by Stone et al. about pure and contaminated surgeries, including colon resections, have shown the antibiotic given prior to surgery was effective in reducing SSI, and further receiving antibiotics after closing the wound has no effect on the SSI rate (33, 34, 35). After these pioneering clinical trials, there were a number of reported studies that further confirmed the benefits of preoperative use with antibiotics. Baum et al. showed the striking results of numerous placebo-controlled studies that showed the benefit of preoperatively prophylactically-applied antibiotics in colon and rectal surgery and concluded that there was no use for further placebo-controlled studies (35). It is important to consider why the antibiotic given after the wound closure does not improve the SSI rate. Bacterial contamination of the environment occurs after tissue injury, bacteria are instantly incorporated into fibrin as part of the inflammatory response to tissue injury. During surgery, contamination of the surgical wound continues from several potential sources. In the act of closing the wound, subcutaneous tissue and skin, the enclosed space is also filled with fibrin that leaves a dense protein matrix with twisted microbes. The fibrin matrix is impermeable to systemic antibiotics from the circulation. The presence of the drug is required at a time when fibrin is produced from the protein serum to act on bacterial strains. Antibiotics administered after contamination of fibrin do not make contact with the surgical site. In addition, edema as well as the activated inflammatory response continues after closing the wound which results in increased hydrostatic pressure in the tissues around the closed incision (36).

The selection of antibiotic for elective surgery on the colon is detailed in Table 1.

Table 1. The most commonly used antibiotics for elective colon surgery

Drug choice (dose)	Advantages	Disadvantages
Cefoxitin (1 g)	Low toxicity cephalosporin with many years of use for prophylaxis, aerobic and anaerobic coverage.	Short biological elimination half-life (45 min); concerns about gram negative resistance.
Cefotetan (1 g)	Low toxicity cephalosporin with many years of use for prophylaxis, aerobic and anaerobic coverage. Long biological elimination half-life (4 hr).	Concerns about gram negative resistance.
Ampicilin/Salbactam (1.5 g-3.0 g)	Extensively used penicillin with a beta-lactamase inhibitor; good anaerobic coverage.	Short biological elimination half-life (1 hr); emerging E. coli resistance in up to 40% of isolates.
Ertapenem (1 g)	Extended gram negative coverage (not Pseudomonas spp.); long biological elimination half-life (3.5 h).	Expense

The selection of antibiotics should have an effect against potential pathogens for contamination of the surgical site. It is expected that this selection will cover staphylococci as the main contaminant of the skin then E. coli of the main enteral gram negative strain in the colon, and Bacteroidesfragilis, which is the primary colonic anaerobic pathogen. This coverage profile was identified in the second generation of cephalosporin antibiotics cefoxitin or cefotetan. This is also seen in semisynthetic penicillins with a β-lactamase inhibitor. Combined antibiotics such as the first generation cephalosporins (e.g., cefazolin) with anaerobic coverage and metronidazole or clindamycin are a choice of antibiotics for prophylaxis, while fluoroquinolone with metronidazole or clindamycin is another option. Another consideration in the use of preventive antibiotics is the biological half-life of the antibiotic elimination. Because of the half-life, i.e., the effects of antibiotics, application immediately before surgery for β-lactam antibiotics (i.e., penicillins or cephalosporins) is recommended. The next generation of antibiotics (cefotetane or ertapenem) are desirable due to the longer period of coverage compared with the second group of antibiotics, for which there is an insufficiently solid evidence of efficacy in elective colon surgery (36, 38).

The second question that is commonly referred to is antibiotic dosing. The traditional dosage was to use the same dose for all patients. A general increase in body mass index (BMI) of patients has raised concerns that the volume of drug distribution in larger patients (37) has been expanded. For bariatric and other operations in patients with BMI > 30, the dose of antibiotic for prophylaxis should be considered. The occurrence of methicillin-resistant Staphylococcus aureus acquired under non-hospitable conditions (CA-MRSA) has caused concern and

led many to advocate the examination of nasopharyngeal and perioperative decontamination as well as the liberal use of vancomycin as a preventive antibiotic (38). The role of this type of prophylaxis is most often performed in large-scale pure surgeries such as coronary bypass or complete joint replacement in orthopedics.

Preparation of the colon. Mechanical preparation of the intestine with the reduction of the intestinal flora in elective surgery of the colon and rectum was soon considered as a standard protocol of preoperative preparation. Mechanical cleansing of the intestine before colorectal surgery depends on the localization of tumor, stenosis, planned surgery (type of procedure) (39). Different techniques and the use of drugs used for the purpose of preparing the intestine depend on the practice of a doctor who leads the preoperative preparation. The use of oral laxatives and skis is combined, as well as local application of chilled 10% solution of Mannitol and skis. However, the application of mechanical cleansing of the intestine with preoperative hunger leads to a disturbance of the balance of volume and electrolytic status, which disrupted the homeostasis of the organism. During mechanical cleaning of the intestine, the patient should be sufficiently hydrated (40). Most recent clinical studies state that there is no statistically significant difference in the incidence of postoperative complications in patients in whom mechanical preparation of the bowel was performed in relation to patients in whom mechanical preparation of the bowel was not performed (41).

Intraoperative preventive measures

Postincision measures. Surgical technique during surgery is a critical issue for preventing SSI in operative treatment. According to Altemire in 1958, "evidence clearly indicates that antibiotic therapy cannot prevent the development of a local infection, unless surgical principles are established or technical details are ignored during the procedure (42)." A poor surgical technique can override the benefits that preventive administration of antibiotics can provide.

Minimizing tissue injury in incision by layers is important to prevent SSI. Rough handling often causes a greater tissue injury further resulting in local inflammation and increasing the risk of leakage and the dehiscence of anastomosis and the development of SSI. Prevention of hematoma formation requires effective hemostasis. Rolled scarves such as silk should be avoided in surgical procedures. Excessive use of electrocautery leaves the necrotic beaches inside the wound and leads to an increased infection rate. Bipolar devices are useful in achieving correct haemostasis, without excessive tissue injury. The electrocautery can be used as an alternative to a surgical knife without increasing the infection rate (42), but should be used with appropriate programs to avoid damaging the tissue. Electrocautery is not recommended for cutting hoses that will be anastomosed due to necrosis of the tissue and loss of perfusion. Placing the drains is done through a newly formed opening, never through the surgical incision itself.

Air handling systems. Bacteria that are transmitted by air as a source of contamination of the wound are long-standing concerns of the surgeon. Lister allegedly aerosolizes carbolic acid into an operating room that prevents the spread of bacteria. Fifty years ago, there has been interest in the use of ultraviolet light in the operating space for the elimination of microbes in the air. Large multicentric studies have been conducted that have proved the unsuitable use of ultraviolet light in this way (43). The use of the Laminar Air Flow System is justified in pure opera-tions (44). Restricting traffic to and from operation reduces the generation of air currents that can significantly reduce bacteria from the floor in the air (45). Given the large number of bacteria from the colon, the dominant operative wound infection in colorectal surgeries will almost always be from the bowel's contents, and in these operations, no major accent is given to the aforementioned procedures, which, however, should not be abandoned.

Antibacterial sewing material. Over the past 15 years, antibacterial sewing material has been developed for closing fascia, subcutaneous tissue, and anastomosis of the organs. The material is coated with antiseptic triclosan. Triclosan is commonly used as an antiseptic in cosmetics and other products and is safe for human use (46). Through *in vivo* experiments, it has been proven that this sewing material reduces the growth of bacteria, but also numerous studies from different countries constitute conflicting evidence of the development of SSI despite the use of sewing material with antibacterial protection (47-51).

Rinse the surgical field. Rinse the surgical area is part of the surgical technique, and is necessary especially for "dirty surgeries" such as surgery of the colon. Depending on the technique, various antimicrobial or antiseptic agents are used.

Core Body Temperature Control. Hypothermia during the operative procedure is associated with haemostasis problems and experimentally proven in the laboratory results in damage to the phagocytic function. Kurz et al. (52) in a randomized study with 200 surgical patients with colorectal disease, intraoperatively examined the body temperature maintained in normotermia (36.6 degrees) compared to patients who were allowed to have a temperature drop (34.7C). SSI was developed by 19% of patients with hypothermia, but only 6% in the group of patients with normothermia. Until recently, there was little evidence to support or reject the merits of maintaining normotermia in colon surgery, but was nevertheless adopted by the US Surgical Care Improvement Project (SCIP) as a process to improve treatment outcomes.

Glycemic control. Complications in surgical patients with diabetes are associated with a risk of developing infections and poor wound healing. Better control of diabetes is closely linked to a better outcome in the treatment. This observation led to the primary research carried out by Furnari et al. (53) for controlling blood sugar < 200mgs/100mL in patients with diabetes by using intraoperative and postoperative insulin infusion. This program included over 2500 patients with diabetes which resulted in a decrease in SSI versus the same rate as

patients who did not suffer from diabetes. Hyperglycaemia has multiple immunosuppressive effects on the host. Accordingly, perioperative hyperglycaemia is practically related to surgical infections including colon resection (54). Generally operated patients with hyperglycemia have a higher risk of postoperative infection including SSI (55).

Delayed primary closure. Delayed primary closure stands as a strategy for the prevention of SSI for possible encounter with an active infection or serious contamination during surgery. Introduced in 1940 (56), this method implies the closure of the abdominal fascia after laparotomy, as well as leaving the skin and subcutaneous tissue open for daily dressing of the wound. In the case of planned surgeries, this method should be considered only in rare cases of contamination of the abdominal cavity with colon contents in an unprepared patient or due to an unplanned occurrence of abscess during surgery.

Postoperative preventive measures

It is known that every operation carries a certain risk of developing a series of complications, some of which may even be life-threatening. The post surgical treatment of surgical site - the type of post surgical care of the surgical wound is determined by the time of closing the incision; any operative site, regardless of the type of closure, must be dressed with the use of sterile gloves, sterile instruments and with respect to the aseptic techniques during the working. Drains placed in the surgical wound could increase the risk of developing an infection because they act as a foreign body and reduce local immunological reaction, i.e. natural mechanisms of tissue defense. In order to prevent and repress intra-hospital infections at all surgical departments, the basic measures of prevention of hospital infections should be consciously and continuously implemented: hand washing, cleaning, washing and ventilation of rooms, cleaning and washing of the related equipment, air quality assurance, early detection and isolation of patients with a hospital infection.

Conclusion

SSI are the most common complications in surgical practice, which contribute to perioperative morbidity, prolonged postoperative stay in the hospital and increased treatment costs. Colorectal surgery is associated with the highest risk of SSI, mainly due to severe bacterial loading of the colon. It is necessary to continuously use all accepted prevention techniques in order to reduce SSI. New methods have to be implemented to reduce SSI in colorectal surgery. There is a great progress in systemic antibiotics in prevention as well as in achieving optimal physiological conditions - intraoperative supplemental oxygen, normothermia, and proper glycemic control. However, despite extensive research in this field and significant progress, SSI will remain a long-standing challenge in surgery.

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INFEKCIJE MESTA HIRURŠKOG RADA NAKON ELEKTIVNIH KOLOREKTALNIH OPERACIJA – PREGLED PREVENCIJE

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Rak debelog creva treći je vodeći uzrok oboljevanja u svetu. U svetu godišnje oboli oko 1.200.000 ljudi. Rak debelog creva vodeći je uzrok morbiditeta i mortaliteta sa oko 500.000 smrtnih slučajeva godišnje. SSI (eng. surgical site infections – infekcije na mestu hirurškog rada) najčešće su komplikacije u hirurškoj praksi. Procenjuje se da oko 2% - 5% bolesnika dobije infekciju operativnog mesta nakon "čistih" neabdominalnih operacija, a čak 20% nakon intervencija u abdomenu. Infekcije operativnog mesta su, u zemljama Evropske unije, najučestaliji tipovi bolničkih infekcija (19,6%). Prijavljena incidencija ovih infekcija, u oblasti kolorektalne hirurgije, iznosi od 5% do 26%. Poznavanje faktora rizika za nastanak hirurških infekcija predstavlja preduslov za njihovu prevenciju. Sprečavanje SSI u oblasti kolorektalne hirurgije zahteva implementaciju mnoštva preoperativnih, intraoperativnih i postoperativnih mera. Sve više izvođena, laparoskopska hirurgija, kod elektivnih operacija na debelom crevu, u odnosu na laparotomije sa velikim incizijama, predstavlja tehniku izbora koja rezultira manjim brojem SSI. Istraživanja pokazuju da odlaganje resekcije kod urgentnih stanja, bilo stomom ili stentom, uz kasniju resekciju, poboljšava rezultate, u smislu manje stope komplikacija među kojima je i SSI, dok je ukupno vreme preživljavanja znatno produženo.

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Ključne reči: *kolorektalni karcinom, hirurške infekcije, antibiotska profilaksa*

Case report

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CUTANEUS AND SUBCUTANEUS METASTASIS FROM HEPATOCELLULAR CARCINOMA - REPORT OF THREE CASES

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Hepatocellular carcinoma (HCC) is the third most common cause of death from all cancers. Metastases of HCC in the skin are very rare and account for only 0.8% of all known cutaneous metastases.

We here present three cases of HCC with metastases in the skin and subcutaneous tissue, which was the first manifestation of the tumor. The diagnosis was based on the characteristics of histomorphological appearance and testing of the immuno-phenotype of tumor cells. In all three cases, within the metastatic tumors, a strong immunohistochemical expression of AE1/AE3, EMA and HepPar1 was confirmed. In two cases, elevated serum levels of AFP were found.

In any diagnosis of cutaneous malignant tumor it is, first of all, important to distinguish any secondary deposits in the skin from the usual skin neo-proliferations. HepPar1 is an excellent marker of hepatocellular differentiation which significantly facilitates the diagnosis of metastatic hepatocellular carcinoma.

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Key words: *Hepatocellular carcinoma, skin metastasis, initial presentation*

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Introduction

Hepatocellular carcinoma is the most common primary malignant tumor of the liver and makes up 7% of all malignant tumors in humans. It is the sixth most common malignant tumor, with the highest incidence in East Asia (China, Korea, Taiwan and Japan) and Western and Central Africa. During 2012, 782,500 new cases of liver cancers were reported worldwide (1, 2) The incidence rate in the USA is growing progressively and in the period between 1975 and 2011, a growth from 2.6 per 100,000 to 8.6 per 100,000 was observed (3).

The increasing incidence of HCC is directly correlated with an increased frequency of hepatitis B (HBV) and hepatitis C virus (HCV) infection (4, 5). It has been also estimated that there is an association of HCC with exposure to aflatoxin, autoimmune hepatitis, steatohepatitis and primary biliary cirrhosis and sclerosing cholangitis (6-8). Some two decades ago it was observed that liver cirrhosis was present in approximately 80-90% of diagnosed patients and it presented the most important risk factor for the occurrence of HCC (9). Other risk factors include chronic alcoholism, cigarette smoking, hemochromatosis and much rarer tyrosinemia (4-6).

Hepatocellular carcinoma often invades blood vessels which can lead to extensive intra - and extrahepatic metastases, usually to the lungs, lymph nodes, bones, kidneys and adrenal glands (10). In this report we examined a case of HCC with cutaneous metastases and two cases of HCC manifested by multiple tumor formations in the subcutaneous tissue. In all three cases, the initial clinical presentation of HCC was the skin and/or subcutaneous tissue involvement.

Case reports

Case No 1

A male patient, aged 63, was referred to a surgeon due to an ulcerative skin tumor on the parietal region of the scalp. The change had been noticed 3

months before he came to the doctor. He described a rapid and constant growth of the tumor.

The resected material was a fragment of skin and subcutaneous tissue, 40 x 27 x 15mm in size, on the surface of which there was an ulceration. The bottom of the ulceration consisted of whitish homogeneous, solid tumor tissue, 25 x 15 x 12mm in size. It was established by histopathological examination that the resected skin and subcutaneous tissue was infiltrated with tumor cells organized in solid clusters and rare adenoid formations. The tumor cells were medium large, atypical, round and polygonal, with abundant, bright, eosinophilic cytoplasm and irregular, hyperchromatic and large nuclei with prominent nucleoli. The stroma of the tumor was scanty. Mitosis were numerous. Tumor growth was infiltrative. Necrosis was present in the tumor tissue (Figure 1).

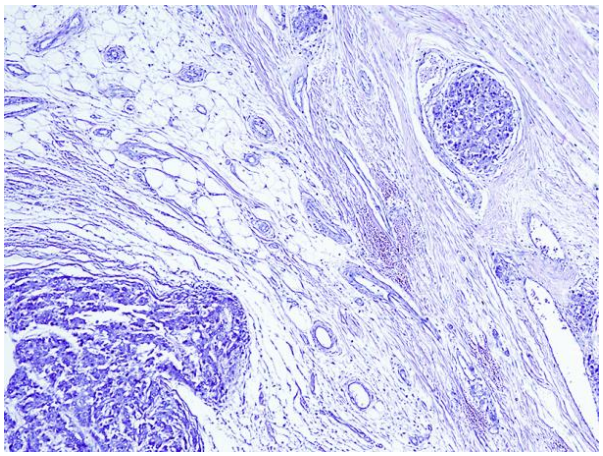

Figure 1. HCC metastases in the skin (HE, 100x)

The tumor cells had the following immunohistochemical profile: EMA (Figure 2), AE1/AE3, CK7 and HepPar1 – positive.

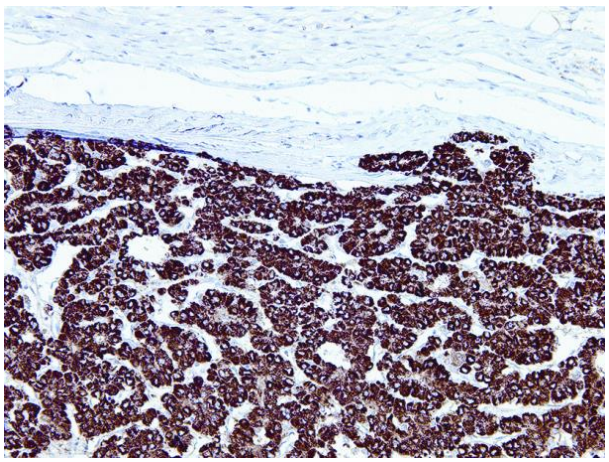

Figure 2. Moderate to strong expression of EMA in the cells of metastatic HCC in the skin (IHC, 40x)

After the performed analysis, a metastasis of hepatocellular carcinoma in the skin was suspected.

As part of the examination, magnetic resonance imaging of the abdomen, bone scintigraphy and chest CT were carried out. After the radiological examinations, a solitary liver tumor, 6cm in diameter, was observed, as well as the changes in the ninth and tenth thoracic and second lumbar vertebra, that corresponded to secondary deposits. Blood analysis and biochemical tests were within normal values. AFP level was higher than normal. The patient underwent oncological treatment; the death occurred 8 months after the hepatocellular carcinoma was first diagnosed.

Case No 2

A male patient, aged 61, was referred to a surgeon due to a subcutaneous tumor on the back, which he had noticed two months earlier, explaining its growth as being progressive.

The patient was treated for tuberculous spondylitis 15 years ago. During the surgical intervention, an unresectable tumor was noticed, and after the biopsy, the material was sent for histopathologic analysis. The obtained sample contained tumor tissue that contained adenoid, trabecular and solid arrangements of large, atypical, cubical and round cells with abundant, bright cytoplasm (eosinophilic or "empty") and irregular, moderately pleomorphic nuclei with prominent nucleoli. The stroma of the tumor was scanty. As it was a case of tumor tissue with malignant morphological characteristics, suspected as being a metastatic deposit, an immunohisto-chemical analysis of the tumor tissue was performed. The immunohistochemical profile of the tumor tissue was: EMA, AE1/AE3, HepPar1 (Figure 3) - positive.

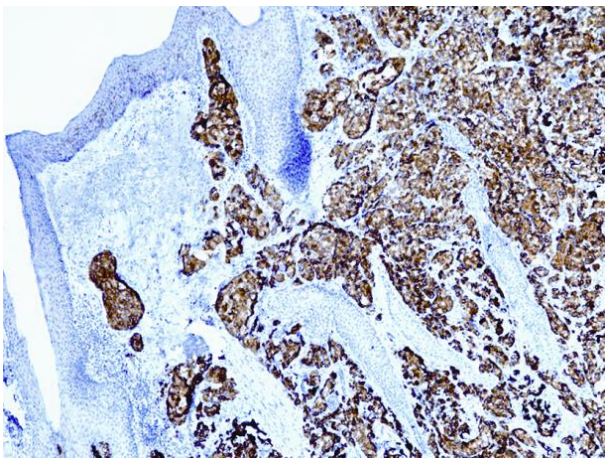

Figure 3. High expression of HepPar1 in the cells of metastatic HCC in the subcutaneous tissue (IHC, 100x)

After the histopathological analysis a subcutaneous metastasis of hepatocellular carcinoma was

suspected. After the histopathological analysis, CT imaging of the chest and abdomen was conducted. The CT scan of the chest revealed a tumor mass that infiltrated the subcutaneous tissue of the back and the sixth, seventh and eighth ribs of the chest wall, with the total size of 6cm. CT scanning of the abdomen revealed a tumor in the liver of 8cm in size. The AFP level was higher than normal. The patient was referred for oncological treatment. Survival period from the time of diagnosis was 19 months.

Case No 3

A patient, 62 years old, was referred to a surgeon due to rapidly growing subcutaneous tumors on the scalp, parotid region of the neck and pectoral region. Due to the clinical suspicion that these may be malignant metastatic deposits in the subcutaneous tissue, a resection of the subcutaneous tumor in the pectoral region was performed. The material obtained was a whitish, firm, nodular tumor of 1.8cm in diameter.

Histopathological analysis revealed tumor tissue composed of scanty stroma and parenchyma, made up of trabecular and solidly arranged large, cuboidal, atypical cells with abundant, eosinophilic cytoplasm and large, irregular, vesicular nuclei with prominent nucleoli and numerous pathological mitoses (Figure 4). The results of an immunohistochemical analysis of the tumor tissue were: HepPar1, AE1/AE3, EMA, Vimentin - positive.

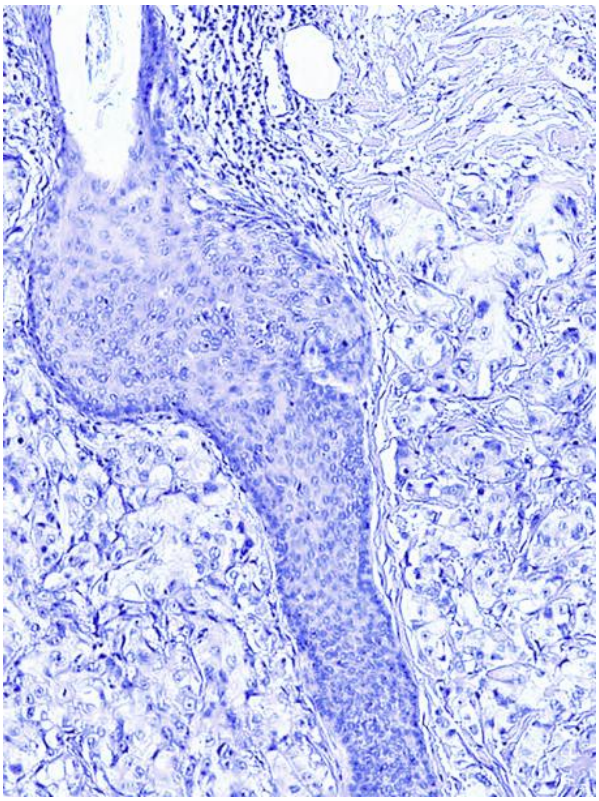

Figure 4. Infiltration of subcutaneous adipose tissue with metastatic HCC (HE, 40x)

After histopathological and immunohistochemical analyses, a metastasis of hepatocellular carcinoma was suspected, and it was later confirmed by radiological and biochemical examinations. The AFP value was within normal range. After the diagnosis, the patient lived for 6 months without any oncological therapy.

Histopathological examination

In all three cases, the samples of tumor tissue were fixed in 10% neutral buffered formaldehyde, routinely processed and embedded in paraffin. From the paraffin blocks, sections of 3-4μm in thickness were made, on which a routine Haematoxylin-Eosin method for histopathological analysis of lesions was applied, as well as immunohistochemical analysis.

The resected tissue samples which were used for immunohistochemical analysis were mounted on highly adherent slides (SuperFrost) and then dried at 60 ^{O}C in a thermostat for up to 24 hours. After the preparation of the sections in the PT link (Dako, Glostrup, Denmark) (reagent: EnVision Flex Target retrieval solution high pH), the further immunohistochemical staining procedure was automated. AUTOIMUNOSTAINER LINK 48 (Dako, Glostrup, Denmark) was used. The Dako EnVision Flex System for visualization was used. Primary Dako antibodies, Flex Ready to use were applied and as a chromogen DAB (Diaminobenzidine) was used. Contrasting in haematoxylin was done manually (for 3 to 5 minutes).

Discussion

Hepatocellular carcinoma is the third most common cause of death from all cancers and the leading cause of death in patients with liver cirrhosis (11, 12, 13). It has an aggressive clinical course and it usually spreads by direct extension into the lumen of the portal vein branches and/or the lumen of hepatic veins. 10 About 30% to 50% of hepatocellular carcinomas develop extrahepatic metastasis (14), most frequently to the lungs, lymph nodes, bones and the adrenal glands (10, 11). In recent years numerous reports have indicated an unusual behaviour of metastatic hepatocellular carcinoma (15-17). This is primarily related to the localization of extrahepatic metastasis, and various authors have described the presence of metastases in the maxillary sinus (15), orbit (16), zygomatic bone (18), mandible (19), parotid gland (20), larynx (21), subcutaneous tissue and skin (22, 23). Subsequently, extrahepatic metastases are often the initial manifestation of hepatocellular carcinoma (18, 19, 21) as was the case with all our three patients, whose first symptoms of hepatocellular carcinoma were related to the presence of metastases in the skin and subcutaneous tissue. The skin is a relatively unusual metastatic site; it is believed that less than 10% of all malignant tumors of the internal organs metastasize to the skin (1, 24) Cutaneous metastases most often originate from cancers affecting the breast and lungs, as well as from melanoma (25). Metastasis of hepatocellular carcinoma in the skin are very rare and are estimated to account for only 0.8% of

all known cutaneous metastases (26), but one should bear in mind that most evaluations are based on autopsy findings or on sporadic case re-ports.

Cutaneous metastases of hepatocellular carcinoma are usually localized in the head and neck region, and rarely on the shoulders and on the trunk (27, 28). Macroscopically, these present as nodules with or without ulcerations (solitary or multiple), as well as a diffuse infiltration (plaque) or as a papule accompanied by hyperpigmentation (29). In the first case, the metastatic tumor was a nodule localized in the parietal part of the scalp and there was an ulceration on the surface of the skin.In the second case, the metastatic tumor presented as a diffuse infiltration. In our third patient, the metastases presented as multiple nodules localized subcutaneously in the areas of the scalp, parotid region and pectoral region.

The mechanism of metastasizing of hepatocellular carcinoma in the skin and soft tissues has not yet been fully clarified. It is believed that cutaneous metastases occur when the tumors spread by a haematogenous route or via implantation during surgical procedures (22), while the possibility of tumor cell seeding after percutaneous needle aspiration is highlighted (23, 30). The appearance of cutaneous metastases suggests that the metastatic disease has become generalized, indicating a poor prognosis (31). Our first patient died 8 months after he had been diagnosed. The survival time for the second patient was 19 months and the third patient died 6 months after his diagnosis.

Hepatocellular carcinoma is almost three times more common in males than females (M:F = 2.7:1). It is the second leading cause of death among men, while the average age at the time of diagnosis is 65. It has been noted that in the countries with a high risk of developing HCC, this tumor may occur even before the age of 20, while in the countries with a low risk, it occurs in the population above the age of 50 (32, 33). All three patients presented in this report were males with an average age of 62 when diagnosed. The differences in the incidence of hepatocellular carcinoma in relation to sex could be the result of the prevalence of alcoholism and chronic diseases in men, but this difference is also associated with increased secretion of the cytokine interleukin-6 (IL-6) in men, which plays a major role in the inflammatory response and exerts pro-proliferative and anti-apoptotic effects (34, 35). In experiments on mice, it has been shown that estrogen inhibits the secretion of IL-6, and suggests that the "estrogen-mediated inhibition" of IL-6 reduces the risk of the development of HCC in women (36).

For a correct diagnosis of cutaneous malignant tumors, it is important to distinguish any secondary deposits in the skin from typical skin tumors. In order to confirm the diagnosis of cutaneous metastasis, a biopsy is necessary. Histopathological diagnosis of metastatic hepatocellular carcinoma can sometimes be relatively simple, with the histological appearance of bright, eosinophilic cells, forming trabecular, pseudoglandular or solid arrangements, while the presence of bile and Mallory's bodies indicates hepatocellular carcinoma (11). In most cases, histopathological diagnosis can be difficult especially as the metastatic cells are often less differentiated than those in the primary tumor. In such cases, immunohistochemical diagnosis is of great importance. HepPar1 is a relatively specific marker for hepatocytes and hepatocellular carcinoma cells. It is the G1K monoclonal antibody which recognizes a mitochondrial antigen present in normal and neoplastic hepatocytes. This marker is very helpful for the diagnosis of occult hepatocellular carcinoma with distant metastases (26).

Our three cases of hepatocellular carcinoma with metastases in the skin and subcutaneous tissue indicate the unpredictable phenotypic characteristics of the tumor, suggesting that every skin lesion should be carefully observed, whereby it is very important that the pathologist identifies a secondary deposit. HepPar1 is a marker of hepatocellular differentiation which greatly facilitates the diagnosis of metastatic hepatocellular carcinoma.

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Prikaz bolesnika

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KOŽNE I POTKOŽNE METASTAZE HEPATOCELULARNOG KARCINOMA - PRIKAZ TRI SLUČAJA

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Hepatocelularni karcinom (HCC) je treći najčešći uzrok smrti, imajući u vidu sve maligne tumore. Metastaze HCC u koži veoma su retke i čine svega 0,8% svih poznatih kutanih metastaza.

Mi prikazujemo tri slučaja HCC sa metastazama u koži i u potkožnom tkivu, koje su bile prva manifestacija tumora. Dijagnoza je postavljena na osnovu karakterističnog histomorfološkog izgleda i ispitivanjem imunog fenotipa metastatskih lezija. U sva tri slučaja je, u metastatskom čvoru, a kasnije i u intraoperativnoj biopsiji, verifikovana jaka imunohistohemijska ekspresija AE1/AE3, EMA i HepPar1. U dva slučaja zabeleženi su i povišeni serumski nivoi AFP.

Za dijagnozu ekstrahepatičnih metastaza važno je, pre svega, razlikovati sekundarne depozite u koži od uobičajenih kožnih neoproliferacija. HepPar1 je odličan marker hepatocelularne diferencijacije, koji znatno olakšava dijagnozu metastaza hepatocelularnog karcinoma.

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Ključne reči: *hepatocelularni karcinom,metastaze u koži, inicijalna manifestacija*

Case report

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POSTPARTUM CARDIOMYOPATHY IN THE CORONARY UNIT: A CASE REPORT

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Postpartum cardiomyopathy (PC) is a form of dilatative cardiopathy, an unclear etiology that occurs in previously healthy mothers. Criteria for diagnosing postpartum cardiomyopathy are based on heart failure from the last month of pregnancy up to 5 months after delivery, left ventricular ejection fraction (LVEF) <45%, absence of a known cause of heart failure, absence of heart disease before the last month of pregnancy and the absence of echocardiographically observed systolic dysfunction of the left chamber before pregnancy. The patient is placed in an intensive care unit. She complains of weakness, malaise, swelling and increased blood pressure. She was treated with medications, diuretics, an ACE inhibitor, a beta blocker and antiarrhythmics due to frequent episodes of ventricular tachycardia (VT) interrupted on 3 occasions by 100 J DC synchronous shock. The installation of a double chamber implantable cardioverter defibrillator was indicated, and it was implanted 5 days after the stabilization of symptoms.

Peripartal cardiomyopathy endangers the health of pregnant women and maternity. A clinical suspicion of the existence of a PC is extremely important for early diagnosis. Echocardiography with a PC is necessary for the diagnosis and monitoring of the course and outcome of treatment. Standard treatment for heart failure is recommended in patients with PC; however, the therapy should be adjusted taking into account the health of the fetus during pregnancy. Further research is needed to determine the pathophysiological mechanisms of this cardiomyopathy, biomarkers specific to the disease itself, effective treatments, and prevention measures for the emergence of a PC.

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Key words: *postpartum cardiomyopathy, implantable cardioverter defibrillator*

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Introduction

Postpartum cardiomyopathy (PC) is a form of dilated cardiomyopathy, an obscure etiology that occurs in a previously healthy woman in labor. It was first described in 1849 (1). So far, many risk factors for PC development have been defined, but the exact etiology is still unknown. Factors associated with the onset of the disease, based on studies to date, are: maternal age, multiparity, hypertensive disorders, pregnancy complications, multiple pregnancies, use of tocolytics, poverty, tobacco use, malnutrition, anemia during pregnancy, inflammation, viral myocarditis, abnormal immunologic abnormalities or hemodynamic response to pregnancy, apoptosis, hormonal abnormalities, impaired angiogenesis, increased oxidative stress. Its incidence shows marked geographical and ethnic variations. It is most commonly reported among women of African descent (2). Previous studies have evaluated the incidence of postpartum cardiomyopathy (PC) in 1 case in 1,455 live births to 1 case in 15,000 live births (3). Recovery rates range from 29% to 72%, while mortality rates range from 0% to 25%. In previous studies, patients were most often monitored only 6 to 12 months after diagnosis, and those who recovered after this time period were not further monitored (4).

Previous work has shown that PC can occur at any age, but its incidence is significant for women over 30 (5).

Criteria for the diagnosis of postpartum cardiomyopathy are based on cardiac weakness from the last month of pregnancy to 5 months postpar-

tum, left ventricular ejection fraction (LVEF) < 45%, no known cause of heart failure, absence of heart disease before the last month of pregnancy, and the absence of echocardiographically observed left ventricular systolic dysfunction before pregnancy (6).

Case report

Patient J. I., 32 years old, was admitted to the Clinical Center Niš, Clinic for Cardiology, because of general weakness, weight gain and high blood pressure, which was first registered the previous day (170/110 mmHg) in the Health Center. The patient denied earlier problems with the cardiovascular system, had three children, the last birth was one month before hospitalization. There were no heart patients in the family. She denied the existence of cardiovascular problems and illnesses until hospitalization. Until then, she had not used cardiac therapy.

After one day of hospitalization, the patient gave an anamnestic account of the appearance of swelling on both lower legs. The islands were doughy and most pronounced around the ankles. In the meantime, the patient reported shortness of breath and coughing with bleeding sputum, nausea, loss of appetite, heart palpitations. Auscultation of the lungs presented with impaired respiratory murmur with audible late-expiratory fissures on both basal sides. An ECG recording recorded sinus tachycardia, a heart rate of about 128/min, without changes in the ST segment, which would indicate ischemia. She was administered 40 mg furosemide injection every 12 h, 100mg Spironolactone orally at noon, 5mg Concor orally twice a day, 10mg Ramipril orally once a day, 20mg Controloc orally twice a day, and 40mg Clexane injection subcutaneously. Laboratory findings: platelet counts, transaminases, bilirubin, sodium, calcium, potassium, urea and creatinine were within the reference values, while B-type natriuretic peptide (GNP) and N-terminal precursor GNP (NT pro-GNP) were triple elevated. The B-type of natriuretic peptide was 1450 pg/mL, while NT pro-BNP was 4560 pg/mL, the C-reactive protein (CRP) was slightly increased 7.6 mg/L.

Echocardiography revealed a dilated left ventricle with diffuse hypokinesia of the left ventricle walls and decreased systolic function; the LVEF was 32%, with mid-level mitral regurgitation and slightly enlarged left atrial dimensions. On the tricuspid flap, moderate to severe regurgitation was observed with normal systolic pressure in the right ventricle, SPDK 30mmHg. A minimal amount of pericardial effusion was recorded in the pericardium.

During ECG monitoring, several episodes of VT duration over 60 sec were observed in the Intensive Care Unit, interrupted twice by a 100J synchronous DC shock in short-term general anesthesia. In addition to beta-blocker therapy, the patient was treated with amiodarone, first parenterally, and then 3x200mg orally a day. The patient was placed in the intensive care unit. The incorporation of a two-chamber implantable cardioverter defibrillator in secondary prevention of sudden cardiac death has been indicated (6), which was done after 5 days of symptom stabilization.

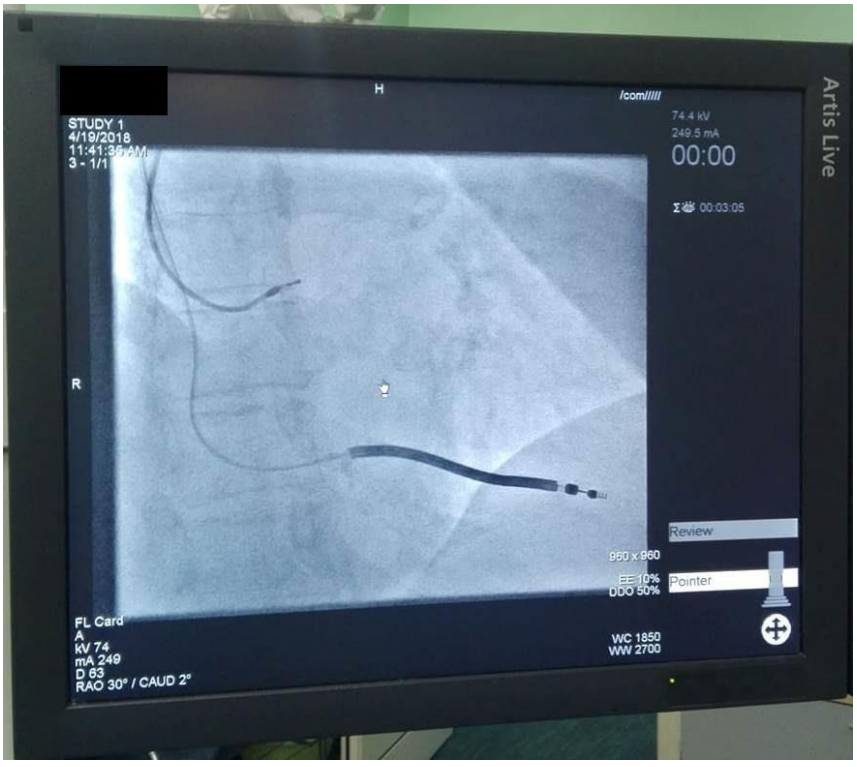

Figure 1. Implantation of a two-chamber implantable cardioverter defibrillator

A St. Judedevice was implanted, St. Jude Fortify Assura DR CD 2359-40C, with active electrode in 65cm long SJM Durata 7122 chamber and with passive electrode in 52cm long SJM Isoflex-Optim 1944 chamber. The fitting parameters were the following p wave 5mV, R wave 13mV, atrium threshold 0.6, ventricular threshold 0.5, atrial impedance 670Ω and ventricular impedance 850Ω (Figure 1).

In the further course of treatment, the patient was hemodynamically and rhythmically stable, cardiac compensated, without significant subjective problems by the cardiovascular system. The control echocardiographic finding is consistent with the previous one. On release, the patient was receiving ramipril at a dose of 5mg, furosemide 40mg on day II, spironolactone 25mg on day II, bisoprolol 5mg twice a day and magnesium once a day.

At the follow-up cardiac examination after 3 months, the patient stated that she was feeling well and had no similar problems after being discharged from the hospital. Routine laboratory assays as well as GNP were in the range of reference values (GNP 37 pg/mL). At the echocardiographic examination of the left ventricle of neat endocavitary dimensions and wall thickness, easily reduced global contractile function, LVEF 50%, left atrium of neat dimensions, mild mitral regurgitation (MR 1+) were present.

Other findings were without abnormalities. A shorter episode of non sustained VT was observed on the PM control. She continued to take 2.5mg Ramipril and 5mg Bisoprolol twice a day. The screening was scheduled for 3 months.

Discussion

Postpartum cardiomyopathy is a rare disease of young women with a poor prognosis. A wide range of prevalence values have been reported in various populations, such as 1 in 1149-4350 woman in labor in the United States, 1 in 1000 in South Africa, and 1 in 300 in Haiti (7, 8).

Diagnosis can often be masked by the fact that dyspnea, palpitations, and lower extremity edema, which are classic findings in PC, are relatively common in healthy pregnant and lactating women. (9).

Postpartum cardiomyopathy has been the focus of attention for the past 10 years. Recently, the first prospective registry for the monitoring of about 100 patients with PC was published. The European Association of Cardiologists (ESC), supporting the formation of the PC HFA Working Group in 2010, has significantly raised awareness of this condition through its website, creating dedicated sessions at the annual ESC Congress and supporting research into this condition. The PC Patient Registry is part of the association's significant EURObservational Research program. Data are collected not only in ESC Member States, but worldwide (10).

Peripartal cardiomyopathy threatens the health of pregnant women and women in childbirth. Clinical suspicion of PC is of great importance for early diagnosis. Echocardiography with PC is necessary to diagnose and monitor the course and outcome of treatment. Standard treatment for cardiac weakness is recommended in patients with PC, and according to the European Association of Cardiologists in the postpartum period includes an ACE inhibitor or angiotensin receptor blocker (ARB), a beta blocker and an aldosterone receptor blocker with diuretic, and ivabradine may be considered. The use of hydralazine, nitrates, beta-blockers and possibly diuretics is recommended in the prepartum period; however, therapy should be tailored to the fetal health during pregnancy (11). Further research is needed to determine the pathophysiological mechanisms of this cardiomyopathy, the disease-specific biomarkers, effective treatments, and prevention measures for the emergence of PC (12).

Conclusion

Our patient registers improvement in LVEF and withdrawal of subjective symptoms of cardiac weakness. Diuretics are excluded from therapy. Controls (including echocardiographic) are planned in 3 months and then every 6 months for up to one year, depending on the condition of the disease. There are no major randomized major studies (due to the low prevalence of the disease itself) that compare the success of treatment with ICD implantation in the primary prevention of sudden cardiac death compared with medication alone. There is consensus that ICDs should be implanted after 3 to 6 months in patients who have no increase in left ventricular ejection fraction (13). Please note that our patient's ICD is embedded in secondary prevention of ISS. Incorporating ICD early in primary prevention can even be detrimental (14). Holland (2009) found that only 0.03% of patients with ventricular arrhythmias had an ICD pacemaker implanted in the early disease period (15). Further research is needed in this direction.

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Faculty of Medicine, University of Niš.

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Prikaz bolesnika

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POSTPARTALNA KARDIOMIOPATIJA U KORONARNOJ JEDINICI – PRIKAZ SLUČAJA

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Postpartalna kardiomiopatija (PC) je oblik dilatativne kardiomiopatije, nejasne etiologije, koja se javlja kod prethodno zdrave porodilje. Kriterijumi za postavljanje dijagnoze postpartalne kardiomiopatije donose se na osnovu srčane slabosti nastale od poslednjeg meseca trudnoće do 5 meseci nakon porođaja, ejekcione frakcije leve komore (LVEF) < 45%, odsustva poznatog razloga za nastanak srčane slabosti, odsustva srčane bolesti pre poslednjeg meseca trudnoće i odsustva ehokardiografski uočene sistolne disfunkcije leve komore pre trudnoće. Bolesnica je smeštna u jedinicu intenzivne nege nakon trećeg porođaja, prirodnim putem, mesec dana pre prijema. Žali se na slabost, malaksalost, otoke i porast krvnog pritiska. Lečena je medikamentno, diureticima, ACE inhibitorom, beta blokatorom i antiaritmicima, zbog uočenih čestih epizoda ventrikularne tahikardije (VT), koje su u 3 navrata prekidane DC sinhronim šokom od 100 J. Indikovana je ugradnja dvokomornog implatabilnog kardioverter defibrilatora, što je nakon 5 dana od stabilizacije simptoma i urađeno.

Peripartalna kardiomiopatija ugrožava zdravlje trudnica i porodilja. Klinička sumnja na postojanje PC je od izuzetnog značaja za ranu dijagnozu. Ehokardiografija kod PC je neophodna za dijagnozu i praćenje toka i ishoda lečenja. Standardni tretman za srčanu slabost preporučuje se kod bolsesnika sa PC; međutim, terapiju treba prilagoditi imajući u vidu i zdravlje fetusa tokom trudnoće. Potrebna su dalja istraživanja radi određivanja patofizioloških mehanizama ove kardiomiopatije, biomarkera specifičnih za samu bolest, efikasnih tretmana, kao i mera prevencije za nastanak PC.

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Ključne reči: *postpartalna kardiomiopatija, implantabilni kardioverter defibrilator*

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MEDICO LEGAL IMPLICATIONS OF HOMICIDE FOLLOWED BY SUICIDE

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Homicide followed by suicide, in the literature known as homicide-suicide (H-S), represents a distinct entity of homicide phenomenon which implies the suicide of a perpetrator after killing one or more persons. Dyadic death (DD) belongs to a special subgroup of H-S and implies the suicide of a perpetrator after killing a single victim. The perpetrator is most often a man in his forties who commits suicide soon after killing his wife or intimate partner because of separation or alienation. The scientific literature has identified various categories of H-S and DD that include killing a victim followed by the suicide of a perpetrator as a part of marital violence caused by jealousy or anxiety due to growing old and/or poor health of marital partners, and more rarely as a part of family violence or when a parent kills his/her child and then him/herself. Groups of the so-called extra-familial H-S include a mixed group of perpetrators composed of dissatisfied workers, members of different cults, religious or political groups who, as a rule, do not kill one, but more victims. Due to the number of victims, such cases do not fall into the DD category from a medico legal point of view.

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Key words: *homicide-suicide, dyadic death, forensic medicine*

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Introduction

Murder is any unlawful violent killing of another human (1). The research into the phenomenology and the etiological characteristics of a murder as a criminal offense and one of the socially most dangerous phenomena represents not only a challenge for scientific disciplines of social, sociological and psychological character, but also a specific curiosity for forensic science. Many homicidal studies provide different classifications of a murder based on the somatic and psychological characteristics of a killer and victim, the time-space connection and their mutual interactions, the way of execution, the consequences, the number of victims, etc. Within this enormous and heterogeneous murder group, a special emphasis is given to a murder followed by suicide, in the literature known as homicide-suicide (H-S), which, as a distinct entity, represents the suicide of a perpetrator after killing one or more persons (2). Depending on whether the act of suicide ensued after the deprivation of life of one or more persons, we are talking about a dyadic death (DD), a triadic death, or an extended murder. Historically, the phenomenon of H-S and DD was even described in Greek tragedy through dyadic family relationships between parents and children (3). The act of suicide most often happens subsequently after the murder on the same day or shortly after it. However, according to some authors, the DD category also includes suicides committed after a long period of time that occur up to one week, i.e. up to 3 months after murdering a victim (1, 2). Certain common epidemiological characteristics between murder, H-S and DD have been determined in the literature dealing with homicide and suicide issues, since H-S and DD share many similarities with family murders, murders of intimate partners, mass murders and suicides. However, there are also special specific features that are characteristic of H-S and DD (1, 2, 4).

H-S and DD groups do not include the cases of murders followed by the suicide of a perpetrator which are conditioned by culturallyspecific circumstances such as, for example, amok in Malaysian culture (a specific traditionallyculturally conditioned sociopathic behavior manifested by the sudden episode of uncontrolled anger of a person, usually male, followed by the killing of a large number of people and then by suicide), as well as cases in which the

perpetrator accidentally got killed or at-tempted but did not commit suicide (2, 4).

The incidence of homicide-suicide

The results of modern research clearly show that, in most developed countries in the world, the H-S rates are low, usually much lower than the rates of murder, whereas suicide represents the leading cause of death (4, 5). Namely, the literature records rates of 0.2 to 0.3 per 100,000 people a year in the United States (4-6) although it is difficult to precisely determine the occurrence of such a phenomenon because there is no internationally standardized classification and cases of H-S are recorded in official statistics as specific cases of suicide or murder. The percentage of H-S, and therefore DD, is usually smaller in countries and regions where there is a high rate of homicide, that is, higher in areas where murders are rare (4-7), for example, the recorded percentage of murder followed by suicide is 42% in Denmark where the rates of murder are low, compared with 4% in the US where murder rates are much higher (5). Although variations in definitions and practices make it difficult to provide accurate rating, it is estimated that between 1000 and 1500 H-S occur in the United States annually, with relative stability in the western countries of the United States (4, 5). Observing official data (8), the murder rate in Atlanta was 38.8 per 100,000 inhabitants from 1988 to 1991, compared to a much lower murder rate of 1.11 per 100,000 inhabitants in England and Wales from 1980 to 1990. In the same countries, H-S rates were 0.46 and 0.07, accounting for 1.4% of the total number of murders in Atlanta and 7.2% in England and Wales (7, 8).

Homicide-suicide classification

Different classifications for H-S are used in the modern literature. Marzuk et al. (2) proposed a basic classification system based on the type of relationship between the victim and the perpetrator as well as the motives of a perpetrator, which was later modified by Hanylick and Koponen (4). According to this classification, H-S is divided into three basic subtypes that include murdering marital/intimate partners, family members and extra-familial people. The group of marital/intimate partner murders includes distinct entities such as cases of H-S of elderly marital/intimate partners, whereas the group of family H-S includes murdering children followed by the suicide of parents (2-4) (Table 1).

Table 1. Classification of murder followed by suicide

Type of H-S*	Perpetrator of H-S	Murder types	Factors and Cofactors of H-S
Marital / partner H-S	Spouse	Murdering a wife (uxoricide), murdering a husband/intimate partner (mariticide)	
	Intimate partner /extra-marital partner		
Family H-S	Mother	Killing a newborn (neonaticide), killing a one year old child (infanticide-filicide), killing a child between 1-16 years of age (pedicide)	
	Father		
	Child (>16 years of age)	Matricide, patricide, familicide	
	Another adult member of the family (> 16 years of age)	Familicide (matricide, patricide, fratricide, sororicide…)	
Extra-marital H-S	Adult male or female	Homicide	
			Love, jealousy
			Merciful murder
			Altruistic or extended suicide
			Family, financial or social stressors
			Retaliation
			Other
			Unspecified

* H-S: homicide-suicide

Since the above classification system provided by Marzuk et al. has certain deficiencies (inaccurate definition of terms "intimate partner", "intimate partner relationship" and relationship between the killer and the victim, lack of data regarding the race and sex of victims and perpetrators, types of weapons/tools, etc.), Hanzlik and Koponen (2, 4) proposed an alternative typological system that enables analyzing and monitoring much more data on the demographic and epidemiological features of individual H-S and DD cases which are listed in the following sections.

The relationship between the victim and the assailant

In a broader context, the term relationship between the victim and the assailant implies a way in which two or more persons are interconnected (2). Regarding this very issue, the term "relationship" implies two people being connected through marriage, extramarital relationship, intimate (love) relationship, parenthood, descendants, and blood relations in the straight and collateral line.

Cofactors

The basic cofactors for the H-S and DD include the upcoming divorce, the earlier divorce, the actual or perceived loss of an unmarried spouse or intimate partner (separation), jealousy, retaliation for a partner's real or perceived infidelity, revenge against a real or perceived lover of a spouse/intimate partner, "mercy'' (merciful murder), altruism ("rescuing" from real or perceived dangers that surround them), financial stressors, family stress, dysfunctional family relationships, alcoholism and/or abuse of psychoactive substances by perpetrators, history of mental illnesses of perpetrators, as well as nonspecific and unknown factors (2, 4).

Motivation

A perpetrator's motivation to kill a victim is rather complex and varies considerably depending on the case. Determining the frequency of mental disorders among perpetrators is further made difficult due to differences in psychiatric diagnostic approaches and classifications of mental illness (2, 4). Nevertheless, there are some generalized and common features that can be observed in most H-S and DD. Although professional literature does not contain such extensive and reliable information about the psychological profiles of perpetrators before the fatal events, psychiatric disorders are considered to have, if not basic then at least a very important role in forming numerous categories of such murders. In cases of family H-S, especially of the marital ones, with the recent separation of intimate partners, problems of a psychic nature are most commonly manifested in the form of paranoia, pathological jealousy and psychosis. Alternatively, depression can play an important role in cases when a parent kills his/her child and it occurs more frequently in H-S perpetrators than the perpetrators of any other type of murder. For this reason, it is assumed that H-S represents only an extension of a suicidal act (9, 10). Also, some H-S perpetrators have a history of previous suicide attempts, as well as a history of a psychiatric treatment (10, 11).

When it comes to killing children, cases of delusional or psychotic disorders with elements of religious devotion also recorded (for example, the case of a child's murder by a father who, before committing suicide with a firearm, cuts his hand off with an axe, because *'if thy hand or thy foot offend thee, cut them off, and cast them from thee: it is better for thee to enter into life halt or maimed, rather than having two hands or two feet to be cast into everlasting fire.'* Gospel of Matthew XVIII) (1). Perpetrators have also been described as impulsive individuals with poor control of aggression and asocial personalities, but also with noted psychological disorders such as loss of self-esteem, frustration, low self-confidence (12). Stressful life events can also trigger H-S in general. Among them, financial or workplace problems, including job losses, are predominant, whereas marital dispute with a feeling of being rejected and alienated, as well as a significant disrupted physical health, can play a significant role in cases of marital and family H-S. The history of family violence of H-S and DD perpetrators towards a victim is very common (10-13), while in the cases of DD of elderly spouses there is usually a prior agreement between the spouses with mutual consent to perform this act which is more desirable when compared to life with an incapacitating disease or unfavorable living conditions (14).

Abuse of alcohol, drugs and other psychoactive substances can also exacerbate the aforementioned factors in cases of H-S, although the presence of these substances is not always revealed by toxicological-chemical analysis during autopsy. This statement is supported by the results of numerous studies that showed lower levels of alcohol in victims and perpetrators compared to the levels of alcohol in those who participated only in the murder (15). According to these researches, abuse of drugs and alcohol was recorded in 17% of perpetrators (11), the presence of psychoactive substances in 10%, alcohol in 21%, and drugs and alcohol in 13% of H-S perpetrators (2, 4, 15). Revenge can be a significant motive in homicide cases of estranged spouses, but also in cases of unsatisfied workers (2, 4).

Significant efforts have been made in numerous studies to clarify the fact whether H-S represents a murder with suicidal elements or a suicide with homicidal elements. However, the results of these researches have shown that such cases are often carefully preplanned by the killer and that demographic characteristics of H-S are significantly different from those of suicide, which has therefore led to a conclusion that H-S is a distinct entity both in relation to suicide and in relation to murder. The H-S special group includes: "family breaker", "triadic death", suicide after mass or serial murders committed by one perpetrator (2, 4).

Types of homicide-suicide

Spousal homicide-suicide

In a group of spousal H-S and DD, the perpetrator is usually a man-a spouse or intimate partner who suffers from "pathological jealousy" or jealous anger caused by frustration (1, 4, 10, 16), aged between 18 and 60, who deprives his own wife or intimate partner of life mainly because of suspicion or knowledge of her infidelity. In some cases, intimate relationships among partners are characterized by real abuse and partner's infidelity. However, cases in which the suspicion of infidelity is unrealistic and is of a delusional character or is present as a part of the psychotic episodes of a perpetrator are more common. The situation in which delusions caused by uncertainty led to irritability, depression and aggression of perpetrators are defined in professional literature as "Othello Syndrome" (17). More than 90% of H-S which involve intimate heterosexual partners was performed by a man who kills a new intimate partner of his wife/partner, which is defined as a triadic death (4, 10). The results of one study showed that the recent separation from a wife or intimate partner increases the risk of H-S and DD by 35.3%, compared with only 21.6% of DD where intimate partners are not separated (18).

One third of all the cases of murdering women by their new intimate partners ended in the suicide of perpetrators (19). The motives of such suicides are the focus of the professionals, and, according to the most widespread opinion, those motives are preceded by the history of partner violence, the history of family violence in childhood, the high level of control and power over a partner during an intimate relationship, as well as the pathological jealousy and possessiveness of a perpetrator. Such H-S is not usually a product of a perpetrator's impulsive decision. On the contrary, the murder of a partner has long been planned and devised, both in terms of time and in terms of the method of performing this act. Based on what has been stated, it can be concluded that the most important strategies for preventing spousal/partner H-S are focused on reducing violence in intimate partner relationships and early identification of cases where there is the highest risk of committing the fatal act. These measures should be undertaken especially in the first months after the separation of partners and they should make it impossible for a perpetrator to own firearms (19).

A subtype of DD, which involves the murder of a spouse accompanied by the suicide of a perpetrator, includes an elderly married couple who have been married for several decades, both of whom suffer from severe illness and/or have existential problems and/or suffer from social isolation (20). In such circumstances, a husband is usually the one who kills his wife by using firearms or by suffocation, and who then commits suicide. This type of activity coincides in some ways with the so-called group of "merciful murders" and "suicide pacts", and it is also described in cases where the partners of victims who suffer from immunodeficiency syndrome (HIV) commit suicide after the "merciful murder" (20, 21). The distinction between the "suicide pact" in which two people consecutively commit suicide and DD within H-S represents a very delicate medico legal problem which makes it particularly difficult to differentiate suicide pacts between parents and children from H-S, i.e., DD (21).

Family homicide-suicide

Family H-S most often involves the murder of one's own child (infanticide) by a parent who commits suicide afterwards. Suicide after infanticide is rare and unusual in most countries, with 10.5% of fathers and 2.3% of mothers who commit suicide after killing a child. In Japan, there is a somewhat higher incidence, with an estimated 500 cases of such deaths being reported annually (22). It is assumed that when it comes to women who kill their own child, it is actually an "extended suicide" where she acts as an altruist in order to "save" her child from specific or potential dangers in their surroundings. The methods that women tend to use when killing their children indicate low intensity violence as oppose to the type of violence applied by men killers, so the most commonly used methods by mothers to murder their children are poisoning, suffocation and exposure to carbon monoxide, whereas fathers tend to use firearms, perform strangulation or stab with a knife (22, 23). Further-more, women often sedate children, and they very rarely kill their spouses/intimate partners or non-biological family members as a part of this act. This phenomenon is contrary to male perpetrators of H-S who, after killing their child/children, very often also kill other children (non-biological or children who were accidentally present at the crime scene), their spouses/ intimate partners and pets. Given the extent of violence to which the victims are exposed, the term "family breakers" has been introduced by some authors to describe these killers (2, 4, 23, 24). Another form of family DD occurs when a parent kills a grown-up child who suffers from a significant physical or mental disability, because he/she no longer feels capable of providing the child with the necessary care due to the old age, illness or financial problems. Such a murder has an "altruistic" character and displays some similarities with DD of elderly married couples (24).

Extra-familial homicide-suicide

Extra-familial H-S includes perpetrators and victims who are not bound in common family life. This type of H-S usually refers to a dissatisfied employee or former employee as a perpetrator who is seeking revenge for actual or perceived insults, damage or abuse at the workplace. This extra-familial H-S is also called "rival" H-S (1, 2). Failure to advance in career, get promotion or gain financial benefits can be motivational factors that reach the level of obsessive delusion that finally results in a cathartic act of killing one or more humans, and then suicide (2, 4).

H-S also includes cases of multiple murders among peers, for example in the US where the media extensively reported on the numerous cases of shooting executed by dissatisfied high school

students. Namely, as a rule, the perpetrators of such acts returned to their previous school (after complet ing their education or being expelled from school), sometimes with lists of potential victims, and used their firearms to kill a large number of victims, including accidental passers by and observers (so-called "secondary targets"). However, the perpetrator most often committed suicide as a result of the police action, i.e., his/her decision to die in the "flame of glory" (2), and not because it was a direct suicide act. This type of H-S belongs to the "pseudo-commando" type of killing. "Pseudocommando" H-S are divided into subtypes of "nonselective" and "pseudo community". In a nonselective (random) type, a perpetrator kills as many people as possible, and the only common feature among the victims is the proximity of the killer. In a subgroup of "pseudo community," a perpetrator targets a particular group of people (for example, the murder of 14 female engineering students at the Montreal Polytechnic School in December 1989, committed by a man in the fight against feminism or terrorist action such as bombing attacks-"kamikaze" attacks, where the death of a perpetrator occurs as a consequence of an explosive device or activity used to kill a large number of other people) (10).

Characteristics of homicide-suicide

Although it has been established that there are some characteristic features of perpetrators, victims and methods of murder and suicide, it has also been observed that there are variations between different social communities and countries. Recent studies have shown a certain similarity in the characteristics of perpetrators, victims and methods of murder and suicide in H-S in general, as well as in DD among various ethnic, racial and cultural groups (25, 26). For example, the most common form of H-S and DD in the United States includes white men who are separated from their marital or extramarital intimate partners and who kill them by firearms. On the other hand, killing children by mothers followed by suicide is more frequent in England (7, 11). The use of firearms (shooting) is the most common form of murder in H-S and DD cases not only in the United States, but also in some parts of England (7, 9, 11). The percentage of female perpetrators ranges from 3 to 8% according to the results of various studies (5-8), whereby this percentage increases in cases of H-S and DD involving children as family members. The H-S and DD perpetrators are generally older than murderers who do not commit suicide and they usually belong to the age group between 40 and 49. When it comes to DD, there is usually a close intimate and personal relationship between a perpetrator and a victim, while the cases where unknown people are killed by a perpetrator who afterwards commits suicide are very rare (5-8, 12). DD is somehow dynamic in the sense that the case scenarios change over time within a single community (12, 27). This has been established by a study conducted in the US over a ten year period in a group of DD committed by white people in urban areas in relation to perpetrators of black race in rural areas (6, 7). Although DD is more likely to occur in population categories of lower socioeconomic status, the results of several studies have shown that DD represents a phenomenon which is more common for the middle class. The place where DD happens is in most cases the bedroom of a family house (5-8, 11). The methods used for murder and suicide vary depending on the availability of firearms, and since they are widely available to citizens in the US, there is a high rate of DD caused by firearms (7). The very act of committing this crime has also shown variations over time, so the results of earlier studies conducted in England indicate a higher incidence of carbon monoxide poisoning (7, 9) compared to the results of recent studies. This can be explained by the fact that households ceased to use gas. Some studies have shown that DD can involve the use of a large number of violent methods and this point to a greater degree of frustration and aggression of perpetrators (11, 12).

The role of forensic medicine in identifying homicide-suicide

The analysis and research of H-S, and in particular DD, are an extremely difficult and delicate medico legal challenge, especially in the cases of murder and suicide of intimate partners and family members, since both the perpetrator and the victim are dead which limits the ability to obtain timely, objective and relevant information (28). A comparative analysis of the results of psychological autopsies and forensic autopsies of H-S perpetrators and victims, as well as the data obtained from relatives and other people who are close to murderers and victims, can be useful for examining the circumstances that preceded the fatal act, but this information is usually insufficient and most often not recorded in the files of official statistical database, autopsy registers and mortuaries (2, 4, 28).

Medico legal expertise, which involves arriving at the crime scene, informing with the circumstances of the case, the autopsy of victims and perpetrators, and then a comparative analysis of all the relevant data obtained and collected during the investigation (autopsy report, traceological analysis, physical evidence, criminal technical data, laboratory analysis, etc.), provides the material evidence and the key proof in clarifying murder cases in general, especially H-S cases.

The basic duty of forensic medicine is not only to determine the nature, origin and cause of the death of the people on whom autopsy was performed, but also to identify other circumstances that preceded the terrible act, the dynamics of the event, the type, number and location of the injury, the order in which injuries were inflicted, the time of death, etc. which all lead to a professional conclusion regarding the roles of the actors in the fatal act (29). Namely, it is often difficult to identify the victim of the murder and the perpetrator of the murder and suicide when two lifeless bodies are found in the same location. Such situations are encountered in cross-killing, two natural deaths, the natural death of one person and the suicide of another, simultaneous suicides, and so on.

Conclusion

H-S and DD represent unusual events that require careful investigation by the competent authorities, and medico legal expertise is one of the key links in the chain of actions that are being pursued for that purpose. The reason for such a fundamental and multidisciplinary analysis of each of these cases lies in the fact that it is possible to mistake double or multiple killings for H-S, and also to view the natural death of one person and the suicide of another or simultaneous suicides as being H-S. Therefore, it is necessary to conduct a thorough research into the phenomenology and etiology of H-S and to identify its features, while the reasons for this phenomenon in each particular case should be sought in the antemortem history of the case at the crime scene and in the autopsy room. Medico legal expertise has a crucial role in resolving all suspicious deaths, as well as in identifying cases of H-S, whether it is a DD, triadic death, or mass murders. In this way, forensic medicine, as an academic discipline dealing with antemortem and postmortem data concerning both perpetrators and victims of murder, has a significant role in predicting such cases and creating preventive measures in terms of identifying, suppressing and preventing such a socially dangerous phenomenon, especially in the domain of marital/partner and family H-S.

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SUDSKO-MEDICINSKE IMPLIKACIJE UBISTVA PRAĆENOG SAMOUBISTVOM

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Ubistvo praćeno samoubistvom, u literaturi poznatije kao ubistvo-samoubistvo (U-S), predstavlja poseban entitet u okviru fenomena ubistva koji podrazumeva samoubistvo izvršioca nakon ubistva jedne osobe ili većeg broja osoba. Dijadična smrt (DS) pripada posebnoj podgrupi U-S i podrazumeva samoubistvo izvršioca nakon ubistva jedne žrtve. Izvršilac je najčešće muškarac u četrdesetim godinama života, koji neposredno pre samoubistva lišava života suprugu ili intimnu partnerku zbog rastavljenosti ili otuđenosti. U stručnoj literaturi identifikovane su različite kategorije U-S i DS, koje uključuju ubistvo žrtve praćeno samoubistvom izvršioca u sklopu bračnog nasilja podstaknutog ljubomorom ili zabrinutošću zbog starosti i/ili lošeg zdravstvenog stanja, a nešto ređe u sklopu porodičnog nasilja kada roditelj lišava života dete a potom i sebe. Grupi tzv. vanporodičnih U-S pripada mešovita grupa izvršilaca koju čine nezadovoljni radnici, pripadnici različitih kultova, verskih ili političkih grupa, koji po pravilu ne ubijaju jednu žrtvu, već veći broj žrtava. Samim tim, zbog broja žrtava, sa sudsko-medicinskog aspekta, ovakvi slučajevi ne spadaju u kategoriju DS.

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Ključne reči: *ubistvo-samoubistvo, dijadična smrt, sudska medicina*

Review article

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THE ROLE OF MESENCHYMAL STEM CELLS IN THE THERAPY OF MYOCARDIAL INFARCTION

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Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into osteoblasts, chondrocytes, adipocytes. During the early 21st century, in vivo studies demonstrated that human MSCs can transdifferentiate into endoderm-derived cells and cardiomyocyte phenotype. Without blood to supply cardiomyocytes (CMs), as in myocardial infarction, the loss of functional CMs progresses as an imbrication of necrosis, apoptosis and autophagy. Besides progressing through different stages of inflammation and healing, the dynamic microenvironment in the infarcted tissue also expresses cardiac cytokines that promote stem cell migration and homing. Given the uncertainty of myocardial salvage, dictated by the degree of necrosis from the sentinel event, it soon became clear that in order to change the long term outcomes of acute myocardial infarction, it is needed to search for a therapy that takes the time to presentation out of the equation. A possible solution to that dilemma appeared in the form of targeted stem cell therapy.

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Introduction

Mesenchymal stem cells (MSCs) are adult, multipotent cells that have the ability to differentiate into tissues of mesodermal origin. Also, they are self-renewable, fibroblast-like cells (1, 2, 3).

First knowledges about stem cells are given by Friedenstein et al. in the 1970s (4). They showed that the bone marrow contains a population of hematopoietic stem cells (HSCs) and an infrequent population known as mesenchymal stromal cells and displayed the capacity of MSCs to differentiate into mesoderm-derived tissue and their significance in regulating hematopoiesis (4, 5). In the 1980s, it was established that MSCs can differentiate into osteoblasts, chondrocytes, adipocytes (6, 7), and into a myogenic phenotype in the 1990s (8). Pittenger et al. demonstrated that adult human MSCs can be expanded to colonies while retaining their multilineage potential (9). In later years, *in vivo* studies demonstrated that human MSCs transdifferentiate into endoderm-derived cells and cardiomyocytes (10, 11) dna *in vitro* coculturing of ventricular myocytes with MSCs induced transdifferentiation into a cardiomyocyte phenotype (12), and discovered their immunomodulatory functions (13). MSCs also suppress T-lymphocyte proliferation, so can be used for allogeneic transplantation and as a potential immunomodulatory therapy (13) (Figure 1).

MSCs are found in the bone marrow, amniotic membrane, synovial fluid, cord blood, adipose tissue, Wharton's jelly, placenta, umbilical vein, amniotic fluid, skeletal muscles, liver and, cord or peripheral blood (14, 15). This wide variety of origins, methodologies, and acronyms prompted standardization in 2005 by the International Society for Cellular Therapy, which set the minimum requirements for MSC definition (16). The minimum criteria for MSCs included plastic adherence, *in vitro* trilineage differentiation to osteoblasts, adipocytes and chondroblasts, cell surface expression of CD105 (endoglin, SH2), CD73 (ecto-5'-nucleotidase, SH3/4), and CD90 (Thy1) and the absence of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR (17).

MSCs have high expansion potential in culture, giving the large numbers of cells within a short period of time, which is of importance in transplant medicine (18, 19, 20). MSCs are characteristic by genetic stability, compatibility with tissue engineering principles, reproducibility of features between different bone marrow isolates, their potential to

trigger regeneration in various fundamental tissues including the myocardium and neovascularization, their ability to home to the damaged tissue or inflammatory sites, and their immunoregulatory properties and so their use as an allogenic treatment (18, 19, 20). It has been shown that MSC transplantation may give benefits in various diseases (21), and one of those diseases in which their ability has been researched a lot was heart attack (MI).

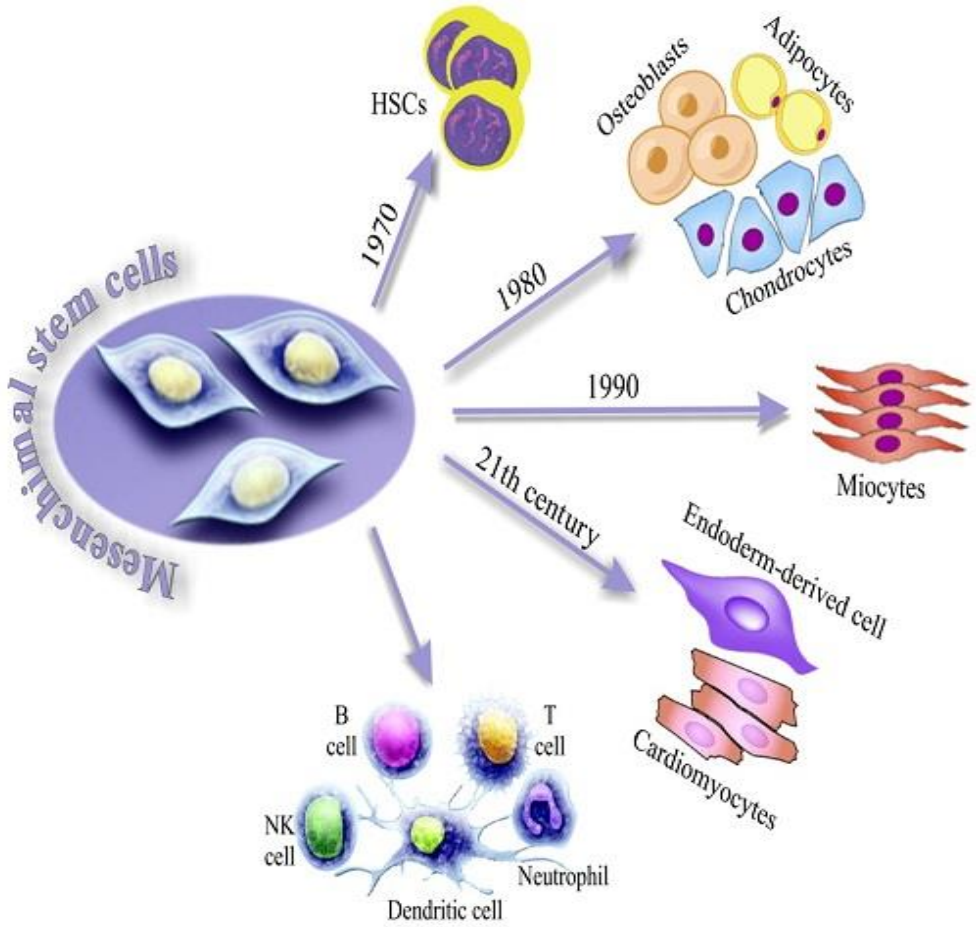

Figure 1. Roles of MSCs

Myocardial infarction and homing endogenous MSCs

Myocardial infarction (MI) is a consequence of the irreversible damage of heart muscle cells, when prolonged ischemia exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms. On this way, functional cardiomyocytes (CMs) pass through processes of necrosis, apoptosis, and autophagy (22, 23).

Besides progressing through different stages of inflammation and healing, the dynamic microenvironment in the infarcted tissue also expresses cardiac cytokines that promote stem cell migration and homing (24).

Stem cells, whether endogenous or exogenous, may reach to the myocardial inflammation, as it has been shown in many studies (25). Also, it has been shown that MSCs homing depends on the nature of MSCs and on the time of their application (25) (Figure 2).

Homing depends on the chemokine receptor CXCR4 which is present on a subpopulation of MSCs and its binding partner, that is, stromal-derived factor-1 CXCL12 (25). Freshly isolated BM MSCs and cultured MSCs also express CCR1, CCR4, CCR7, CCR10, CCR9, CXCR5, and CXCR6 which also participate in MSC migration (25). Adipose-derived MSC-like cells express integrins, cell surface molecules that participate in migration of variety of cells, while neutralizing antibodies against integrins (integrin-beta1 integrin, but not integrin-alpha 4 which is involved in MSC migration) inhibit MSC homing to infracted myocardium (25).

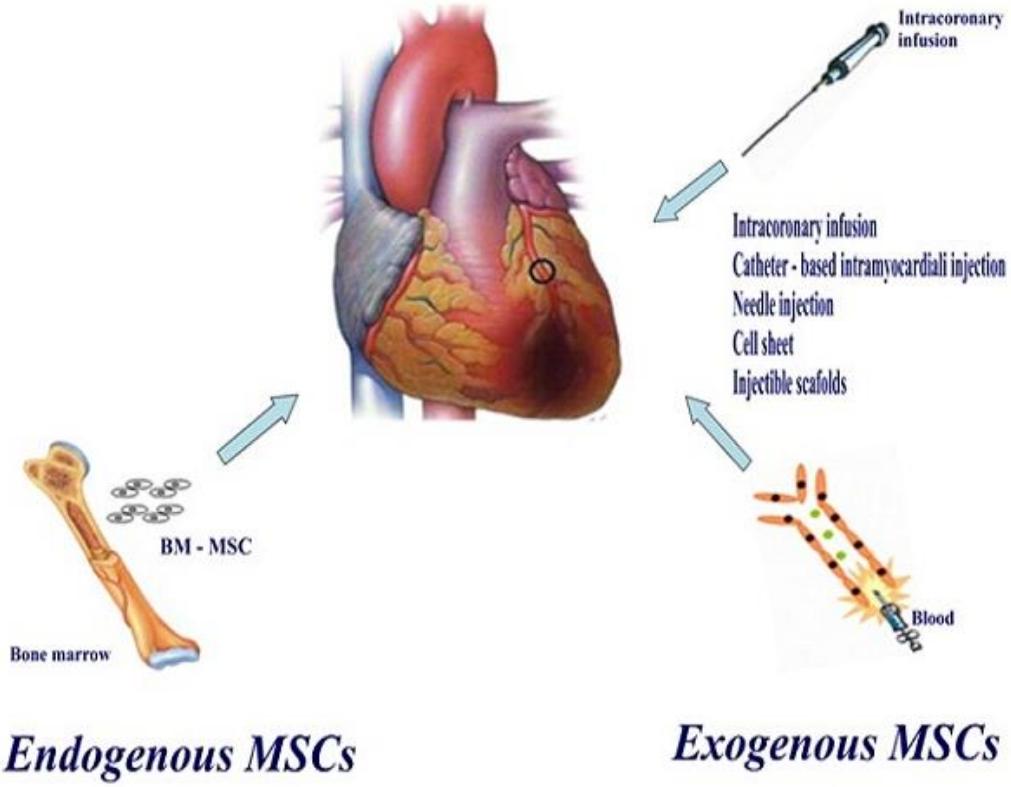

Figure 2. MSCs therapy in myocardial infarction

MSCs transplanting to the post-infarct myocardium

Transplanting stem cells to the post-infarct myocardium augments the cytokine effect to attract endogenous stem cells, via their paracrine mechanisms (anti-inflammatory and immunomodulatory mechanisms) and on that way modulate the regenerative environment (26). MSCs secrete an array of growth factors and anti-inflammatory proteins in response to inflammatory molecules such as interleukin-1 (IL-1), IL-2, IL-12, tumor necrosis factor-α (TNF-α) and interferon-gamma (INF-γ). These factors have feedback mechanisms among the many types of immune cells (27). The key immunomodulatory cytokines include prostaglandin 2, TGF-β1, HGF, SDF-1, nitrous oxide, indoleamine 2,3-dioxygenase, IL-4, IL-6, IL-10, IL-1 receptor antagonist and soluble tumor necrosis factor-α receptor (27). MSCs prevent proliferation and function of many inflammatory immune cells, including T cells, natural killer cells, B cells, monocytes, macrophages and dendritic cells (27).

After the transplantation to the post-infarcted myocardium, MSCs decrease protein production and gene expression of inflammation cytokines TNF-α, IL-1β and IL-6, inhibit deposition of type I and III collagen and gene and protein expression of MMP-1 and TIMP-1 (27). So MSCs preventing myocardial remodeling after MI, due to their capability to attenuate LV cavitary dilation and transmural infarct thinning, and to increase EF, FS, LVESP and dp/dtmax, decrease LVDd, LVEDV, LVEDP (27). MSCs also have the cardiac protective effect in ischemic heart disease thanks to their anti-inflammatory role (28).

Endogenous stem cells, beside the paracrine signaling effects, have the potential to differentiate into functional myocardium (24). Together, the delivery and differentiation of stem cells replenish the lost cardiomyocytes from MI and provide increased vascularity in the post-injury zone to prevent further ischemic tissue damage (29).

MSCs as novel therapeutics

In response to acute MI, MSCs, as novel therapeutics, can be developed to promote CMs survival and improve cardiac function after MI (30, 31). Actually, the gold standard for resolving acute MI is percutaneous coronary intervention (PCI) (32). The aim of any medical or surgical therapy is to establish revascularization and limit the degree of myocardial injury, so targeted stem cell therapy is a possible solution (33, 34).

In comparison with the other cell types, MSC therapy can be promising option in AMI treatment (11, 35). The border zone between necrotic myocardium and viable myocardium is part of interest, and depends on the reaction of viable myocardium to the

area of infarct (20). It is stem cells home to the injured myocardium, in order to produce a therapeutic response. They adhere to myocardium and transmigrate through the endothelium, invade the interstitium and at the end engraft the damaged myocardium (36).

Goals of stem cell therapy

The main goal of cell-based therapies for cardiac diseases is to stop damaging of myocardial tissue and establish its revascularization by accelerating the normal healing process, improving vascularization, inhibiting apoptosis and potentially regenerating cardiac muscle (37, 38). MSC therapy has found application in myocardial repair whether in ischemic heart diseases or in heart failure patients (20).

Limitation and improvements of cell-based therapy

Because of the low retention of cardiac stem cells regardless of the delivery method used, it is needed to improve their engraftment and differentiation in the future.

There are several limitations based on most previous clinical trials of cell-based therapies: low engraftment of BMCs, poor survival of transplanted cells in ischemic tissue, failure of adult stem cells to differentiate efficiently into mature and functional cardiomyocytes, inadequate recruitment of circulating or resident cardiac stem cells, anomalous electromechanical coupling between the transplanted cells or between the transplanted and host cells with consequent arrhythmias (39).

There exist some difficulties that interfere with the evaluation of the effect of cell-based therapy, like the use of LVEF for assessing the effects of cell therapy, incorrect target population of not very sick patients with baseline LVEF 50% and existence of alternative therapeutic strategies like PCI and standard medicament treatment (39).

Homing of stem cells can be improved by using extracorporeal shockwaves of the target organ or tissue. Hydrogels, cell sheets, prefabricated matrices, microspheres and injectable nanomatrix have been used for improving retention of transplanted cells (39). Also, correction of environment conditions can help in cell retention as well as using genetic engineering tools, including overexpression of pro-survival genes or by transplanting the cells together with pro-survival or pro-angiogenic factors or transplantation of preconditioned cells that suppress inflammatory factors and immune responses, and promoted heart function (39).

Questions remain about whether adult stem cells in the heart truly undergo functional and electrical integration and whether this may have hypocontractile and proarrhythmic consequences.

Conclusion

Many preclinical and clinical studies performed on animal and human models have showed that MSC therapy is safe and effective therapy for cardiac regeneration, although their healing mechanism is not precisely defined. MSCs do not appear to be rejected by the immune system, have great growing potential and the ability to enhance tissue repair. Understanding adult stem cells such as MSCs is to provide further development of this field and eventual use of other stem cells in the treatment of many other human diseases.

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ULOGA MEZENHIMALNIH MATIČNIH ĆELIJA U TERAPIJI INFARKTA MIOKARDA

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Mezenhimalne matične ćelije (eng. *Mesenchymal stem cells* (MSCs)) su multipotentne stromalne ćelije koje mogu da se diferenciraju u osteoblaste, hondrocite i adipocite. Početkom 21. veka, *in vivo* studije su pokazale da humane MSCs mogu da transdiferenciraju u ćelije dobijene od endoderma i kardiomiocitni fenotip. Kada kardiomiociti nisu snabdeveni krvlju, kao što je slučaj sa infarktom miokarda, gubitak funkcionalnih kardiomiocita odigrava se putem nekroze, apoptoze i autofagije. Pored prolaska kroz različite faze inflamacije i ozdravljenja, dinamično mikrookruženje u infarciranom tkivu takođe eksprimira citokine, koji promovišu migraciju matičnih ćelija i njihov *homing*. S obzirom na neizvesnu sudbinu miokarda diktiranu stepenom nekroze, postaje jasno da je u cilju boljeg ishoda infarkta miokarda, neophodno pronaći adekvatnu terapiju. Moguće rešenje ove dileme je ciljana terapija matičnim ćelijama.

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Ključne reči: *mezenhimalne matične ćelije, infarkt miokarda, terapija*

Review article

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THE IMPORTANCE OF MAGNETIC RESONANCE IMAGING IN THE DIAGNOSIS OF TEMPOROMANDIBULAR DISORDERS

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The addition of new imaging modalities, specifically mangnetic resonance imaging has and will continue to increase the amount of diagnostic information available to the clinician. Imaging is the only method of obtaining visual information on the status of the joint tissues short of arthroscopy or open joint surgery. The magnetic resonance imaging capacity to contrast soft tissue types makes it an ideal tool for investigating internal derangements. Its ability to image in multiple planes is well suited for examining the three-dimensional nature if internal derangements of temporomandibular joints. A major bonus is its freedom from ionizing radiation and other known health hazards making it a low-risk method for research and clinical investigation. A major disadvantage is its cost and associated limited availability. Magnetic resonance imaging examinations of the temporomandibular joints are part of the current standard of care in the evaluation of temporomandibular disorders, providing the clinician anatomic information that can guide treatment decisions.

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Key words: *magnetic resonance imaging, temporomandibular joint, temporomandibular disorders*

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Introduction

The radiologic investigation of the temporomandibular joints may bring to light pertinent information. However, only after relating these findings to the clinical symptoms will the diagnostic significance become apparent. Since some osseous changes may not be detectable in the radiologic examination, the final diagnosis becomes a clinical procedure. The addition of new imaging modalities, specifically magnetic resonance imaging (MRI) has and will continue to increase the amount of diagnostic information available to the clinician (1). Imaging is the only method of obtaining visual information on the status of the joint tissues short of arthroscopy or open joint surgery. Its primary purpose is to provide information to assist the diagnosis and treatment planning process. Despite the temporomandibular joint imaging long history of research and clinical application, the quality of information gleaned from imaging is often less than desired (2). The small size of the TMJ, the widely varying fossa and condylar morphology and the surrounding dense osseous structures make clear und undistorted imaging of the joint hard tissue technically difficult (3).

TMJ anatomy and function

Major components of the temporomandibular joint include the mandibular condyle, the articular disc, the glenoid fossa, and the articular eminence of the temporal bone. Unlike most joints, the articulating surfaces are fibrous and not cartilaginous. The fibrocartilaginous articular disc is biconcave, dividing the joint space into superior and inferior compartments; this relationship is well seen in the presence of joint effusion (4). The anterior and posterior portions of the articular disc, which are thickened by the morphology of the disk annulus, are designated the anterior band and the posterior band, respectively, with a thinner intermediate zone in between. The disc is attached to the temporal bone and condyle posteriorly by elastic and loose connective tissue; this tissue is also known as the retrodiscal soft tissue or the bilaminar zone. The lateral pterygoid muscle, the only muscle of mastication serving to open the jaw, inserts on the mandibular condyle inferior to

the articular surface but can partially insert on the joint capsule and disc as well (5).

Magnetic resonance imaging technology

Magnetic resonance imaging technology exploits the varying proton content of different tissues. The protons in tissue fluids are polar, analogous to tiny bar magnets, with their magnetic fields or dipoles aligned in random fashion (6). When exposed to the strong magnetic field of the Magnetic resonance imaging scanner, some of the protons align parallel to the direction of the external field. Radio waves of a specific frequency (similar to broadcast signals) are directed at the tissue inducing proton precession, a motion similar to toy top winding down. The magnitude of precession is proportional to the amount of radio frequency energy absorbed. When the radio frequency excitation is stopped, the protons relax to their original low energy state and, in the process, emit the absorbed energy which can be detected by receiver antennae placed over the areas of interest (7).

Magnetic resonance imaging examination of the temporomandibular joint has gained an important role in the diagnosis of internal derangement, because it allows direct visualization of the articular disk in both the open- and closed-mouth positions. Nuclear magnetic resonance was introduced 40 years ago as a research tool in chemistry and physics. The development of large superconducting magnets and high speed computers has paved the way for adaptation of the technology to clinical diagnosis. The technology as applied to medicine was renamed "magnetic resonance imaging" to avoid the stigma attached to the term "nuclear" (8). Its key advantages over other imaging technique are elimination of ionizing radiation and the capacity to produce high resolution images in most anatomic planes (axial, sagittal, frontal and oblique), without positioning of the patient as required wit direct sagittal CT scanning.

Specially small coils placed over both temporomandibular joint areas enhance the clarity of received signals and allow both joints to be imaged in a single exam sequence (9). The strength of the emitted signals is proportional to the amount of protons in the tissue. Signals are location coded as a result of strength gradation in the primary magnetic field (10). This allows the computer to assign an intensity and location values to the emitted signals which are then manipulated by the computer into cross sectional images.

Magnetic resonance imaging is most commonly applied to the diagnosis of internal derangements (11). However, it has potential to diagnose hard tissue lesions. Some authors reported that number of bony abnormalities were noted on coronal views that were not appreciated on sagittal views nor in some case, on tomography. Magnetic resonance imaging has been reported to be 95% accurate in assessment of disk position and form, and 93% accurate in assessment of osseous changes (12). However, several authors have noted a lack of correlation between magnetic resonance imaging findings of disk displacement and the extent of pain and dysfunction of the temporomandibular joint in patients with painful limitation of mandibular opening (13, 14). Moreover, disk displacement was found in a substantial number of asymptomatic volunteers. For example, Ahmad and colleagues reported a 21% prevalence of internal derangement on magnetic resonance imaging evaluation of 57 asymptomatic people (15).

The clinical significance of imaging findings of internal derangement is controversial (16). The prevvalence of displacement of the temporomandibular joint disk among asymptomatic volunteers was previously reported as nearly 33% and the prevalence of normal articular disk in symptomatic joints was reported to be 16%–23% (17). Moreover, arthroscopy and magnetic resonance imaging have shown that the temporomandibular joints with anteriorly displaced disks have the capacity to form remodeled retrodiscal tissue that resembles cartilage (i.e., pseudo-disk formation) (18). Furthermore, the retrodiscal tissues have adaptive capacity and often respond appropriately to the functional loads placed on the tissues (19, 20).

Conclusion

Magnetic resonance imaging capacity to contrast soft tissue types makes it an ideal tool for investigating internal derangements. Its ability to image in multiple planes is well suited for examining the three-dimensional nature if internal derangements of the temporomandibular joints. A major bonus is its freedom from ionizing radiation and other known health hazards making it a low-risk method for research and clinical investigation. A major disadvantage is its cost and associated limited availability. Magnetic resonance imaging examinations of the temporomandibular joints are part of the current standard of care in the evaluation of temporomandibular disorders, providing the clinician anatomic information that can guide treatment decisions. This article has reviewed some of the key findings and imaging appearances of the degenerated temporomandibular joint. Further research will continue to enhance our understanding of the potential contributions of contrast-enhanced studies and dynamic imaging.

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ZNAČAJ MAGNETNE REZONANCE TEMPOROMANDIBULARNOG ZGLOBA U DIJAGNOSTICI TEMPOROMANDIBULARNIH POREMEĆAJA

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Uvođenje novih načina rendgenskih snimanja, posebno magnetne rezonance, pruža sve veći broj dijagnostičkih informacija koje su dostupne kliničarima. Magnetna rezonanca je jedan od načina dobijanja vizuelnih informacija o stanju zglobnog tkiva bez artroskopije ili otvorene operacije zgloba. Magnetna rezonanca ima sposobnost da pomoću kontrastnih supstanci određuje tipove mekih tkiva, što je čini idealnim alatom za istraživanje unutrašnjih poremećaja. Njena sposobnost da daje slike u više ravni pogodna je za ispitivanje trodimenzionalne prirode unutrašnjih poremećaja temporomandibularnih zglobova. Glavna prednost je odsustvo jonizujućeg zračenja i drugih agenasa opasnih po zdravlje, što je čini metodom niskog rizika za istraživanje i kliničko ispitivanje. Glavni nedostaci su njena cena i ograničena dostupnost. Ispitivanja temporomandibularnih zglobova magnetnom rezonancom deo su standarda u proceni temporomandibularnih poremećaja, pružajući kliničarima anatomske informacije koje mogu uticati na donošenje konačne odluke o lečenju.

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Ključne reči: *snimanje magnetnom rezonancom, temporomandibularni zglob, temporomandibularni poremećaji*

Review article

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MID-RANGE HEART FAILURE: A NEW KID ON THE BLOCK?

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Heart failure may be defined as a clinical syndrome with a great range of left ventricle abnormalities, in its function and/or its structure. In 2016, with reference to the ejection fraction, the European Society of Cardiology guidelines, for the first time, introduced a separate clinical entity, called heart failure with mid-range ejection fraction (HFmrEF). The introduction of the mid-range heart failure into the clinical practice and its involvement into the current ESC guidelines led to the inclusion of these patients into great clinical trials as a separate cohort of patients. The biomarker panel, the exact pathophysiological mechanism and the most effective therapy approach are yet to be determined and most probably depend on the underlying etiology of the heart failure. Identification of the proper pathophysiological mechanism of mid-range heart failure will probably answer the current question about whether this type of heart failure is a transitional form between reduced and preserved ejection fraction or represents a distinct and a brand new clinical entity.

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Introduction

Heart failure (HF) may be defined as a clinical syndrome with a great range of left ventricle abnormalities, with regard to its function and/or its structure. However, the dimensions of the left ventricle (LV) may vary, from the normal size, presented with the preserved ejection fraction (EF), up to significant LV chamber dilatation, presented with the reduced EF (1). Ever since it was introduced into the clinical practice, left ventricular EF has been considered an important clinical parameter with respect to the classification of heart failure patients, regarding their demographics, response to therapies and general outcomes (2).

According to the measuring EF, current American heart failure guidelines divide patients into two main cohorts: heart failure patients with reduced ejection fraction - (EF < 40%) and those with preserved EF - (EF > 50%) (3). However, this kind of patient division poses a very important question how to define and how to categorize patients with EF in between (4). This so-alled grey area, or borderline ejection fraction, involves patients with ejection fraction that ranges from 40%-49% and may be considered pathophysiologically, biochemically and clinically as a completely distinct group of patients. At the beginning, these borderline patients were classified as heart failure with preserved ejection fraction (HFpEF) patients who had isolated diastolic dysfunction, with the declined LVEF secondary to the systolic dysfunction development (5). However, the knowledge that heart failure with reduced ejection fraction (HFrEF) patients may recover after medical or device therapy (6) implies that these patients in the gray zone may represent a separate and heterogeneous group of patients, sharing similar pathophysiological and biochemical features. Understanding that the prevalence of these borderline patients is increasing, with no current guidelines referring to this particular group, the European Society of Cardiology (ESC) recognized the need to create a new subgroup of patients with heart failure (7). Therefore, the 2016 ESC HF guidelines created a separate clinical entity for patients who were previously in the borderline zone, called heart failure with mid-range ejection fraction (HFmrEF).

This new division will raise the opportunity for the research to be conducted, aiming to better understand their underlying pathophysiology, possible biomarkers and management strategies (8).

Epidemiological consideration

There is not much data regarding the exact prevalence of HFmrEF, since most of the trials are stratifying patients into EF below or above 50% (4). Some of the studies reported that heart failure with mid-range EF constitutes at least 10%-20% of all heart failure patients (4) and that it may be more prevalent in less selective cohorts. The others (9-11) reported that their portion stands between 13% and 24%, implying that in United States approximately 1.6 million people have heart failure with EF between 40% and 50%. Nevertheless, many researchers agreed that patients with HFmrEF may make up one-quarter of all patients with HF (12-15) and about 10% of newly diagnosed heart failure patients (12). However, after the analysis of the trends, the portion of HFmrEF was reported to be pretty steady, remaining between 13% and 15%, while the portion of HFrEF was decreasing (from 52% to 47%) and HFpEF was increasing (33% to 39%) (16). Similarly, the Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM) study reported that 17% of included patients had EF between 43%-52% (17), the same percentage seen in Cardiovascular Heart Study (18) and Chinese Study (19). Given that 10%-20% of any heart failure cohort represents patients with mid-range EF, this group should not be easily neglected.

Pathophysiological and biochemical consideration

The current knowledge on the exact pathophysiological mechanism of the HFmrEF is very limited. Patients' signs and symptoms may vary, from the ones seen in HFrEF to those presented in patients with preserved EF (4, 8). However, it is most likely that the major underlying feature is ischemia, presented in more than 40% of HFmrEF (9, 20). This high percentage is more similar to those with reduced EF and much higher compared to HFpEF. Ischemia was the most probable cause, twice as likely, for HF admission in HFmrEF and HFrEF than in HFpEF (9), as well as new and prior ischemic events (21). Pathophysiologically speaking, it may be that patients with mid-range HF represent a subgroup of patients with preserved EF who have a coronary artery disease and are therefore in an early stage of HF with reduced EF. Thus, the pool of potential HFmrEF patients may consist of all who had limited or re-vascularized myocardial infarction, cardiac remodeling, myocarditis or cardiomyopathy, partially recovered or in the early stages (4).

The assessment of functional capacity, measured on CPET by peak VO2 and Ve/VCO2, turned out to be similar in HFmrEF and HFpEF and is much better compared to HFrEF (22). This was the first study that documented the heterogeneity of patients with mid-range heart failure, coming to the conclusion that the patients who recovered from HFrEF had a more favorable phenotype.

If we consider mid-range heart failure as an overlapping phenotype of systolic and diastolic dysfunction (23), it may be hypothesized that generalized endothelial dysfunction represents the base for abnormal diastolic properties of the heart (24), while initial cardiac disease is responsible for impaired systolic yet diastolic properties of the heart (25). It was proposed that comorbidities may be a key factor accelerating general inflammation that then leads to diastolic dysfunction of the heart. However, systemic inflammation involves microvascular endothelial dysfunction, therefore myocyte hypertrophy, increasing resting tension and fibrosis (26). Accordingly, endothelial dysfunction and inflammation may have a crucial role in the pathogenesis of HFmrEF, so their targeting may be beneficial for the general outcome.

There is not much evidence about biomarker profiling in HFmrEF. By measuring different biomarkers, according to the known pathophysiology of heart failure (myocardial stretch, inflammation or oxidative stress), it was demonstrated that patients with HFmrEF had an intermediate biomarker profile interacting between cardiac stretch and inflammation (27). Furthermore, biomarkers related to inflammation and cardiac remodeling had predictive value for HFmrEF and HFpEF, but not for HFrEF. However, natriuretic peptides, cystatin C and high sensitivity troponin were all good predictors for HFmrEF among the patients who were followed for a median of 12 years (28). Natriuretic peptides were stronger predictors of HFrEF compared to HFmrEF and did not differ in their association with incident HFmrEF and HFpEF. Moreover, lower levels of NT-proBNP during the monitoring of patients with HFmrEF were positively associated with reduced risk of HF hospitalization or death of any cause (29).

Many different pathophysiological mechanisms may account for the development of the HFmrEF, suggesting that this type of heart failure is very diverse and that the underlying etiology may be crucial for the future outcome.

Clinical consideration and risk factors

European Society of Cardiology guidelines define this group as patients with EF between 40% and 49%, positive natriuretic peptides levels and structural heart disease or diastolic dysfunction (7). According to the literature, their demographic characteristics stand in between those with HFrEF and HFpEF, but are more similar to HFpEF. Furthermore, mid-range heart failure patients are more likely to be females, having a hypertensive disease or a history of atrial flutter/fibrillation (13, 30-32). However, some researchers confirmed that HFmrEF was more prevalent in males and younger patients compared to those with HFpEF (33). The likeliness of having a coronary artery disease was documented to be much higher compared to those with preserved ejection fraction (34). Mid-range heart failure patients also had a greater risk of a new ischemic heart disease

(34). Nevertheless, prior myocardial infarc-tion and revascularization were more likely to be present in patients with HFmrEF and HFrEF than in those with preserved EF (21,35). The atrial fibrillation prevalence seen in HFmrEF (60%) was estimated to be somewhere between HFpEF (65%) and HFrEF (53%) (36), while dilatation of both left ventricle and atrium was significantly lower in patients with mid-range EF compared to those with reduced EF (12). After comparison of HFmrEF patients with atrial fibrillation (AF) and those who had sinus rhythm, it was noted that those with AF were older, more hypertensive, had different cerebrovascular events or longer history of heart failure, but the prevalence of ischemic heart disease was lower (36). The analysis of risk factors for hospitalization in HF patients revealed that HFmrEF stood in between HFrEF and HFpEF, and the most significant factors were: medication incompliance, lung infections, arrhythmias and myocardial ischemia (9, 21). The assessment of comorbidities demonstrated that renal disease, diabetes mellitus, hypertension and anemia had similar prevalence in HFmrEF and HFpEF, which was higher than in those with reduced EF (33).

The usage of beta blockers has been observed in a few studies and was similar in all three groups of heart failure patients. It should be noted that those with reduced EF were using more digoxin and agents that block renin–angiotensin–aldosterone system, while calcum-chanel blockers were more used as therapy in patients with preserved EF (11, 37, 38).

Outcome consideration

However, studies have shown that the outcomes for HFmrEF were different when compared to those with reduced or preserved EF. Cardiovascular Health Study (18) demonstrated that the mortality rate for HFmrEF was between those with reduced and preserved EF and that the all-cause mortality rate in HFmrEF was higher compared to the control group. It should also be noted that an inverse relationship between EF and risk of events was documented, es-pecially when EF was between 40% and 45% (17). Therefore, in patients with EF below 45% the hazard ratio for all-cause mortality was by 39% higher for every 10% reduction of the ejection fraction (17). However, when ejection fraction over 45% was as-sessed, all-cause mortality and all respective elements of cardiovascular events were steady (17). These facts may lead to the conclusion that when analyzing the outcomes, the stable form of chronic HFmrEF corresponds to HFpEF. Still, in terms of outcomes, these findings cannot be applied in acute heart failure hospitalization nor the therapies that should be used (4).

The Acute Heart Failure Global Registry of Standard Treatment (ALARM-HF) demonstrated that HFmrEF patients had hazard ratio of all-cause in-hospital mortality or 30 days mortality lower than that of HFrEF, but close to that of HFpEF (39). The Get With The Guidelines-HF (GWTG-HF) Registry documented similar five-year mortality in all patients with HF (37), whereas HFmrEF had a statistically significant re-admission rate compared to the other groups of heart failure. Furthermore, in the Rica registry, one-year mortality was highest for HFrEF, while it was similar for HFmrEF and HFpEF with no differences in the 30-day or one-year re-admission rate (40). Network for the Study of Heart Failure (REDINSCOR I) and the Muerte Súbita en Insuficiencia Cardíaca (MUSIC) (41) assessed all-cause mortality during the 41-month follow-up and found out that it was higher for HFrEF than for HFmrEF and HFpEF, where the rate was very similar. However, the likeliness of cardiovascular death or sudden cardiac death was higher for patients with HFmrEF compared to HFpEF. In the other, similarly designed study, REDINSCOR II registry, in over one-year prospective follow-up no statistical significance in all-cause mortality, cause of death or HF re-admission was demonstrated in the analyzed groups (42). The most frequent cause of death among all the groups was refractory HF. All-cause mortality after 30 days, one-year and three-year follow-up in all three groups was assessed in the Swedish Heart Failure Registry (14) with a statistically higher rate in favor of HFrEF compared to HFpEF and HFmrEF, where it was similar and without significance. However, the existence of coronary artery disease raised the three-year mortality rate in HFmrEF compared to HFpEF. This study also confirmed that chronic kidney disease was a risk factor for mortality of patients with mid-range heart failure and HFrEF, but not in HFpEF (14). Heart failure with mid-range EF, chronic obstructive pulmonary disease and having an age over 85 years all positively correlated with higher mortality in the first year after hospital discharge, compared to the other groups of heart failure (12, 21). The ESC Heart Failure Long-Term Registry (12) observed a one-year follow-up in ambulatory patients with heart failure and demonstrated that mortality rate of HFmrEF was intermediate between HFrEF and HFpEF, but with no statistical significance. Non-cardiovascular mortality also did not differ between evaluated groups. However, the proportion of patients who underwent hospitalization was higher in the group with reduced EF compared to HFmrEF or HFpEF.

The post hoc analysis in the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial (TOPCAT) (17) documented that the primary end point of the study (mortality due to cardiovascular death) was reduced, but only in patients with preserved ejection fraction who had EF 45%-49%.

The Chronic Heart Failure Analysis and Registry in the Tohoku District-2 (CHART-2) study (10) assessed patients with mid-range heart failure according to whether they improved or deteriorated from HFrEF (16%) or HFpEF (44%) during one year. The mortality rate of patients with mid-range heart failure was similar to those with HFpEF, but it significantly increased if they transitioned to HFrEF. Similar results were documented at Washington University Heart Failure Registry (1) where 73% of patients improved their EF from below 40%; 17%

deteriorated from EF that was over 50%, while 10% kept their EF between 40%-50%, remaining within HFmrEF. Accordingly, patients with improved HFmrEF had statistically significant cardiovascular clinical outcomes compared to those who deteriorated or those who remained mid-range (1). The most recent data from the CHARM study mostly confirmed the previous findings. That post hoc analysis confirmed that the incidence rates for different cardiovascular events, including cardiovascular death and all-cause death were both similar and lower in patients with HFmrEF and HFpEF, after comparison with HFrEF, indicating that HFmrEF may be a milder form of HFrEF (43).

All of the data indicate that in the context of HfmrEF, it is worth noting whether patients experienced worsening or improving of their EF during the follow-up period. It is documented that patients with ischemic heart disease or with an acute ischemic episode will be more prone to a deterioration of EF instead of an improvement (21). Therefore, patients who transitioned from reduced to mid-range ejection fraction had better outcomes in general, compared to patients who had stable HFmrEF. However, patients who impaired their EF, from preserved to mid-range, had a worse prognosis when compared to the ones with stable HFmrEF. So far, no conclusions can be drawn from the data about whether HFmrEF is a transitional form of heart failure or an independent clinical entity.

The treatment considerations

The current European Society of Cardiology guidelines on Heart Failure (7) suggest that treatment of HFmrEF should be equal to HFpEF rather than HFrEF, but so far no therapies have conclusively been shown to improve outcomes in HFmrEF (1, 3, 7). The analysis of data from different clinical registries (11, 14, 37, 40-42) indicate that the most prescribed agents are angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), beta-blockers and mineralocorticoid receptor antagonists (MRA). Accordingly, diuretics are recommended when signs of congestion are present (7). The CHART-2 study assessed the prognostic characteristics of agents commonly prescribed in heart failure patients (10). It was found that therapy with beta blockers positively correlated with improved mortality in patients with reduced or mid-range heart failure, contrary to those with HFpEF (10). However, the use of diuretics was a negative prognostic factor in mid-range and reduced EF, but not in patients with preserved EF (10), while the usage of lipid lowering therapy demonstrated reduced mortality only in HFpEF (10). In general, the outcomes when traditional heart failure disease-modifying agents were prescribed differed in HFmrEF compared to HFpEF patients (10). The prognosis, however, was likely to be equal to those with HFrEF. Moreover, the therapy with beta blockers was found to be very effective in improving mortality when patients presented with sinus rhythm, but not with atrial fibrillation, in all those with EF below 50% (44).

The results from the Swedish Heart Failure Registry (14) also documented the beneficial effects of beta-blocker therapy in decreasing mortality in a one-year follow up, but only in HFmrEF patients who had coronary artery disease. However, the therapy with ACEI and ARB was proven to be beneficial in reducing mortality, whether patients had coronary artery disease or not. Moreover, the use of diuretics in HFmrEF had negative impact on their prognosis. The CHARM study found that candesartan may be beneficial for HFmrEF in the same way for HFrEF, since it was proven to reduce cardiovascular and heart failure events to the same extent (43).

When arguing about the most potential therapy approach in patients with mid-range heart failure, it should be worth mentioning that the treatment of coronary artery disease, as a possible underlying factor of HFmrEF, may be a key factor for improving prognosis in this group of patients. The management of risk factors and cardiovascular and non-cardiovascular comorbidities is also highly recommended.

Conclusion

It can be observed that the introduction of the mid-range heart failure into the clinical practice and its involvement into the current ESC guidelines has gained sufficient attention for them to be included in the clinical trials as a separate cohort of patients. Briefly, they are likely to be older and females, clinically resembling patients with heart failure with preserved ejection fraction. However, regarding the presence of coronary artery disease they are more similar to those with heart failure with reduced ejection fraction. The biomarker panel and the most effective therapy approach are yet to be determined and most probably depend on the underlying etiology of the heart failure. Identification of the proper pathophysiological mechanism of mid-range heart failure will probably answer the current question about whether this type of heart failure is a transitional form between reduced and preserved ejection fraction or represents a distinct and a brand new clinical entity.

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SRČANA SLABOST SA GRANIČNOM EJEKCIONOM FRAKCIJOM – TRANZITORNA ZONA ILI ZASEBAN KLINIČKI ENTITET

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Srčana insuficijencija može se definisati kao klinički sindrom sa različitim spektrom abnormalnosti leve komore, njene funkcije i/ili strukture. Evropsko udruženje za kardiologiju je 2016. godine, u vodiču za lečenje srčane slabosti, po prvi put, kao posebnu kategoriju uvelo srčanu slabost sa graničnom ejekcionom frakcijom, EF 40% - 49%. Uključivanje u evropski vodič, a samim tim i prepoznavanje bolesnika sa graničnom srčanom slabošću, u kliničkoj praksi dovelo je do toga da oni budu uključeni u velike kliničke studije kao zasebna grupa bolesnika. Biomarkerski profil, tačan patofiziološki mehanizam i najefektniju terapiju za grupu bolesnika sa ejekcionom frakcijom 40% - 49% tek treba utvrditi i najverovatnije zavise od same etiologije srčane slabosti. Identifikacija pravog patofiziološkog mehanizma srčane slabosti sa graničnom ejekcionom frakcijom najverovatnije će odgovoriti na aktuelno pitanje da li je ovaj tip srčane slabosti tranzitna forma između srčane slabosti sa redukovanom i očuvanom ejekcionom frakcijom ili predstavlja poseban klinički entitet.

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Ključne reči: *srčana slabost, ejekciona frakcija, granična ejekiona frakcija, očuvana ejekciona frakcija*

Review article

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BURNOUT SYNDROME AT WORKPLACE AMONG DOCTORS

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Burnout syndrome at workplace is a very present problem over the last few decades in our country and worldwide. It is defined as a gradual loss of motivation and emotional weariness which develop at workplace as a result of special demands of a workplace, individual characteristics and expectations of a worker, as well as work results that are not in accordance with invested efforts. The consequences are emotional weariness, depersonalization and the experience of reduced personal achievement in workers. This syndrome is characteristic for humanistic and helping professions. Burnout syndrome at workplace is especially characteristic among doctors because of the specificity of their profession and potential discordance of demands and achievements at work. The most common consequences of this syndrome among doctors are chronic weariness, cognitive functions disorder, sleep disorder, depression; as far as somatic symptoms are concerned, there are headache, stomachache, arrhythmia, tachycardia, hypertension and so on. According to the research results, burnout syndrome at workplace most commonly appears among doctors younger than 35, who have less work experience and work more than 40 hours a week, who are not married and do not have children. The prevalence of this syndrome is higher among women, as well as among doctors who work at intensive care and surgery unit. Primary and secondary prevention is necessary: organizational change in a collective that lead to better redistribution of tasks in the collective and the improvement of interpersonal relations, as well as systemic work on removal and reduction of symptoms of this syndrome among doctors.

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Introduction

Burnout syndrome at workplace is a very present problem over the last few decades in our country and worldwide. It is defined as a gradual loss of motivation and emotional weariness which develop at workplace as a result of special demands of a workplace, individual characteristics and expectations of a worker, as well as work results that are not in accordance with invested efforts (1-2). The consequences are emotional weariness, depersonalization and the experience of reduced personal achievement in workers. This syndrome is characteristic for humanistic and helping professions.

Burnout syndrome at workplace is especially characteristic among doctors because of specificity of their profession and potential discordance of demands and achievements at work (3-4).

Burnout syndrome at workplace as a medical term

Burnout syndrome at workplace is mentioned for the first time in literature in the middle of the last century as unadjusted and undesirable behavior of workers within certain occupations, which occurs in mentally unstable individuals. Only later was this phenomenon connected to conditions in which employees work. In 1947, Herbert Freundenberger was the first to introduce the term burnout syndrome as a state of weariness or frustration ensued at a workplace, since it did not lead to the expected reward. At the end of the twentieth century this term was introduced in medical dictionary and was acknowledged in professional literature, where it was described as gradual loss of motivation and emotional weariness due to specific demands of a workplace, personal characteristics and expectations, as well as discrepancy between invested effort and achieved results (4-6).

The most quoted and the most accepted definition of burnout syndrome at workplace is "emotional weariness, depersonalization and the experience of reduced personal achievement within humanistic and helping professions" (7). In a number of papers, experts point out that some workers who intensively work with people, after many years of dedication to work, in the end, metaphorically speaking, burn out. Within certain professions that are connected to intense work with people (such as doctors, nurses, teachers, professors, social workers, psychologists, lawyers, police officers) this syndrome leads to reduced professional commitment and attention, as well as to many symptoms of health disorders (8-11).

Some authors point out that burnout syndrome at workplace develops as a result of long term stress at work. Stress at work represents a phenomenon that appears when the capacities of an employee to adjust to work demands are in discrepancy. It represents temporary (momentary) adaptation process, which is followed by certain psychological and physical symptoms. If adaptation mechanisms are not sufficient to overcome the discrepancy between demanded and achieved, it leads to a breakdown which represents the final phase of the adaptation process (7, 12-13).

Burnout syndrome at workplace among doctors

Burnout syndrome at workplace among doctors is especially characteristic because of the specificity of their profession and potential discordance of demands and achievements at work. Humanistic professions, which certainly include doctors' profession, include different ways of helping people, as well as different interpersonal relations, which can have crucial importance for success at work. In some cases, patients with whom doctors work are in terminal stadiums of illness, potential possibilities for recovery are minimal or non-existent, their life quality is bad (14-16), which can burden doctors, no matter how much they try to deal with patients and their loved ones professionally.

Doctors can be exposed to great psychological pressure from different interest groups, such as patients, managers, media, social environment. If demands of a workplace, that is, certain interest groups, go beyond possibilities and capacities of a doctor to realize them in a satisfying way, it leads to extended, chronic state of stress which results in burnout at workplace (7, 17-20).

Researches have shown that some doctors who work in the same field and do the same jobs under the same conditions, do not react in the same way to prolonged stress factors of a workplace, that is, it does not lead to burnout syndrome at workplace in everyone. Some doctors are exposed to different health risk factors in the environment and at workplace, which influences their life quality and occurrence of different disorders. There is an assumption that certain personal characteristics are very significant for potential occurrence of this syndrome, that is, discordance between nature of a job and personality can lead to burnout at workplace (13).

Risk factors for the occurrence of burnout at workplace among doctors

Factors that contribute to the occurrence of burnout syndrome at workplace can be situational and individual. Situational factors are related to burden of a workload, duration of working hours, bad interpersonal relationships, lack of solidarity and support among colleagues. As far as individual factors are concerned, there are personality, demographic characteristics and attitude toward work. Many researches have shown that interpersonal factors and lack of social support at workplace much more often represent risk factors for burnout syndrome at work place among doctors than demographic factors and sole properties of a job.

According to research results, burnout syndrome at workplace most commonly appears among doctors younger than 35, who have less work experience and work more than 40 hours a week, who are not married and do not have children. The prevalence of this syndrome is higher among women, as well as among doctors who work at intensive care and surgery unit (21-25).

Symptomatology and consequences of burnout syndrome at workplace

Experts consider that burnout syndrome at workplace occurs gradually and symptoms are unspecific and hardly recognizable in the initial phase. At first, great commitment to work and enthusiasm occur in a worker, but after a while there is stagnation and disappointment, since the invested effort did not result in a suitable reward. It is followed by the next phase, which includes emotional withdrawal and isolation, so that the person can save themselves from the previous phase. Very often, this phase precedes the following phase that is characterized by apathy. If the change does not come, the state of health can be very afflicted (26-33).

The authors, who provided the basis and described basic symptoms of burnout syndrome at workplace, classified them in three basic groups: emotional weariness, depersonalization and personal unfulfillment. Emotional weariness among doctors includes cumulative influence of many factors from the working environment which lead to gradual loss of physiological and emotional reserves, so they lead to the state of physical and emotional weariness. They are no longer capable of the same commitment to their job and patients, they cannot provide high standards of their profession and are chronically stressed. As a consequence, it leads to depersonalization of an employee, that is, specific disorder of personality perception, so they withdraw from the social environment and their relationship toward patients, colleagues, personnel at workplace can be changed. It happens that some of them become less committed to patients, less humane, even insensitive at workplace. The third form of burnout syndrome

at workplace is the sense of personal unfulfillment, which is characterized by personal dissatisfaction and dissatisfaction with their own work results, and can be manifested in different ways and create many consequences.

Most commonly, the consequences of this syndrome among doctors are fatigue, cognitive functions disorder, sleep disorder (difficulties when falling asleep, restless dream, awakening too soon), and the somatic symptoms are most often headache, stomachache, arrhythmia, tachycardia, hypertension and so on. Biochemical tests can show increased secretion of stress hormones, but also other hormonal disorders can occur. Psychological symptoms are chronic anxiety, apathy, anger, frequent mood swings. Sometimes there are problems with concentration and memory, which additionally makes a very demanding doctors' job difficult. Sometimes they have the sense of sorrow and emptiness; they seem disappointed and deal with the events in their environment pessimistically. Situations that they solved easily and efficiently, now become burden for them, and sometimes they deal with the events in their environment cynically. Beyond work activities, they cannot relax anymore, have fun and enjoy spending time with friends, family and other joys of life. Disturbed interpersonal relationships with the environment can lead to almost complete isolation. In certain cases, those people are prone to depression, they take psychoactive substances and can even be prone to suicide (26-29).

Persons with this syndrome are often prone to other illnesses, common cold and allergy.

A person that has burnout syndrome at workplace shows reduced performance on the job, reduced productivity and bad quality of work. Such people are often absent from work, take sick leave, have reduced life quality and general wellbeing (30-31).

In some European countries with high standards of social and health insurance and well organized health system, burnout syndrome at workplace is defined as medical diagnosis and detailed instructions for treatment and prevention are given. Psychiatrists, psychologists, social workers and doctors of different specialization have especially important part in the team that deals with this problem and they contribute through counseling, educational seminars and individual work with people. Support is given to employed doctors, as well as employers and also to all interested individuals.

Prevention of burnout syndrome at workplace

Primary and secondary prevention is necessary: organizational change in a collective that lead to better redistribution of tasks in the collective and the improvement of interpersonal relations, as well as systemic work on removal and reduction of symptoms of this syndrome among doctors (32-34).

Primary prevention certainly includes improvement of physical fitness and health through different sport and recreational activities, adjusted to certain population groups of doctors, development of good habits (regular sleep and rest, regular nourishment) and eliminating habits that negatively influence health of individuals (smoking, alcohol consumption and the abuse of unauthorized substances).

Secondary prevention includes implementation of measures and procedures that will directly influence prevention and alleviation of the symptoms of burnout syndrome at workplace. The prevention work includes activities at the level of individual factors, as well as the level of organizational risk factors that can lead to burnout syndrome (6, 25, 32, 35).

Determination, evaluation and work on the improvement of doctors' life quality, as well as elimination or alleviation of preventable health risk factors (36, 37), and specially improvement of constructive anger management strategy skills, as well as relaxing skills (6, 32) can greatly contribute to prevention of this syndrome among doctors.

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SINDROM SAGOREVANJA NA POSLU KOD LEKARA

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Sindrom sagorevanja na poslu ("burnout sindrom") je poslednjih decenija veoma aktuelan problem, kako u svetu tako i u našoj zemlji. Definisan je kao postepen gubitak motivacije i emocionalna istrošenost, koja nastaje na radnom mestu usled posebnih zahteva radnog mesta, individualnih osobina i očekivanja samog radnika, kao i rezultata rada koji nisu u skladu sa uloženim naporima. Posledice toga su emocionalna iscrpljenost, depersonalizacija i doživljaj umanjenog ličnog postignuća kod radnika. Ovaj sindrom je karakterističan kod humanističkih i uslužnih zanimanja. Sindrom sagorevanja na poslu kod lekara je posebno karakterističan zbog specifičnosti posla kojim se oni bave i potencijalne neusklađenosti zahteva i ostvarenog učinka na poslu. Najčešće posledice ovog sindroma kod lekara su hroničan umor, poremećaj kognitivnih funkcija, poremećaj spavanja, depresija, a od somatskih simptoma se češće javljaju glavobolja, bolovi u stomaku, aritmije, tahikardija, hipertenzija i dr. Sindrom sagorevanja na poslu se, prema rezultatima istaživanja, najčešće javlja kod lekara mlađih od 35 godina, koji imaju manje radnog iskustva i rade više od 40 sati nedeljno, koji nisu u braku i nemaju dece. Prevalencija ovog sindroma kod žena lekara je veća, kao i kod lekara koji rade na odeljenjima intenzivne nege i hirurgije. Neophodna je primarna i sekundarna prevencija: organizacione promene u kolektivu koje dovode do bolje preraspodele poslova u kolektivu i poboljšanja međuljudskih odnosa, kao i sistematski rad na otklanjaju i ublažavanju simptoma ovog siindroma kod lekara.

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Ključne reči: *sindromi, sagorevanje na poslu, lekari*

Review article

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MUSCULOSKELETAL BIOMECHANICS IN THE HUMAN JAW

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Most computer simulations treat the mandible as a rigid or as a flexible beam acted upon by muscles capable of differential contraction, and predict dental and articular reaction forces at selected sites. The more advanced models employ finite element analysis to include estimations of local skeletal stress, strains and deformations. It seems certain that the jaw bends three-dimensionally in a complex manner when loaded by muscle action, and how it does so depends on the clenching task. In addition, compressive loads on the mandibular condyles vary with the bite point, and are unevenly distributed between them with asymmetric biting on the dental arches. The problem of morphological definition is difficult to overcome, since this varies so much between individuals, especially when accompanied by pathology. Variations in jaw motion are common too, making it hard to define normal patterns of behavior.
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Key words: *temporomandibular joint, temporomandibular movemant, articulatio in tmj.*

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Introduction

Muscle fibers generally attach directly to the bone, or to cylindrical, ovoid, or elongated tendons. Complex skeletal muscles often contain large internal aponeuroses to which muscle fibers attach, and it is common for these aponeuroses to differ in orientation and size within the same muscle. Fibers may lie parallel to the line of action of the muscle if it is simple, or at angles to its aponeuroses if it is complex. When one of the attachments is to a tendon sheath and the other to an area of bone, translation and rotation of both bone and aponeurosis are possible due to the action of an induced force couple (1). The relationship between the anatomical form of skeletal muscle, tensions during contraction, tendon properties, muscle shape, and local intramuscular pressures are not well understood. Conceptually, any interactions between these variables have usually been established by modeling.

In order to describe new knowledge about the structure and biomechanical properties of the musculoskeletal system, we used more recent literature (more than three-quarters of the references are from the last ten years). Older references were used to the extent that reflects knowledge that is still current in this area. Several databases were used for the literature search, and some references came from personal collection. All studies were potentially included, but we used only available data. In the literature review, we used original articles, retrospective studies, data from appropriate textbooks and a few review articles. The authors of some papers have used different biomechanical models, especially the finite element method (2).

Finite element analysis (FEA) has become a popular tool for simulating the effect of feeding loads on the skull. However, modeling the function of the masticatory system in a realistic way is a challenging task. One difficulty is to decide on specific values for the essential model properties because the literature often reports large ranges for these values and indeed sometimes they are unknown (3). Another difficulty is to decide on the degree of simplification of the model, because software and time limitations do not allow modeling of the masticatory system in all its known complexity. It is therefore not surprising that previous FEA studies of the masticatory apparatus differ significantly regarding basic input variables such as material properties, constraints, and applied forces (4)

Jaw mechanics

Computer simulation has emerged as a practical way to demonstrate the principles of muscle and jaw mechanics. It has been used to predict force

distribution in the system, and to explain the relative contributions of variables such as muscle size and angulation to reaction forces at anatomical sites resisting the effects of muscle contraction (5).

The mandible as a rigid beam

A central assumption in many simulations is that the mandible is a rigid beam, acted upon by isometric muscle forces at key points, and resisted by reaction forces at other sites. Two-dimensional models can simulate bilaterally-symmetrical forces only, e.g., midline (incisal) bites or bite forces assumed to be equally distributed on both sides of the dental arch (6). All models are designed to solve the magnitude and/or direction of a number of unknown force vectors when others are specified. It is possible to assign likely tensions to all the muscles, and solve for reaction forces at the teeth and joints. Conversely, a modeler can place forces at the teeth and joints, then calculate muscle tensions and their levels of presumed activation (7). For example, coaxial, transverse forces acting through the mandibular condyles may have to be divided arbitrarily in different proportions between the right and left condyles (8).

Irrespective of how they are used, rigid beam models ensure that any forces which are not coplanar are made so by expressing them as vector components in two or three orthogonal planes, and that all translational forces and all torques about any axis sum to zero (9). Rigid beam models presently cope with presumed asymmetrical magnitudes and orientations of forces at both condyles by solving for single vectors at each condylar site. With few exceptions, bite point force is also represented by a single vector passing through some point in the dental arch (10). In fact, however, occlusal forces are usually multiple since dispersed contacts on cuspal facets have different orientations. Single-point bite forces are important oversimplifications in models, because any under-representation of non-parallel forces at the dental arch requires the introduction of compensatory forces and torques at condylar reaction sites.

In summary, it can be assumed that during normal functional loading of the dentition, bilateral, though usually asymmetrical, condylar loading occurs according to the side of the dental arch used (11). The magnitudes of these loads fluctuate with changing muscle tensions and bite forces, and the loads are usually directed at angles commensurate with the natural resistance provided by local anatomy during tooth clenching, condylar forces are developed which are aligned approximately perpendicular to the eminence when the jaw leaves the intercuspal position, and roughly parallel to occlusal plane, or more anterior than this when the condylar head is in the fossa. Under unusual conditions, perhaps involving unique combinations of musculoskeletal architecture, dental arch form, unilateral posterior tooth contact, and symmetric muscle contraction, it seems possible to apply traction to the articulation on the side of the bite point (12).

The mandible as a flexible beam

Finite element analysis (FEA) models of the jaw depend on the static equilibrium theory for their operation, but differ from rigid beam models because they permit analysis of physical changes within the system, which is considered to be elastic. This is accomplished by dividing the mandible and its articulation into its component tissues, e.g., cortical and cancellous bone, fibrous connective tissue, enamel, dentine, etc. The tissues are each represented by thousands of small geometric elements with unique elastic properties which may differ along element axes (13). Each element is connected to neighboring ones by nodes, thereby linking the entire system. When muscle force vectors are applied at optional sites, rigid boundary constraints introduced at others prevent the network from moving. The system deforms elastically in between, permitting detailed analysis of such properties as displacements, stresses, strains, element forces, and reaction forces (14).

FEA modeling has many advantages. The lower jaw behaves as an object capable of deforming in a nonlinear manner, copying its response in life. It does so under the influence of jaw muscle tensions which can be applied to wide areas of the bony cortex instead of isolated points. FEA models can be built for different purposes, e.g., to study the effects of different muscle activation strategies, altered musculoskeletal morphology, the design and probable behavior of prosthetic and surgical procedures. They can also be used to evaluate devices, implants or other biomaterials (15).

Conclusion

Most computer simulations treat the mandible as a rigid or as a flexible beam acted upon by muscles capable of differential contraction, and predict dental and articular reaction forces at selected sites.

The more advanced models employ finite element analysis to include estimations of local skeletal stress, strains and deformations. It seems certain that the jaw bends three-dimensionally in a complex manner when loaded by muscle action, and how it does so, depends on the clenching task. In addition, compressive loads on the mandibular condyles vary with the bite point, and are unevenly distributed between them with asymmetric biting on the dental arches. The problem of morphological definition is difficult to overcome, since this varies so much between individuals, especially when accompanied by pathology. Variations in jaw motion are common too, making it hard to define normal patterns of behavior.

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MUSKULOSKELETALNA BIOMEHANIKA LJUDSKE VILICE

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Većina kompjuterskih simulacija tretira mandibulu kao krutu ili kao fleksibilnu gredu na koju deluju mišići sposobni za različite oblike kontrakcija i predviđaju efekte sila na zube i temporomandibularni zglob. Napredni kompjuterski modeli koriste metode analize konačnih elemenata u objašnjenju lokalnih stresnih uticaja na skeletni i mišićni sistem. Pretpostavlja se da se donja vilica savija trodimenzionalno, na složen način pod dejstvom mišića, zavisno od mesta delovanja sile stezanja. Pored toga, kompresivna opterećenja na mandibularnim kondilima variraju sa tačkom zagriza, u kom slučaju su neravnomerno raspoređena na zubnim lukovima. Problem morfološke definicije teško je prevazići, jer se veoma razlikuje između pojedinaca, posebno kada se utvrde patološki procesi. Varijacije u kretanju vilice su uobičajene, pa je teško definisati normalne obrasce ponašanja.

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Ključne reči: *temporomandibularni zglob, pokreti u temporomandibularnom zglobu, artikulacija viličnog zgloba*

Original article

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MONITORING OF HEMOSTASIS DISORDERS IN CARDIAC SURGERY

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Bleeding during and after cardiopulmonary bypass is a multifactorial and potentially lethal complication. That is why one of the most difficult tasks in cardiac surgery is the establishment of a timely, physiological hemostasis. The aim of this study was to diagnose the most common coagulation disorders in patients who underwent surgical revascularization of the myocardium (their frequency, follow-up complications) and therapeutic care of them. The prospective survey included 100 respondents (22 female females-22.0% and 78 male respondents -78.0%), who were subjected to single, double and triple surgical revascularization of the myocardium. Preoperative as well as 3 hours and 24 hours postoperatively determined the following parameters: blood count, coagulation status, platelet function parameters, rotational thromboelastometric parameters, blood and blood product use, use of synthetic hemostatic agents. The most commonly diagnosed hemostasis disorders are preoperative and postoperatively disrupted platelet function (up to 31% of patients), postoperative extrinsic coagulation factor concentration depletion (postoperatively) (21% of patients), intrinsic factor coagulation activity disorder (23% of patients after surgery) and disturbed concentration of functional fibrinogen and impaired fibrin clot polymerization in 17% of patients following surgery. During the study, 13% of patients received a cryoprecipitate transfusion after surgery, 10% of patients received frozen fresh plasma, 22% were transfunded with platelet concentrates, 20% of patients received desmopressin acetate, while 3 patients received a prothrombin complex concentrate in the postoperative course.

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Key words: *cardiac surgery, hemostasis, bleeding*

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Introduction

The aortocoronary bypass is usually performed under conditions of extracorporeal circulation (ECC). Cardiopulmonary bypass (CPB) is a method that replaces cardiac arrest and pulmonary circulation during cardiac surgery. The primary role of this technique is to provide tissue oxygenation and thermoregulation during surgery. The extracorporeal circulation machine is used for this purpose (1, 2).

Bleeding during and after cardiopulmonary bypass is multifactorial. Namely, prolonged contact of platelets with the intestinal surfaces of the extracellular bloodstream interferes with their function, leads to activation of platelets and coagulation cascade, which in the final outcome has a thrombocytopenia in more than 30%, as well as consumable coagulopathy. Contact activation of the coagulation cascade occurs upon contact of the artificial surface with blood, primarily through the activation of factor XII. Platelets of patients undergoing cardiac surgery are very sensitive, as patients are generally preoperatively receiving mono or dual antiplatelet therapy, which inhibits their function and further disrupts perioperative and postoperative hemostasis. Dilution within the primer, hypothermia during the intervention, prolonged duration of the extracorporeal blood flow procedure, also disrupt systemic hemostasis. A number of patients have used oral or parenteral anticoagulation therapy, prior to the surgery which inhibits successful hemostasis by inhibiting the activation of factors II, VII, IX, X, XI. Increased perioperative blood loss, combined administration of heparin and protamine, preoperative anemia, renal failure, liver disease, age over 70 years, female gender, congenital or acquired coagulopathies are all risk factors that disrupt the coagulation cascade and hemostasis.

One of the most difficult tasks in cardiac surgery is the establishment of timely, physiological hemostasis. Bleeding usually occurs during and after cardiac surgery. During this emergency, routine laboratory testing of coagulation using PT, APTT, platelet counts is usually slow and insufficient. The point of care (POC) devices for monitoring the hemostatic system play a real role in these acute conditions: rotational thromboelastometry, impedance aggregation, activated coagulation time, functional fibrinogen and percentage of clot lysis (3).

Systemic hemostatic therapy is crucial for the timely management of bleeding complications.

The aim of the study

The aim of this study was to diagnose the most common coagulation disorders in patients undergoing surgical myocardial revascularization (their frequency, associated complications) and their therapeutic management, as well as the selection of adequate hemostatic therapy in the management of coagulation disorders in cardiac surgery patients.

Materials and methods

This prospective study included 100 patients who underwent single, double and triple surgical myocardial revascularization at the Clinic of Cardiac Surgery, Clinical Center Niš, from 15.06.2018. to 15.12.2018. One hundred patients were included in the research (22 female 22.0% and 78 male 78.0%). The mean age of the study population was 64.6 ± 7.5 years (min 43, max 80 years).

All patients enrolled in the study underwent preoperative mono or dual antiplatelet therapy (acetylsalicylic acid ± clopidogrel/ticagrelor), which was discontinued 5 days before surgery.

Following standard cardiac surgery preoperative patient preparation, patients were operated on according to standard cardiac surgery protocols.

The following parameters were determined preoperatively as well as 3 hours and 24 hours postoperatively:

1. blood count (erythrocyte-Rbc count, hemoglobin-Hgb, hematocrit-Hct, leukocyte count-Wbc, platelet count-Plt) on a Horiba CRP 200 automatic counter, from 4ml whole blood sampled in a EDTA anticoagulant tube.

2. coagulation status (prothrombin time-PT, International Normalized Ratio-INR, activated partial thromboplastin time-aPTT, fibrinogen-F I, antithrombin III-AT III, D dimer, on ACL Elite Procoagulome ter), from the whole blood sampled using Na-citrate anticoagulated tube, centrifuged immediately after sampling for 15 min at 3500 rpm and then pipetted 250 microlitre samples from the serum were released for coagulometer testing.

3. parameters of platelet function (platelet activation by adenosine diphosphate (ADP test)–registers residual effect of clopidogrel/ticagrelor on platelet function; platelet activation by arachidonic acid (ASPI test)-registers residual effect of acetylsalicylic acid and TRAP test-represents the natural potential of platelet-independent therapy, performed on a MULTIPLATE Roch Germany impedance aggregator). Blood was sampled in 4ml lithium-heparin anticoagulant tubes and within 30 min of sampling, analyzes were performed.

4. basic parameters of rotational thrombelastometry (parameters of internal-INTEM test and external coagulation pathway-EXTEM test, namely: (coagulation time-CT which depends on the concentration and activity of plasma coagulation factors, maximum clot strength-MCF whose value is conditioned by number and platelet function as well as fibrinogen concentration, the amplitude clots after 10 minutes-A10 also depends on platelet count/ function and fibrinogen concentration, alpha angle, maximal lysis-ML registering pathological lysis of the clot), functional fibrinogen-FIBTEM test, A10 depending on the persistence and polymerization of fibrin clot), on a ROTEM rotational platelet device Roche Germany, whole blood 4ml in sodium citrate tube as anticoagulant, and blood was also analyzed within 30 min of sampling.

5. The use of blood and blood products, perioperatively and postoperatively, was recorded in the medical records kept in the operating room (operating list), as well as in the intensive care unit (shock list).

6. The use of synthetic hemostatic agents (prothrombin complex concentrate-PCC complex, desmopressin acetate-DDAVP, tranexamic acid) was recorded in the medical records kept in the operating room (operating list) as well as in the intensive care unit (shock list).

Multiplate test parameter values indicating an increased risk of increased perioperative and postoperative bleeding, which may result from impaired platelet function, are based on recommendations and guidelines as follows: ADP test ≤ 310 (reference value 570-1130) aggregation units per minute (AU/ min), ASPI test ≤ 400 AU/min (ref. values 710-1490 AU/min) and TRAP test ≤ 500 AU/min. (ref. values 923-1509 AU/min).

The basic parameters of ROTEM tests that are related to various disorders of hemostasis (accompanied by increased bleeding) and the consequent use of hemostatic therapy are:

- Coagulation time-CT Extem ≥ 100 s (ref. value 38-79s), indicates disturbance of the coagulation factor of the external coagulation pathway (II, V, VII, IX factor).
- Coagulation time-CT Intem ≥ 300 s (ref. value 100-240s), refers to disruption of the coagulation factor of the intrinsic pathway (all factors except VII and XIII) or the presence of residual effect of high molecular weight heparin.
- Maximum clot strength-MCF Extem/Intem ≤ 45mm (ref. Value 50-72mm), occurs with impaired function and platelet count or fibrinogen deficiency.
- Clot amplitude after 10 minutes-A10 Fibtem ≤ 8 mm (ref. Value 9-23mm) registers at low concentration and poor polymerization of fibrin clot, which depends on the concentration of functional fibrinogen.
- Maximum clot lysis-ML ≥ 15% (ref. Value

< 15%) indicates pathological hyperfibrinolysis, which also results in increased bleeding in patients.

Statistical data processing

Data were presented in the form of arithmetic mean and standard deviation, minimum and maximum values, as well as absolute and relative numbers.

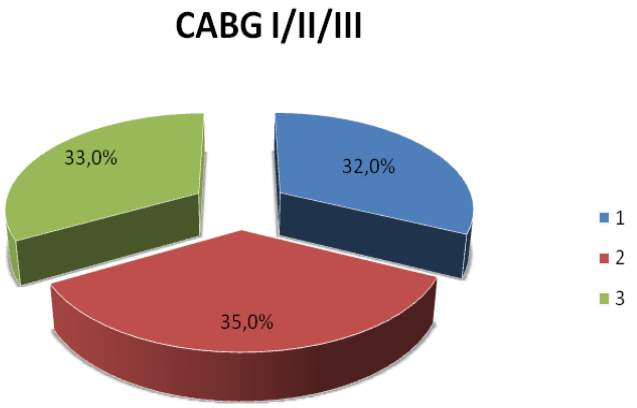
The normality of continuous variables was tested by the Kolmogorov-Smirnov test. If the data distribution was a normal comparison of values preoperatively and postoperatively at two moments (3 h and 24 h after surgery), an ANOVA was performed for repeated measurements. If data distribution was not normal, Friedman's test was used for this comparison. If the data distribution was a normal comparison between the two groups, the t-test was performed, if the data distribution was not normal this comparison was performed by the Mann-Whitney test.

The hypothesis was tested with a significance threshold of $p < 0.05$. Data analysis was performed in SPSS 16.0 software package.

Results

The number of surgical revascularization types (single, double, triple cardiopulmonary bypass-CABG I, II/III) was uniform in the study population. Double cardiopulmonary bypass (35.0%) was the most common; it was followed by triple (33.0) and single (32.0%) (Graph 1).

The number of erythrocytes monitored in the three measurements decreased significantly ($p < 0.001$). Also, hemoglobin and hematocrit values declined statistically significantly over the follow-up period ($p < 0.001$ and $p < 0.001$, respectively). The number of leukocytes 3 h after surgery increased, compared to the period before surgery and then up to 24 h after surgery. There was a statistically significant difference in the number of leukocytes during the follow-up period ($p < 0.001$). Platelets dropped abruptly up to 3 hours after surgery, then we had a slight increase in the 24-hour period after surgery. Platelet counts changed statistically significantly during the follow-up period ($p < 0.001$) (Table 1).

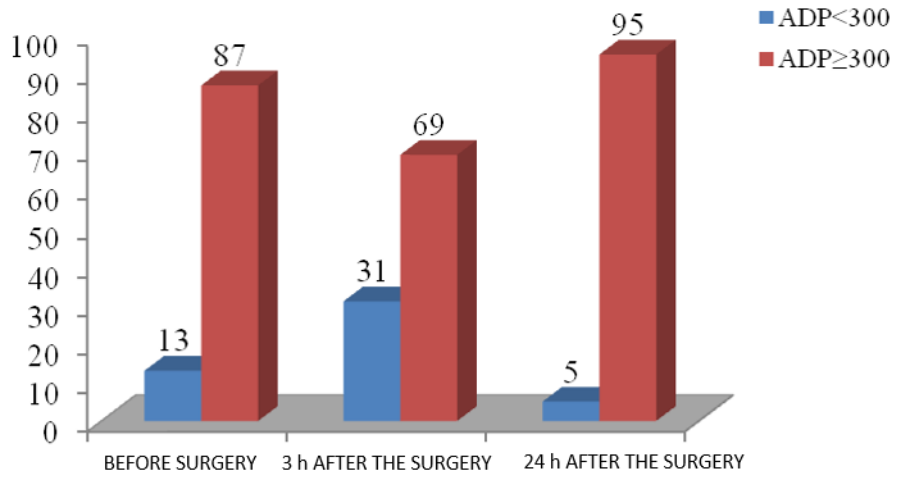


Graph 1. CABG I/II/III distribution

Table 1. Red blood cells number (RBC), hemoglobin values (HGB), hematocrit (Hct), white blood cells (Wbc) and platelet number (PLT) before and 3 h and 24 h after surgery

Parameter	Preoperatively	3 h postoperatively	24 h postoperatively	p-value[1]
RBC	4.46 ± 0.47	3.86 ± 0.55	3.76 ± 0.49	< 0.001
Hgb	137.74 ± 10.93	114.98 ± 11.32	109.96 ± 8.87	< 0.001
Hct	39.18 ± 4.17	32.99 ± 3.95	31.86 ± 3.10	< 0.001
WBC	7.11 ± 1.28	10.07 ± 3.07	9.14 ± 3.34	< 0.001[2]
PLT	236.22 ± 63.88	148.13 ± 56.65	167.01 ± 50.96	< 0.001[2]

[1]Aritmetic mean ± standard deviation, [1]ANOVA for repeated measurements, [2]Friedman test

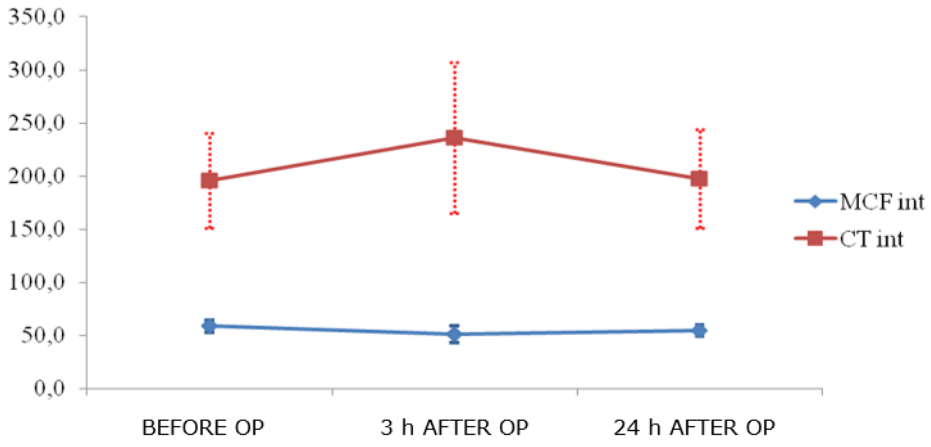


Graph 2. Distribution of patients with low ADP before surgery and 3 h and 24 h after surgery

Table 2. Values of CT int and MCF int at follow-up in the study population

Parameter	Preoperatively	3 h postoperatively	24 h postoperatively	p-value[1]
CT INTEM	195.96 ± 44.63	236.11 ± 70.65	197.43 ± 46.83	< 0.001
MCF INTEM	58.89 ± 5.70	50.91 ± 7.87	54.70 ± 7.87	< 0.001

[1]Aritmetic mean ± standard deviation, [1]Friedman test



Graph 3. Values of CT int and MCF int during the monitoring period in the study population

Preoperatively, 13 persons had ADP < 300 aggregation units per minute-AU/min (13.0%), after 3 hours of surgery 31 persons had ADP < 300 (31.0%), and after 24 hours of surgery 5 persons had ADP < 300 (5.0%) (Graph 2). CT intem values increased in the first 3 h of surgery compared to the preoperative period and then returned to the preoperative values in the period up to 24 h. It was found that there was a statistically significant difference in CT intem values between the three measurements ($p < 0.001$) (Table 2). MCF intem values declined in the first 3 hours after surgery compared to the preoperative period, and then began to increase slightly up to 24 hours after surgery. It was

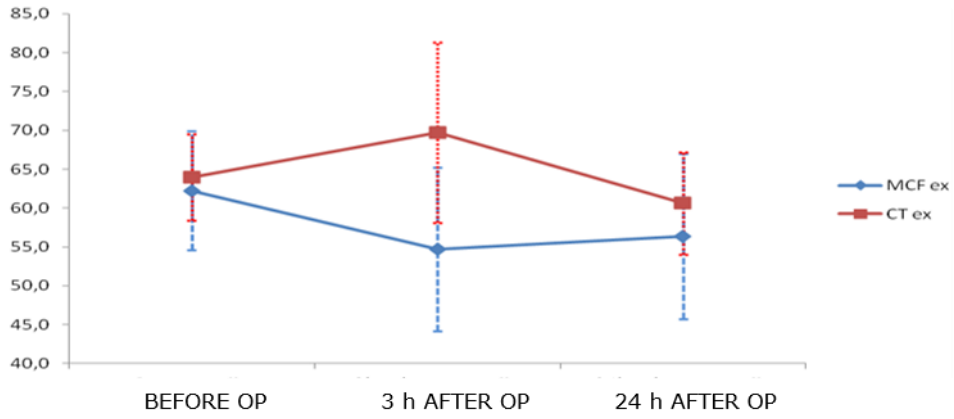
found that there was a statistically significant difference in the values of this parameter between the three measurements ($p < 0.001$) (Table 2, Graph 3).

CT extem values increased in the first 3 hours after the surgery compared to the preoperative period, and then fall to lower values compared to the preoperative period up to 24 hours. It was found that there was a statistically significant difference in CT extem values between the three measurements ($p < 0.001$) (Table 3). MCF extem values decline in the first 3 hours after surgery relative to the preoperative period, and then begin to increase slightly within 24 hours of surgery. It was found that there was a statistically significant difference in the values of this parameter between the three measurements ($p < 0.001$) (Table 3, Graph 4).

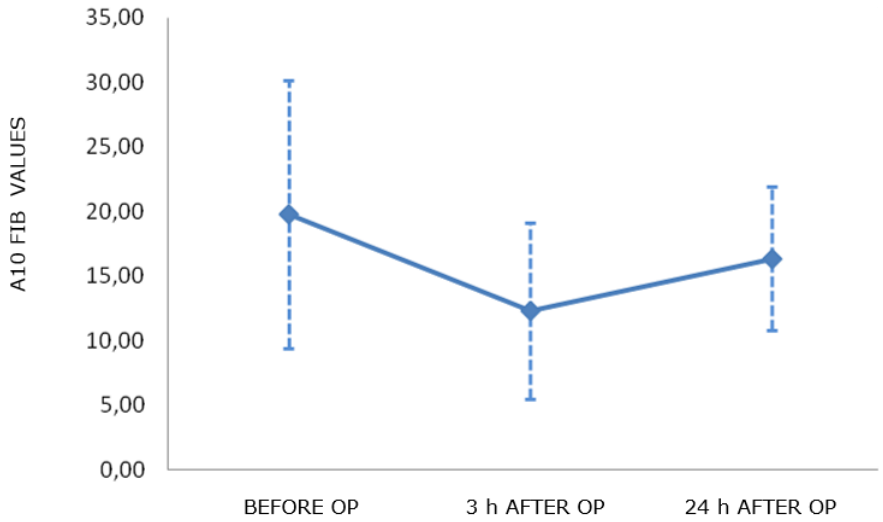
Table 3. Values of CT ex and MCF ex in the monitoring period in the study population

Parameter	Preoperatively	3h postoperatively	24h postoperatively	p-value[1]
CT extem	63.96 ± 7.67	69.71 ± 10.56	60.62 ± 10.61	< 0.001
MCF extem	62.25 ± 5.58	54.73 ± 11.63	56.38 ± 6.59	< 0.001

[1]Aritmetic mean ±standard deviation, [1]ANOVA for repeated measurements



Graph 4. Values of CT ex and MCF ex during the monitoring period in the study population



Graph 5. Values of A10 fibtem during the follow-up period in the total population

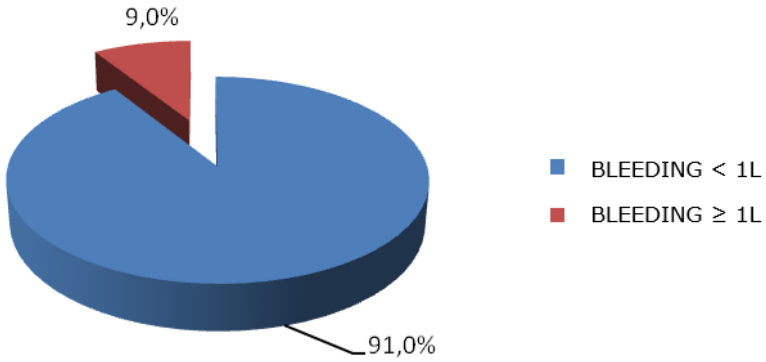
A10 fibtem values decreased within 3 hours after surgery (12.31 ± 6.82) in comparison to preoperative values (19.79 ± 10.35) and that was followed by a slight increase (16.36 ± 5.89) (Graph 5). It was found that there was a statistically significant difference in the values of this parameter between the three measurements ($p < 0.001$).

MCF extem of less than 45mm was measured in 21 patients (21.0%) 3 hours after surgery and in 3 patients (3.0%) 24 hours after surgery. CT intem greater than 300 was measured in 23 patients 3 hours after surgery and there were no such measurements after 24 hours. MCF intem of less than 45 was measured in 17 patients (17.0%) 3 hours after surgery and in 5 patients (5.0%) 24 hours after surgery. A10 fibtem ≤ 8 was measured in 8% of patients before surgery, in 15% of patients 3 h after surgery, and in 2% of patients 24 h after surgery (Table 4).

In the study population, 9 patients (9.0%) had bleeding of over 1L in the drain (Graph 6).

Table 4. Parameters indicating increased peri and postoperative bleeding

	Before operation	3 h after operation	24 h after operation
CT ex > 100	0 (0.0%)	4 (4.0%)	0 (0.0%)
MCF ex < 45	0 (0.0%)	21 (21.0%)	3 (3.0%)
CT int > 300	0 (0.0%)	23 (23.0)	0 (0.0%)
MCF int < 45	0 (0.0%)	17 (17.0)	5 (5.0%)
A10 fib ≤ 8	8 (8.0%)	15 (15.0%)	2 (2.0%)
INR > 2	0 (0.0%)	6 (6.0%)	0 (0.0%)
APTT > 50	0 (0.0%)	8 (8.0%)	5 (5.0%)
ML > 15	0 (0.0%)	0 (0.0%)	0 (0.0%)



Graph 6. Frequency of bleeding in the drain ≥1L after 24 h after surgery in the study population

Blood and blood products (plasma, cryoprecipitate, platelets), as well as systemic hemostatic agents (desmopressin acetate, prothrombin complex concentrate) were not received by any patient prior to surgery.

Three hours after surgery, 12 patients (12%) received allogeneic resuspended erythrocytes, while 24 hours postoperatively 8 patients received one unit (350ml) of resuspended donor erythrocytes.

Three hours after the operation, 13 patients (13.0%) received cryoprecipitate. Five patients (5%) received 10 doses of cryoprecipitate, 6 patients (6%) received 15, and 2 patients (2%) received 20 doses (A10 fibtem </ = 8). After 24 hours of surgery, no one received cryoprecipitate.

After 3 hours, 10 patients (10.0%) received fresh frozen plasma-CT Extem/Intem ≥ 100/240s. One patient received 3 doses and nine patients 4

doses of plasma. Within 24 hours of myocardial revascularization, fresh frozen plasma was not received by any of the patients.

Platelet transfusion after 3 hours from surgery was received by 22 patients (22.0%)-MCF Ext / Int ≤ 45mm, A10 Fibtem ≥ 10mm, ADP test ≤ 300 AU /min, ASPI ≤ 400 AU/min, TRAP ≤ 500 AU/min. An average of 11.14 ± 4.45 doses was administered. After 24 h of intervention, there were no patients requiring platelet concentrate transfusion.

With regard to systemic hemostatic agents, desmopressin acetate (DDAVP) and prothrombin concentrate complex (PCC), 3 hours after surgery, 13 patients received one ampoule of DDAVP (20mcg)-ASPI test ≤ 300 AU/min, while 24 hours after surgery another 7 patients received the same medication.

Each of 3 patients received PCC of 1000 IU postoperatively, and all patients' INR level was 5 or greater before therapy, as well as CT Extem ≥ 100s.

Discussion

According to the National Blood Collection & Utilization Survey (NBCUS), blood transfusions and blood derivatives, with the exception of platelet transfusions, declined between 2008 and 2011 in the United States of America. A reduction of 8.2% in red blood cell (RBC) transfusions and a decrease in plasma transfusion by 13.4% were observed.

In the United States, contrary to the general trend, there was an increase in blood and blood transfusions during cardiac surgery during 2010, when a total of 34% of operated patients received transfusions of erythrocytes or other blood derivatives (4). Moreover, cardiac surgery was the branch of surgery that consumed the largest amounts of blood flow, while orthopedic surgery was in the second position (5, 6). Transfusion is often necessary during cardiac surgery to correct coagulopathy, blood loss, and hemodilution due to priming. Very often, patients undergoing cardiac surgery have numerous comorbidities, such as anemia or previous myocardial infarctions, which increase the risk of complications and therefore the need for blood transfusions is greater (7).

It is important to note that in most patients who underwent cardiac surgery and who had an increased rate of morbidity and mortality, an average of one to two units of blood were compensated (1, 7, 8). These patients are generally treated for anemia but have generally been hemodynamically stable (8, 9).

The study we performed included 100 cardiac surgery patients, of whom 20 patients received 24 h postoperatively an average of one unit-350ml of resuspended erythrocytes, exclusively at Hgb ≤ 85 g/l, with the aim of correcting postoperative anemia to avoid hypoxia, what is consistent with the results described previously.

The authors performed rigorous statistical analysis for their data and were able to show, using multiple logistic regression, that decreased activity due to ADP activation, predicted increased bleeding. In platelet mapping, the percentage of platelet inhibition, which subtracts the contribution of fibrin to the curve, but also the maximum amplitude due to platelet activator (MAADP), can be examined. The authors showed that both parameters were equally predictive in this data group. In addition to the predictive effect with respect to blood loss, platelet activation due to ADP predicts the need for platelet transfusion. It appears that the value of MAADP may have the ability to determine which patients on clopidogrel are likely to require platelet transfusion (10, 11). The results in this study also indicate the importance of preoperative and early postoperative testing of ADP as an independent predictor of increased bleeding in cardiac surgery.

Regarding frozen fresh plasma transfusions, as well as platelet concentrate transfusions, this study was performed solely on the basis of hemostasis therapy algorithms guided by ROTEM and Multiplote (12, 13). A total of 10 patients received an average of 3.5 units within 3 hours of surgery, with an average of 3.5 units of 220ml per patient, exclusively after detection of coagulation factor deficiency by ROTEM assay parameters (Extem and Intem). Donor platelet concentrate was obtained by 22% of patients early after surgery, also based on analysis of point-of-care (POC) hemostasis tests, both on ROTEM parameters and on Multiplate analyzer (ADP, ASPI, TRAP test). The use of the synthetic hemostatic agent desmopressin acetate to correct platelet function was performed on the basis of ASPI test values and on the comparison of ROTEM values related to blood clot strength (a total of 20 subjects received the drug after surgery).

In order to reduce the amount of blood loss and blood transfusions required during and after cardiac surgery, it is important that antiplatelet drugs such as acetylsalicylic acid and clopidogrel, routinely prescribed for patients before cardiac surgery, especially those undergoing surgical myocardial revascularization, are suspended before surgery. There is no precise information when to and on what day antiplatelet medication should be discontinued in order to obtain the most optimal results in terms of reducing postoperative drainage and the need for reimbursement of blood and blood derivatives (14). However, the literature data show that discontinuation of antiplatelet medication 2 days before cardiac surgery results in a significant reduction in the need for reimbursement of platelets (15).

The connection between discontinuation of antiplatelet drugs, and preoperative Multiplate and ROTEM values and major adverse cardiovascular and cerebral prothrombotic events could not be demonstrated, which is in agreement with the results obtained in our study.

Thromboelastography is a dynamic qualitative and quantitative assessment of clot formation that consists of three stages: clot initiation, clot strength, and clot stability (fibrinolysis). Performing thromboelastography using ROTEM is very useful, but it does not eliminate the need for other POC techniques that allow the evaluation of platelet function (e.g., Aggregometry-Multiplate), all of which are incorporated into numerous algorithms that are constantly being modified and developed (16, 17).

Despite the improvements made with existing new techniques, most surgeons are still inclined to accept a significant amount of blood loss as a characteristic of cardiac surgery. The research conducted at our institution also indicates that only 9% of patients had an average postoperative drainage loss of more than 1000ml, primarily due to the timely administration of so-called hemostatic agents. Targeted hemostasis therapies are guided by point of care devices for testing hemostasis.

It is very important to ensure adequate drainage and removal of blood from the pericardium and pleura cavity (it has high fibrinolytic activity and tissue coagulation factor). Removing this blood and clot probably not only reduces the chance of excessive blood loss by preventing systemic coagulopathy, but also has beneficial effects on several other factors associated with surgery, such as inflammation, atrial fibrillation, pericardial effusions (tamponade), and development of adjuncts (18). Our results indicate that no patients with pathological hyperfibrinolysis were registered as a result of early administration of anti-fibrinolytic-tranexamic acid at a dose of 1000mg immediately after the sternotomy.

After careful evaluation, hemodilution appears to be the most prominent factor associated with the development of coagulopathy after cardiac surgery, and probably plays an important role in the onset of blood loss after cardiac surgery (19). Prothrombin Complex Concentrate (PCC) is a hemostasis agent used for vitamin K dependent coagulation factor deficiency (II, VII, IX, X), which occurs especially in dilution coagulopathy during and after cardiac surgery. The advantage is on the PCC side compared to the plasma. There is currently no consensus on the dosage and timing of PCC administration, and the increased risk of thromboembolic complication must always be balanced for PCC administration. Three patients in this study received an average PCC dose of 1000 IU postoperatively because of the deficiency of these factors, which was registered by the CT extem parameter 100 ≥ s on ROTEM, as well as the values of prothrombin time or INR over 5.

Fibrinogen is one of the most important coagulation factors and it is possible for the clotting process to fall below a critical level during hemodilution, so care should be taken when it is necessary to administer fibrinogen concentrate (20). Since we did not possess fibrinogen concentrate following the FIBTEM test on ROTEM, and especially the clot amplitude after 10 min (A10), each of 13 patients received an average of 15 doses of cryoprecipitate early postoperatively (3 h after intervention).

Fibrinogen is an acute-phase protein whose level gradually increases during and after surgery in response to surgical trauma and the use of extracorporeal circulation. Increased concentrations of D-dimer and prothrombin fragment 1 + 2, together with increased thrombin production, indicate that the hypercoagulable state develops up to 5 days after cardiac surgery. The interaction of these two factors (hypercoagulable state and administration of fibrinogen concentrate) may increase the risk of thromboembolic complications in the postoperative period. Therefore, adequate anticoagulant and/or antiaggregation therapy to prevent the occurrence of thromboembolic complications in the postoperative course is required, especially in patients not receiving vitamin K antagonists, even in patients without prior bleeding. The delicate balance between bleeding tendency and hypercoagulable state must be maintained.

Point of care coagulation management with ROTEM and impedance aggregometry is now often used to determine first-line therapy with specific coagulation factor concentrates such as fibrinogen concentrates and prothrombin complex concentrates (PCC).

Roberts HR and colleagues published the results of a prospective randomized trial aimed at studying the effects of hemostatic therapy, guided by either conventional coagulation analysis or POC testing, in cardiac surgery patients (21). Patients diagnosed with diffuse bleeding after heparin reversal or increased blood loss for the first 24 hours were included and randomized to the POC group. The hemostatic therapy algorithms in combination with POC testing reduced the number of erythrocyte transfusion units compared with conventional laboratory coagulation testing. Moreover, POC-guided therapy was associated with decreased use of fresh frozen plasma (FFP) and platelet concentration and cost, as well as improved clinical outcome.

The use of POC evaluation can provide a faster and more complete insight into this delicate balance, creating a more individualized, patient-centered treatment. The wide variation in the patient's sensitivity to the use of clopidogrel, often produces very different individual results before surgery, necessitating the continued use and determination of POC before, during and after cardiac surgery. A patient-centered, individual approach can help reduce perioperative and postoperative blood loss and minimize the need for transfusion.

Conclusion

Due to the complexity and duration of cardiac surgery procedure, hemostatic changes occur in patients undergoing CABG. Moreover, other hemostatic abnormalities may already be detected in patients preoperatively due to their type of disease and/or their pharmacological treatments (oral anticoagulants, antiplatelet aggregation drugs).

Because of all of the above, it is a priority to diagnose the most common coagulation disorders in patients undergoing surgical myocardial revascularization and to choose appropriate hemostatic therapy to timely manage coagulation disorders in cardiac patients.

In this context, the most common disorders of the hemostatic system in this study were diagnosed with preoperatively and postoperatively impaired platelet function (up to 31% of patients), impaired activity and concentrations of extrinsic coagulation factors postoperatively (21% of patients), impaired activity and concentrations of intrinsic coagulation factors (23% of patients after surgery) and impaired concentration of functional fibrinogen and impaired polymerization of fibrin clot in 17% of

patients after surgery. Described disorders resulted in bleeding which, in an extreme case, can cause a lethal outcome.

Of particular clinical importance are the devices for POC testing of the hemostatic system, which allow for the correct and timely diagnosis of these disorders of coagulation, the prediction of possible bleeding that has not yet manifested clinically, and the choice of targeted hemostatic therapy that will prevent or stop the bleeding. Due to laboratory and clinical detection of hemostatic disorders recorded during the study, 13% of patients received cryoprecipitate transfusion after surgery, 10% of patients received frozen fresh plasma, 22% were transfused with platelet concentrates, 20% of patients received desmopressin acetate, while 3 patients received prothrombin complex concentrate in postoperative flow. It should be noted that 20% of patients received transfusion of resuspended erythrocytes (an average of 1 unit) after cardiac surgery, and all operated patients received autologous transfusion of their own blood through the intraoperative blood salvage device during the intervention.

Because of all this, only 9% of surgery patients had drainage greater than 1L within the first 24 hours of surgery, with no consequent complications regarding surgical reintervention.

Thanks to the described protocols of the POC application of coagulation system testing, the speed of the tests themselves, and rapid clinical decisions regarding hemostatic therapy, the mortality rate of operated patients included in the study was about 1% (not due to hemostatic disorders), which ranks among the world's eminent institutions that deal with cardiac surgery.

Modern methods combined with proven clinical protocols, extensive clinical experience of staff, respecting the principle "time is life", enables the best possible care for patients with detected hemostatic disorder in cardiac surgery.

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MONITORING POREMEĆAJA HEMOSTAZE U KARDIOHIRURGIJI

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Krvarenje tokom i posle ugradnje kardiopulmonalnog bajpasa je multifaktorijalna i potencijalno letalna komplikacija. Zato je jedan od najtežih zadataka u kardiohirurgiji uspostavljanje pravovremene, fiziološke hemostaze.

Cilj ovog istraživanja bio je dijagnostikovati najčešće poremećaje koagulacije kod bolesnika koji su podvrgnuti hirurškoj revaskularizaciji miokarda (njihovu učestalost, prateće komplikacije) i terapijsko zbrinjavanje istih. U prospektivno istraživanje uključeno je 100 ispitanika (22 osobe ženskog pola - 22% i 78 muških ispitanika – 78%), koji su bili podvrgnuti jednostrukoj, dvostrukoj i trostrukoj hirurškoj revaskularizaciji miokarda. Preoperativno, kao i tri sata i 24 sata postoperativno, određivani su sledeći parametri: krvna slika, koagulacioni status, parametri funkcije trombocita, parametri rotacione trombolastometrije, upotreba krvi i produkata krvi, upotreba sintetskih hemostaznih agenasa. Najčešći dijagnostifikovani poremećaji hemostaznog sistema su preoperativno i postoperativno poremećena funkcija trombocita (do 31% bolesnika), poremećaj aktivnosti i koncentracije faktora spoljašnjeg puta koagulacije postoperativno (21% bolesnika), poremećaj aktivnosti i koncentracije faktora unutrašnjeg puta koagulacije (23% bolesnika posle operacije) i poremećena koncentracija funkcionalnog fibrinogena i poremećena polimerizacija fibrinskog ugruška kod 17% bolesnika posle hirurške intervencije. Tokom istraživanja, 13% bolesnika je primilo transfuziju krioprecipitata posle operacije, 10% bolesnika je primilo zamrznutu svežu plazmu, 22% je transfundovano koncentratima trombocita, 20% bolesnika je dobijalo dezmopresin-acetat, dok su tri bolesnika primila koncentrat protrombinskog kompleksa u postoperativnom toku.

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Ključne reči: *kardiohirurgija, hemostaza, krvarenje*

Review article

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CONTACT DERMATITIS – A REVIEW OF THE LITERATURE WITH THE CONNUBIAL TYPE IN FOCUS

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Contact dermatitis (CD) is an acute or chronic skin inflammation induced by exogenous exposition and direct contact with chemical, biologic, or physical agents. Classic types of CD are irritative CD (acute and cumulative with various subtypes), allergic (acute, subacute and chronic, with specific subtypes and noneczematous variants) and photoreactive CD (phototoxic and photoallergic). A specific form of CD is connubial CD which is caused by indirect exposure to substances via physical contact with marital partner or some other person with whom he or she lives. The agent which causes dermatitis is not used by the patient himself.

As CD is frequently encountered in everyday practice, with polymorph clinical picture and various etiology, it is very important to discover the cause of the disease and its elimination, if possible, is of greatest importance. Connubial dermatitis is frequently unrecognized, which creates difficulties in treatment.

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Key words: *contact dermatitis, frequency, causes, connubial dermatitis, diagnosis, treatment*

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Introduction

CD is an acute or chronic skin inflammation induced by exogenous exposition and direct contact with chemical, biologic, or physical agents (1).

Four to seven percent of dermatologic consultations are the consequence of CD. It most frequently affects hands - in about 75% of all cases (2), and in 90% of occupational CD (2).

CD accounts for more than 77% of all occupational skin diseases (3).

The Aim

The aim of this research was to present the most recent data about CD and its various types, causes, types of allergens, changes of localization, etc. Studies on connubial dermatitis are specifically analyzed since this disease is not frequently discussed and it is difficult to diagnose.

Types of CD

Classic types of CD (4, 5) are: irritant CD, acute-simplex and cumulative-detritiva (6-8), allergic CD (acute, subacute and chronic) and photoreactive CD (toxic and allergic).

There are various subtypes of irritant CD (9): acute irritant, late acute, traumatic, pustular, irritant reaction, cumulative, asteatotic, acneiform, non-erythematous, subjective or sensory, airborne and frictional.

Depending on duration, the clinical picture of ACD varies (10).

Acute ACD usually appears suddenly and can be manifested by a clinical picture characterized by several stages: erythematous, papulous, vesicular, bullous, pustulous, madidans, squamous, crustous. Usually there is more than one center which is confluent with others. It is itchy and appears upon repeated exposure to the allergen in sensitized persons.

Subacute ACD is characterized by less pronounced erythema, exudation and edema with dominant inflammatory infiltration.

In chronic stage, the skin is dry, thick, brownreddish, lichenified with rare papulae, squama, sometimes with rhagades. Itching is also pronounced.

Phototoxic CD is provoked by tar derivatives, drugs, furocoumarin colors (11), while photoallergic CD is induced by antimycotic drugs, perfumes, halogenated salicylanilides, phenothiazines, sulfonamides, and sunscreen agents.

Specific CD types are (12, 13): airborne CD, generalized/systemic, pigmentary, ectopic, connubial (consort, paradox and protein CD).

Noneczematous CD variants are (14-18) lichenoid CD, erythema multiforme-like dermatitis, cellulitislike, contact leucoderma, contact purpura, erythema dischromicum perstans-like dermatitis, inflammatory, granulomatous, actinic prurigolike, follicular CD.

Contact allergic dermatitis can be provoked by (19) plants, metals, perfumes, garments and fabrics, rubber, plastic, preservatives, cosmetic products, computer mouse, drugs, and it can also be work-related.

ACD can appear in any part of the body (20-22) but it most frequently appears on the hands, feet, in axilla, on the eyelids, in anogenital and diaper region, perorally (contact stomatitis and cheilitis).

The most frequent allergens (13) are nickel, neomycin, Balsam of Peru, perfumes, thimerosal, gold, formaldehyde, quaternium-15, cobalt and bacitracin.

There is no difference between genders when it comes to the reaction to primary irritants (23). Also, prevalence of contact sensibilization does not depend on gender but on the intensity of the previous exposure to the specific allergen (24).

Contact sensibilization in children is a serious problem (21). ACD can be registered in 3-5 year old children, while the prevalence grows with age (25-28) and is the same as in the adults. Patch testing is safe and efficient (29, 30).

"Connubial" and "Consort" contact dermatitis

Connubial dermatitis is caused by indirect exposure to substances via physical contact with a marital partner (31). It is not necessary for the contact to be of a sexual nature (32, 33). Connubial ACD appears when the agent which causes dermatitis is not used by the patient himself, but by the partner or some other person with whom one lives (33) since it occurred through a sexual contact (34).

Although the term connubial is mainly used, it would rather mean that marital partners are involved, while "consort" refers to a partner or a friend (which would be more relevant to the actual behavior) (11) and other family members.

Sexual contact or other sexual activities could also be the source of irritation, trauma, allergic or nonimmunologic contact urticaria (34).

First reports on connubial contact allergic dermatitis date back to 1975 when Wilkinson (35) determined that ACD or photodermatitis can be the consequence of home-based activities, marital contacts or drugs usage.

In 1976, Caro (36) presented a case of a patient who had a rash on the right side of the neck and the front part of the right axilla. The sensibilization occurred due to the contact with bed sheets contaminated with benzoyl peroxide used by his wife for acne treatment.

In a 55 year old patient, sensibility to propylene glycol was registered after having used a specific cream (37). One year later, 24 hours after the sexual intercourse with his wife, he had ACD on his penis and scrotum. Sensitivity to vaginal lubricant, which his wife had used, was proven and it contained propylene glycol (38). A similar situation occurred in a 40 year old patient who developed pruritus, erythema, erosions and edema on glans and prepuce 24 hours after sexual intercourse. He had had similar changes three months earlier. The cause was a lubricant that his wife had used prior to the intercourse. Sensitivity to the readymade preparation was registered as well as to chlorhexidine gluconate (39).

A 30 year old male always got dermatitis on his penis, scrotum, and lower abdomen after the intercourse with one of his girlfriends. There were no similar changes after the intercourse with other women.

Testing proved a positive reaction to Balsam of Peru which was one of the ingredients of a hygienic spray that the girl used before the intercourse (40).

A 20 year old woman would always get rash on her face, neck, sometimes on hands after the intercourse with her husband. After it was proven that her partner had acne which he treated with benzoyl peroxide and testing showed positive results, the treatment was changed and the rash subsided (11).

A young woman had diffuse follicular rash on the upper arms, front part of the trunk and the inner sides of her thighs. When her boyfriend was absent, there were no changes. When he came back, rash reappeared after going to the beach. It was proven that the cause was a sunscreen lotion (Coppertone), which her light skinned boyfriend used for protection and after that they would have the intercourse (14).

Contraceptive rubber diaphragms, rubber condoms and spermicides can produce ACD in sensitive men and women. Women can get vulvitis and vaginitis while men can get balanitis. It is recommended to use nonrubber condoms or some other material underneath (41).

A 22 year old male had erythematous edematous dermatitis corpus on the penis and balanoposthitis several hours after the intercourse. He used condoms. Testing proved allergic reaction to thiuram mix and benzocaine. Benzocaine was incorporated in the gel used for the enhancement of the sexual intercourse (42).

Semen can also cause allergic reactions like contact urticaria and anaphylaxis in sensitized women (43-45).

A case of a 25 year old woman with the familiar atopic history was described. After a sexual intercourse she would get urticaria, swelling of her eyelids and abdominal cramps, and once she had circulatory collapse. When a condom was used, there were none of these symptoms. Sensitivity to semen plasma was proven. It is supposed that the cause is the protein which is found in the normal semen sample (46).

A seven months pregnant woman had clearly bordered dermatitis of the lower abdomen and back as well as on her legs after wearing her husband's trousers. He had psoriasis and was treated with dithranol. He did not take a shower 30 minutes after

the application of the medicine and wore the trousers. With consequent relevant behavior the appearance of irritation was avoided (44).

A 40 year old woman had repeated dermatitis eruptions of the left hand side.

The changes were related to the contact with her husband's perfumed skin. Testing proved sensitivity to the perfume ingredients. These changes stopped occurring when her husband stopped using that specific perfume (47).

A 50 year old male had three years old history of rash and itching on the left side of his chest, back and left hand. Patch testing proved a positive reaction to paraphenylene diamine and orange dispersion. It was a reaction to his wife's dyed hair, as she slept on his left side (48).

Two marital partners were treated for follicular pruritic rash and erosions without success. The treatment became successful only when it was realized that the problem was due to the contact with fiberglass with which her husband worked, and the wife washed her clothes together with his contaminated clothes, so that she got in contact with the same substance (33).

A 35 year old woman got erythema, vesicles and bullae on her face, back of the neck, breasts, bottom and upper extremities, after her husband had cuddled with her the previous night. The case became clear with positive test results to urushiol, wood extract in ethanol and crushed wood, the fruit of which her husband had eaten in the restaurant on the previous day (49).

A 52 year old female got androgynous alopecia due to the contact with her husband who was applying testosterone gel on his upper arm for hypogonadism for 18 months. Androgynous alopecia was confirmed on the basis of clinical and dermoscopic findings. Laboratory analysis showed high testosterone levels and free testosterone (50).

Connubial ACD can appear due to propylene glycol, hygienic sprays used by women, perfumes and contraception, Balsam of Peru, benzoyl peroxide and hair dyes, sunscreen lotions, rubber, benzocaine, paraphenylenediamine, drugs-corticosteroids etc. (42, 43, 47, 48).

In a 46 year old woman who worked with hop for 30 years and who had skin problems in the form of dermatitis and conjunctivitis, an allergic reaction to hop leaves was proven. Although she had stopped working, she had several relapses. It turned out that they occurred after she had slept with her husband in the same bed who had worked with hop and did not wash up. It was a simultaneous connubial and occupational dermatitis to hop (51).

Airborne agents can induce skin reactions. They can be irritant CD, allergic CD or photoallergic and phototoxic reactions, or photocontact urticaria, acne-like lesions, erythema fixum due to drugs, lichenoid rash, etc. (52, 53). Various pharmacologic classes of drugs can produce different reactions, either after direct contact or via inhalation (54).

A four year old boy was treated for asthma with Pulmicort aerosols (budesonide) and Bricanyl (terbutaline) in the inhalation chamber. After 4 days of treatment, his mother had an itchy swelling on her face with conjunctivitis. After the treatment with Tridesonit creme (desonide) it got worse. Prick tests proved sensitivity to budesonide and Pulmicort and positive tests to Tridesonit creme and triamcinolone acetonide. It was connubial ACD caused by corticosteroids, which is rare (55).

A 51 year old male had skin changes at the time when his four year old daughter was receiving corticosteroid inhalation therapy for her asthma. Here, besides sensitivity to budesonide and triamcinolone, sensitivity to prednisolone, hydrocortisone, tixocortol pivalate, hydrocortisone 17-butirat and amcinonide was reported (56).

Diagnosis, treatment and prevention

CD diagnosis is set on the basis of history, complete clinical checkup, elimination and exposition testing, functional skin ability determination and immunologic analyses (57).

Causal and symptomatic therapy is performed (58-61).

Occupational CD prevention is primary, secondary and tertiary (62).

Conclusion

Contact dermatitis is frequently registered in dermatologists' everyday work. Clinical picture is polymorphic and of extremely different etiologies. Discovering the cause of the disease and eliminating it, if possible, is of greatest importance. In order to accomplish that, it is necessary to perform an entire and conscientious checkup of the patient. If it is not the case, CD can remain unrecognized and unclear, which frequently happens when it comes to connubial contact dermatitis.

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The work has not been published so far.

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KONTAKTNI DERMATITIS – PREGLED LITERATURE SA KONUBIJALNIM TIPOM U FOKUSU

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Kontaktni dermatitis (KD) je akutna ili hronična inflamacija kože, koja nastaje usled egzogene ekspozicije i direktnog kontakta sa hemijskim, biološkim ili fizikalnim agensima. Klasični tipovi KD su iritativni (akutni i kumulativni sa raznim subtipovima), alergijski (akutni, subakutni i hronični sa specifičnim tipovima i neegzematoznim varijantama) i fotoreaktivni KD (fototoksični i fotoalergijski). Poseban oblik KD je konubijalni KD, koji je izazvan indirektnom ekspozicijom na supstance preko fizičkog kontakta sa bračnim partnerom ili drugom osobom sa kojom bolesnik živi. Agens koji je izazvao KD nije upotrebljen od strane obolelog.

Kako se KD često registruje u svakodnevnoj praksi, sa polimorfnom kliničkom slikom i raznovrsnom etiologijom, vrlo je važno otkriti uzrok bolesti i po mogućstvu ga ukloniti. Konubijalni dermatitis često ostaje neprepoznat, što stvara poteškoće u lečenju.

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Ključne reči: *kontaktni dermatitis, uzroci, konubijalni dermatitis, dijagnoza, tretman*

Original article

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COMPARATIVE CLINICAL AND HISTOPATHOLOGICAL STUDY ON COLLOID MILIUM OF THE SKIN

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Colloid milium (CM) is unusual cutaneous disorder with unknown prevalence. The disease usually present clinically by the development of yellowish translucent or flesh-coloured papules on the sun-exposed skin. Histologically, it is characterized by the presence of colloid in the dermal papillae, with mistakenly diagnosed either keloid or facial amyloidosis. Microscopical findings showed atrophic or ulcerous epidermis with a large deposition of amorphous eosinophilic material containing fissures which expand the dermal papillae with extension into deep dermis (papules or plaques on the sun-exposed skin). Histologically, it is characterized by the presence of CM. We have studied the most frequent, classic adult type. The diagnosis was established after an examination of a skin biopsy under light microscopy. For distinguishing colloid from amyloid, differential stain had to be used. The other three recognized variants (juvenile colloid, pigmented colloid milium (hydroquinone related) and colloid degeneration (paracolloid)) are very rare and were not analysed.

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Key words: *coloid milium, skin cancer, histological characteristics*

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Introduction

Colloid milium (CM) was first described by Wagner in 1866 as "Das Colloid-Milium Der Haut" and has been known historically as colloid pseudomilium, colloid infiltrativum, hyalinoma (1-5). CM is an unusual rare cutaneous degenerative process with unknown prevalence, linked to excessive sun exposure and to petroleum products and hydroquinone (6, 7). It was pointed out that CM in the nodular type represents a degeneration product of elastic fibres which is most frequently induced by solar radiation (8-10). Colloid milium includes at least four distinct clinicopathological conditions (5); classic, the adult variant, typically affects areas of sun damage on fair skinned persons (6-9). It is characterized by the presence of multiple, dome-shaped or flesh coloured papules developing on the light-exposed skin and the observance of dermal colloid under light microscopy. The following variants are also pointed out: recognized adult type; juvenile colloid milium; pigmented colloid milium (hydrquinone related) and colloid degeneration (paracolloid), gray to black areas on the face and neck, probably heterogenous group (10-14).

Aim

Both histopathologic mimicker of Colloid Milium and its cutaneous deposition on the light-exposed skin are the reasons for this retrospective clinical, morphological, and histochemical study. We must make a distinction between histopathologic mimicker, dermatopathologist's initial impression of nodular amyloidosis, calcinosis cutis, milia cystis, multiple syringoma and keratosis. The purpose was to determine further therapy and prognosis of this difficult to treat entity.

Materials and methods

We analysed 12 surgical biopsies of the CM for clinical diagnosis of adult type (head skin tumours) taken from the nose (4), upper lip (1), eyelid (1), forehead (3), chin (2) and scalp (1). The taken surgical biopsies were fixed during 24 h in 10% formaldehyde solution. Treatment of fixed material

was performed in autotechnicon in "HUMAN POLICLINIC". Paraffin sections of 4 micrometer thickness were stained with conventional H&E technique for histopathological diagnosis of the present process. Specific histochemical PAS, Van Gieson and Congo red methods were also used to confirm the presence of CM.

Results

Clinical characteristics

CM is a rare cutaneous deposit disease that usually presents clinically by the development of yellowish semitranslucent or flesh-coloured papules or plaques on the sun-exposed skin.

Of 86 patients operated for "malignant tumours formations" on the skin of the face, CM was detected incidentally in 12 (13.9%) patients, more frequently in females (8:4), in mid-adult life (54 years), 1-5mm in diameter, dispersed in the cheeks, nose, upper lip, eyelid, periocular region forehead, chin and scalp.

Macroscopical characteristics

CM is a rare cutaneous condition with four subtypes, characterized clinically by development of yellowish translucent papules or plaques on sun-exposed skin. In some of patients, the lesions were increasing in the summer and decreasing in the winter. Only CM occurring in the palpebra and conjunctiva (1 case) was presented like gelatinous, small translucent dome-like amber papules. Patients describe a gradual eruption of papules or nodules on sun–exposed areas.

Microscopical characteristics

In the epidermal/papillary dermis, histology examination of paraffin sections revealed the pale, homogenous eosinophilic material expanding the epidermal papillae and extending into deep dermis (Figure 1). Scattered lymphocytes and plasma cells were observed at the periphery. Colloid material was concentrated in the upper and middle parts of the dermis (Figure 2) with sparing subepidermal layer of the papillary dermis (Grenz zone) (Figure 3). In this manner, the dermis was filled throughout with fissured eosinophilic colloid material showing characteristic long and horizontal artifactual clefts (Figures 4 and 5). Sometimes, subepidermal Grenz zone was partially lined with fibroblastic cells (Figure 6).

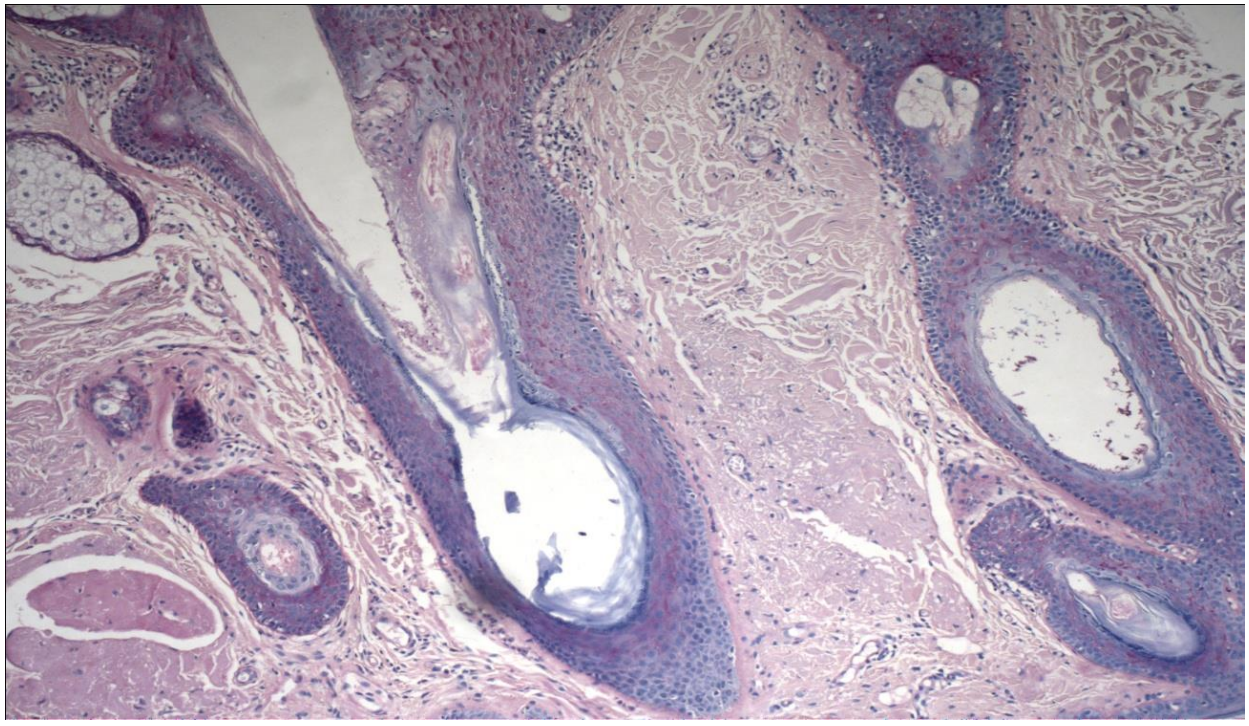

Figure 1. Homogenous Colloid material expanding papillary derm, HE x 200

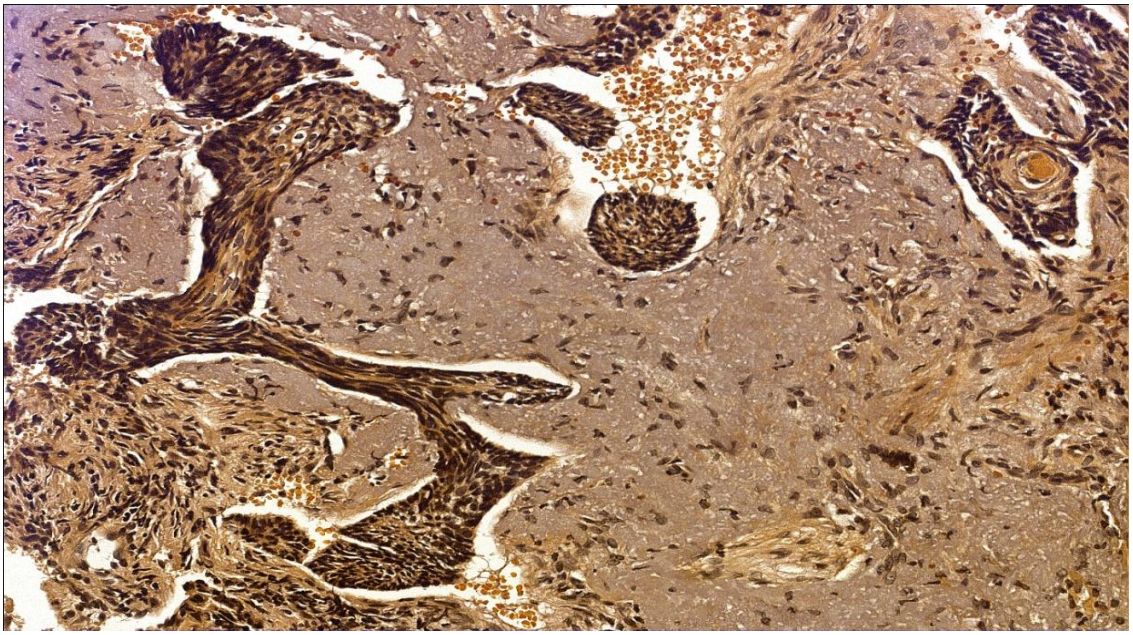

Figure 2. Basocellular Ca, with homogenous Colloid Milium in deeper derm and with inflammation, HE X 200

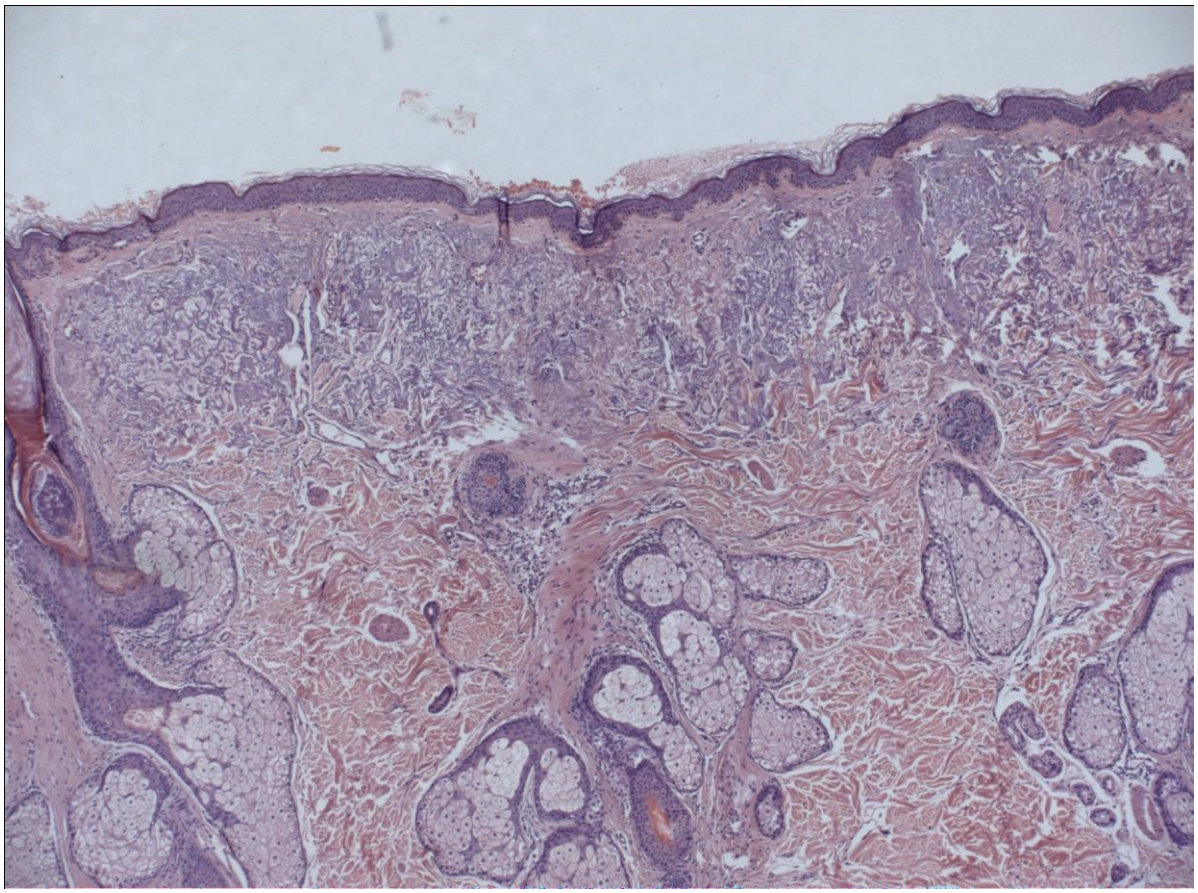

Figure 3. CM spared subepidermal Grenz zone, HE x 200

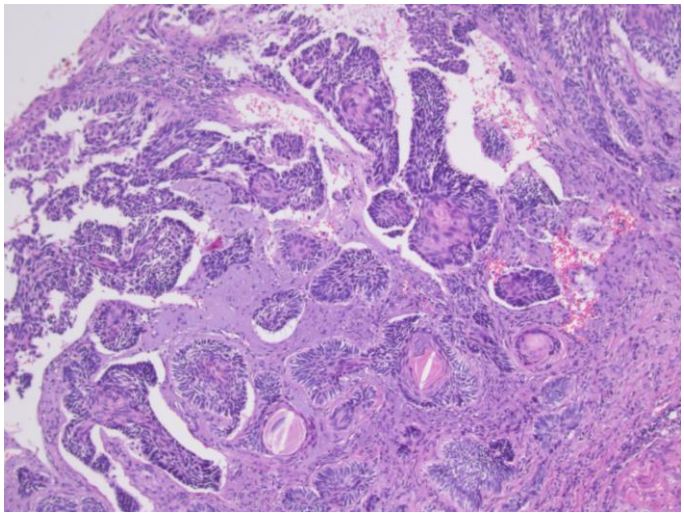

Figure 4. Fissured CM in squamo-basocellulare carcinoma cutis, PAS x 200

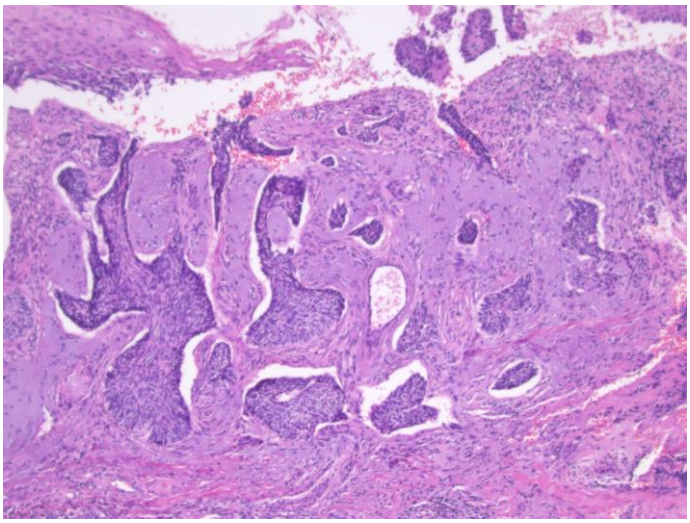

Figure 5. Ulcerous basocellular cancer, with fissured dermal Colloid Milium, PAS x 200

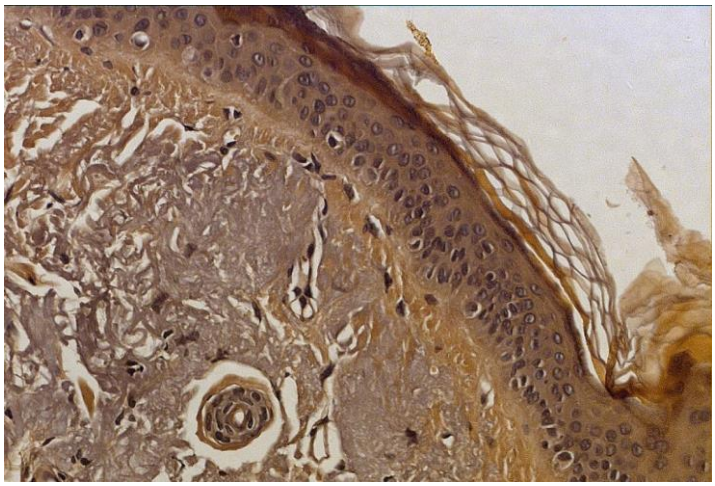

Figure 6. Colloid with subepidermal Grenz zone, partially lined by fibroblasts: HE x 300

Discussion

The material in the dermis represents a degeneration, product of elastic fibres which is induced by solar radiation and colloid degeneration (5). Colloid degeneration includes at least four distinct clinicopathological conditions (7-10):

- classic adult type CM (adult form develops in sun exposed parts of the body in patients who have actinic-damaged skin); it develops in mid-adult life; plenty of cells- brown semitranslucent papules, or plaques, 1-4mm in diameter, seen in the cheeks, ears, neck and dorsum of the hands. Chronically sun-damaged skin, whether it was that of actinic elastosis, or actinic keratosis, basal cell epithelioma, polymorphic light eruption, discoid lupus eritematosus or colloid millium, showed striking staining characteristics. This involved predominantly the upper one-third of the dermis, but often extended, with progressively decreasing intensity, into the mid-dermis. The intensity of staining was directly proportional to the extent of actinic damage clinically. At-tempts to remove these lesions are generally unsuccessful, but either dermabrasion or long-pulsed YAG laser has been reported to be effective (14). Dermatopathologists have recorded and described the basophilia associated with actinically damaged skin for many years. In some instances it is an aid to diagnosis.
- juvenile colloid milium (exceedingly rare prior to puberty: papules or plaques are seen on the face and neck);
- pigmented type CM: hydroquinone related
- colloid degeneration (paracolloid): gray to black areas on the face and neck, it is probably a heterogeneous related group. The lesions of colloid milium tend to reach a peak within three years, after which few new papules occur. The lesions do not resolve and occasionally may be pruritic (12).

There are some cases in the literature where severe adult colloid milium presented as papillomatosis cutis associated with vitiligo (9); further, there is a case of a patient who was habitually exposed to UVA-radiation twice a week for 7 years for aesthetic reasons.

A mucoid or gelatinous substance can sometimes be expressed from the papules by applying pressure or puncture. The lesions are often easily hemorrhagic with minor trauma. Involved skin may be thickened, furrowed, and hyperpigmented. The male-female ratio is 4:1 (14).

Conclusion

Colloid milium is a rare degenerative condition with unknown prevalence and with the presence of multiple dome-shaped amber or flesh-coloured papules or plaques on sun-exposed skin. This prevalence was about 13% in analysed operative skin specimens from tumorous formations of the face, and more common in women. Diagnosis is based on light microscopy study of a skin biopsy, which shows fissured eosinophilic colloid masses in the papillary dermis, with sparing subepidermal layer of the papillary dermis. Amyloid stains are negative. Differential diagnosis has an important role in the categorization of lesions.

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KOMPARATIVNA KLINIČKA I HISTOPATOLOŠKA STUDIJA KOLOIDNOG MILIJUMA KOŽE

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Koloid milijum (CM) je neobično kožno oboljenje nepoznate prevalencije. Bolest se obično klinički ispoljava žućkastim prozračnim ili papulama boje mesa na koži eksponiranoj suncem. Histološki, karakterisana je prisustvom koloida u dermalnim papilama, sa pogrešno dijagnostifikovanim keloidom ili facijalnom amiloidozom. Mikroskopski nalaz je pokazivao atrofični ili ulcerozni epidermis sa velikim depozitima amorfnog eozinofilnog materijala koji sadrži fisure koje šire dermalne papile prema dubljim delovima derma (papule ili plakovi na koži eksponiranoj suncem). Histološki je karakterisano prisustvom CM. Proučavali smo najčešći klasični adultni tip. Dijagnoza je postavljena nakon ispitivanja biopsije tkiva pod svetlosnim mikroskopom. Za razlikovanje koloida od amiloida upotrebljavane su različite boje. Ostale tri prepoznate varijante (juvenilni koloid, pigmentni koloid milijum (povezan sa hidrohinonom) i koloidna degeneracija (parakoloid) su veoma retke i nisu analizirane.

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Ključne reči: *Koloid milijum, kancer kože, histološke karakteristike*

Original article

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FRACTURES OF THE FIBULA ABOVE THE LOWER TIBIOFIBULAR SYNDESMOSIS

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Fractures of the ankle are common injuries. According to Lauge Hansen's classification, there are five types of ankle fractures. In certain types of fractures tibiofibular syndesmosis injury occurs, a fracture of the fibula above the syndesmosis and a fracture of the medial malleolus or a rupture of the deltoid ligament, and these types are: supination-eversion (SE), pronation-eversion (PE) and pronation-abduction (PA).

We presented a group of 46 patients who were treated at the Department of Orthopedic Surgery and Traumatology due to the ankle fracture and rupture of the distal tibiofibular syndesmosis. All underwent surgery immediately after the injury, on average, 28 hours after the injury occurred when osteosynthesis of malleolus and transfixation of syndesmosis with spongiosum screw placed above it were performed. Postoperatively, we placed a plaster splint, which was worn for 3 weeks, and support on the injured leg was banned for up to 6 weeks. After removing the plaster, all patients were included in physical therapy. Osteosynthesis material was removed after 6 months.

We have evaluated the results of the treatment according to the Olerud and Molander score. By tracking the subjective and objective signs we have acquired the following results: in the group of excellent and good results there were 34 (73.9%) patients, in the group of satisfactory, there were 7 (15.2%), in the group with poor results there were 5 (10.9%) patients.

Lateral malleolus and tibiofibular syndesmosis are key to the anatomical reduction of displaced fractures, and restoring the integrity of the lateral malleolus establishes stability of the ancle.

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Key words: *tibiofibular syndesmosis, fibula fracture, osteosynthesis, transfixation of syndesmosis*

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Introduction

Fractures of the ankle joint are common in everyday life. Almost 2% of the general population will sustain an ankle fracture during their lifetime. Eponym of Dupuytren implies an indirect fracture of the fibula above the distal tibiofibular syndesmosis, syndesmosis rupture and rupture of the deltoid ligament or fracture of the medial malleolus. This results in the destruction of articular forks which causes the dislocation of the talus.

Tibiofibular syndesmosis consists of the fibular incisure of the tibia and fibula protrusion lying in the incisure. Front edge of incisure of the tibia is much more developed than the rear one and crosses over the medial two-thirds of the fibula to serve as a support to the fibula in order to restrict movement forward (1-4). Between the bone structure of syndesmosis there is no cartilage, there are adipose tissue, blood vessels and synovial recess of the upper ankle with length up to 1.5cm, and through it syndesmosis communicates with the joint cavity.

The movements of plantar and dorsal flexion of the ankle joint are determined by the mechanical complex of the foot which consists of fibular malleolus, the tibiofibular syndesmosis and pars dorsalis of tibia - these are the key elements to the stability of the ankle. The ligaments of the tibiofibular syndesmosis with their elasticity allow the movement of the

fibula and move it into the syndesmosis; there are four of them: lig. tibiofibulare anterior, lig. tibiofibulare posterior, lig. tibiofibulare interosseus and interosseus membrane. Rupture of all four ligaments causes diastasis of tibiofibular syndesmosis up to 7.3mm (5-7).

The aim of this paper was to highlight the role and importance of the fibula and syndesmosis in the movements of the ankle, the types of injuries and the necessity for anatomic surgical reduction.

Materials and methods

With the permission of the ethical committee, a research was conducted on 46 patients with fractures of the ankle and the disruption of the distal tibiofibular syndesmosis who were treated at the Department of Orthopedic Surgery and Traumatology, Clinical Center Niš, in the period from January 2011 to January 2013. Within the two year period, there were 85 ankle joint injuries of this type, but in this paper we have only shown patients with this type of injury, who were regularly monitored during followups three, six and twelve months after the surgery.

For the classification of ankle fractures, we used the Lauge Hansen classification, which is based on the mechanism of injuries, and there are 5 types: supination-eversion (SE) type, supination-adduction (SA), pronation-eversion (PE), pronation-adduction (PA) and pronation-dorsiflexion (PD) type (5, 8, 9).

The criteria for including the test subjects in the study were: a fracture above the lower tibiofibular syndesmosis, a rupture of syndesmosis and an injury of medial compartment of the ankle joint– a rupture of lig. deltoideum or a fracture of medial malleolus. This study includes test subjects with the upper part fibula fractures, the so-called Maisonn fractures and open fractures of the ankle joint.

In this study, we include three types of indirect fractures of the fibula above the distal syndesmosis. They are the result of different mechanisms of injury, and they are characterized by different lines of fracture in the distal and middle part of the fibula. In supination-eversion (SE) type, the supination and external rotation of the foot occur; fractures of the fibula are spiral, where the front top of the fracture line is positioned at 4cm or more above the top of the malleolus and then goes backwards and up. The fracture of the medial malleolus or the rupture of deltoideum ligament occur in the final, fourth phase of the injury. In pronation-abduction (PA) type, deltoideum ligament is stretched and ruptures first, then rupture syndesmosis and fibula, the fracture line is curved and it is located at 6 centimeters or more from the top of malleolus. In pronation-eversion (PE) types of fracture, malleolus fracture occurs, or ruptures of the deltoideum ligament and then fibula fractures. The fracture line of the fibula is 5 centimeters or more from the top of malleolus (9, 10).

For the purposes of real insight and objective assessment of the fracture, as well as the assessment of response to treatment, radiography was performed in two basic projections, and stress radiography for diagnosing the rupture of lig. Deltoideum (11, 12). Standard AP radiographs were obtained with the leg in 20 degree of internal rotation in men and 15 degree of internal rotation in women (13). We have measured the tibiofibular clear space (syndesmosis AB), and the overlap of the tibia and fibula (syndesmosis BC) (Figure 1). We observed that syndesmosis AB is normally < 5mm and BC normally > 10mm (14). We have measured the talocrural angle, it is normally 83 ± 4 degree (15) and medial clear space, > 4mm is abnormal (16) (Figure 2). Radiological control was done at the time of admission, during and after the operation, after the first, third and twelfth month.

The surgical procedure was performed immediately after the admission and preparation for surgery, on average, after 28 hours (from 1 to 6 days). The medial malleolus was fixed with malleolar screw, and rarely with Kirschner pin. Lig. deltoideum had to be shown, it could be interpolated in the line of malleolus fracture or between the malleolus and the talus-in this case, its reposition and suture with bioresorptive screw was done. For the purposes of operative approach to the lateral side, the shin was placed on a pillow not including the heel, since this would cause the frontal subluxation of the talus and malreduction. The foot was free and enabled the orthopedic repositioning of the fracture, positioning of the talus and the control over talocrural angle of the injured side which was compared to the counterlateral, normal angle, and the length of fibula was thus confirmed. The fibular fracture was displayed by lateral incision over the lover fibula half. The reduction of the fracture was achieved through foot manipulation, a one-third wrap around board was placed at the height of lateral malleolus, and it was then modeled to follow the axis with valgus position from 10 to 12 degrees. The board was centered between the front and rear bone edge, it was adjusted with Kirschner needles and an additional radiological shot was done. If the right length of fibula was achieved, the talocrural angle was reduced properly and the talus centered, then the fibula fracture was fixated (17).

Tibiofibular transfixion screw was placed individually or through one of the lower holes on the plate above the tibiofibular syndesmosis-i.e., 1.5 to 3.5cm above the hinge line (4). The bolt could affect four cortices-i.e., it could pass through the fibula and tibia, and it could pass through three cortices and the entire metaphyseal tibia (Figure 3). It was not necessary to tighten it greatly, or to place the foot at an angle of ninety degrees in relation to the lower leg while setting the screws (18). The stability and quality of reduction of the fracture were assessed roentgenographically.

Postoperatively, plaster splint or a brace was placed for three weeks, and support on the operated leg was banned for 6 weeks (19, 20), afterward followed physical procedures and gradual support on the operated leg.

After 12 months of monitoring, functional results of the respondents were evaluated. There are

many scoring systems, Joy Gregory (16), William Phillips (21), Foot and Ankle Outcome Score (FAOS) (22), we used Olerud and Molander's scoring system (23). The subjective symptoms of pain, deterioration in walking, difficulty in climbing the stairs, aggravation in doing sports and limited working ability as well as objective symptoms were evaluated: skin condition, deformity, muscle atrophy, loss of motion in the ankle joint and loss of subtalar inversion-eversion.

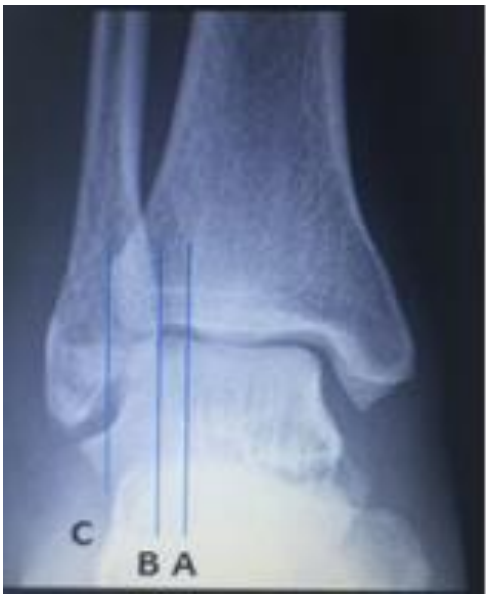

Figure 1. AP radiograph of the ankle *Tibiofibular clear space* (syndesmosis AB) is the space from the lateral border of the posterior tibial malleolus to the medial border of the fibula. *Overlap* of the tibia and fibula (syndesmosis BC)

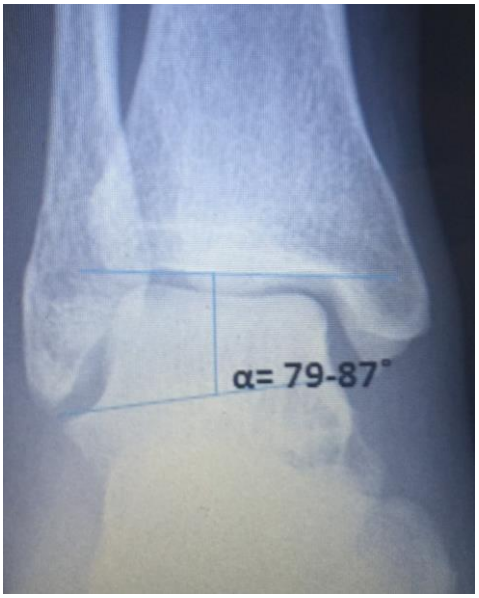

Figure 2. The talocrural angle is formed by a line perpendicular of the distal tibial articular surface and a line joining the tips of both malleoli. The medial clear space is the distance from the lateral border of the medial melleolus to the medial border of the talus.

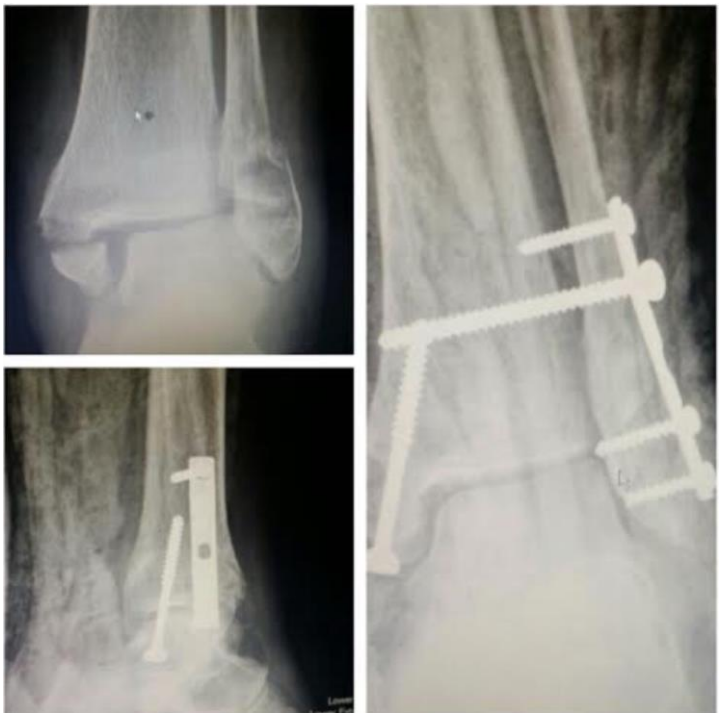

Figure 3. Fibula fracture with tibiofibular rupture of syndesmosis and postoperative treatment

Results

In the group of 46 patients with disruption of the distal tibiofibular syndesmosis and fibular fractures, there were 28 (60.9%) men and 18 (39.1%) women, and the age of patients ranged from 16 to 79 years (average 36.7 years). Among the causes of injury, slip and fall were dominant in 32 (69.6%) patients, fall from a height in 9 (19.6%) and traffic accidents in 5 (10.9%) patients.

According to the Lauge Hansen classification, 24 (52.2%) patients had supinatio-eversion type of injury. Pronation-abduction type of injury was in second place with a total of 17 (37%) patients, and 5 (10.9%) injuries resulted in pronation-eversion type of ankle injury (Table 1).

Table 1. Display the respondents by sex, cause of injury, type of fracture and the final results of the treatment

Sex		Injury cause		Fracture Type		Injury Type		Evaluation system	
Male	28	Slipping	32	bimaleolar	30	Supination-eversion	24 (52%)	Excellent+good results	34 (74%)
Female	18	Fall from height	9	trimaleolar	9	Pronation-abduction	17 (37%)	Satisfactory results	7 (15%)
		Traffic accident	5	unimaleolar	7	Pronation-everson	5 (11%)	Poor results	5 (11%)

In our series, average dorsiflexion of the ankle was 16 degrees (angle, 8 to 30 degrees) and the average plantar flexion was 40 degrees (range, 15 to 60 degrees). The difference relative to the contralateral side averaged a loss of 5 degrees of dorsiflexion and 4 degrees of plantar flexion.

Based on the final functional result of treatment by Olerud and Molander's scoring system, the average ankle score for these patients was 87 points (range 55 to 100 points).

In the group of excellent and good results, there were 34 (73.9%) patients, 24 patients (52.2%) had an excellent result (a score of 90 to 100 points), and 10 (21.7%) had a good result (an ankle score of 80 to 89 points). They had no pain in the ankle, range of ankle joint motion was not restricted and there was no deformity. They were able to perform all work activities and to engage in sports.

In the group of satisfactory results, there were 17 (37%) patients. They had occasional pain, which intensified during work, long standing and walking. They had a swollen ankle and foot and could not do the same job as before the injury. In the group with poor results, there were 5 (10.9%) patients. They had constant pain in the ankle and took painkillers. They had a swelling and deformed ankle and foot. The movements of the ankle joint were reduced by more than 60%. Even with the help of a stick, it was hard for them to move and they were unable to work and do sports. In the monitored group of patients there were complications to:

- the dislocation of the fibula fracture and syndesmosis due to the short plate on the fibula and a short screw with which tibiofibular syndesmosis was fixed - two cases,
- a fracture of the syndesmosis screw because of the incorrect fixation and early loading of the operated leg – one case,
- the appearance of synostosis at the height of tibiofibular syndesmosis – one case,
- bad reposition and fixation of the medial malleolus - two cases,
- postraumatic arthrosis of the ankle after one year - three cases.

We followed radiologically the size of tibiofibular space (syndesmosis AB), preoperative average value was 7mm (from 5 to 13mm), post treatment the average value was 4.5mm (from 3 to 6 mm). The overlap between tibia and fibula (syndesmosis BC) after the ankle joint injury was 4mm (from 0 to 11mm), and after the treatment the average was 10.5mm (from 10 to 13mm). Talocrural angle was compared to the healthy side, the average postoperative was 84 degrees (from 80 to 86). All the malleous fractures were healed on average within 3.5 months. There was no widening of the free medial ankle space-that is, its average postoperative value was not larger than 4mm.

Discussion

Healthy distal tibiofibular syndesmosis is responsible for biomechanics and congruency of the concave part of the upper ankle joint. Together with the lateral malleolus, it is the key to the stability of the ankle. Lauge-Hansen described the sequence of stages and the pathological mechanism of the fractures of the fibula above the syndesmosis based on his extensive experiments in cadavers. From the present study it is clear that there are three types of fractures of the fibula proximal to the syndesmosis each one produced by a typical sequence of stages in the development of a complete lesion. From the pattern of the fibular fracture, it is possible to conclude which lesions always accompany it (4, 6, 11, 19, 21, 24). In our series supination-eversion type is the dominant one, and other authors confirm it as well (10, 24).

Rupture of the tibiofibular ligaments, which constitute syndesmosis, results in separating the

tibia and fibula, and the development of the conditions for the anterolateral rotary instability of the ankle joint, talar tilt and signs of anterior talus drawer (1, 5, 25).

Close (26) has shown that normal movement of the ankle depends on the precise relationship determined by the syndesmosis. If the syndesmosis is disrupted, there may be widening of the tibiofibular joint and lateral shift of the talus. Ramsey and Hamilton (27) reported that when the talus moved laterally by 1 mm the contact area of the tibio-talar articulation was decreased by 42%. Burns et al. (28) has shown that a complete disruption of the syndesmosis combined with a tear of the deltoid ligament caused a decrease of 40% in the tibiotalar contact area, an increase of 36% in the tibiotalar contact pressure.

Today's attitude towards the treatment of tibiofibular syndesmosis rupture is surgical treatment, because the anatomic reduction with the closed method is almost impossible. The problem of diastase of syndesmosis is not treatment but diagnosis. For this reason targeted stress x-ray images, CT, NMR of the ankle are done, where one can see the degree of damage to the syndesmosis. This applies to the medial side of the ankle as well-if the medial malleolus is intact, and there is swelling, soreness and bruising then we need to look for the rupture of lig. deltoideum radiologically or during the surgery (10, 11, 16).

Transfixed syndesmosis screw can be metal or bioresorptive (20, 29), we used a metal one. Some authors (16, 20) have recommended placing the screw across only three cortices so that normal motion can occur at the syndesmosis. In our series of treated patients we have also applied the same method of the stabilization of tibiofibular syndesmosis. It can be placed independently or as a part of the panel that is used for osteosynthesis of fibular fractures. It is always placed transversely at 1.5 to 3.5cm above the wrist line, it is not recommended to place it through the syndesmosis, because it has a synovial recess through which it communicates with the joint cavity. On that level, there are also blood vessels of the front and rear tibiofibular ligaments that make up the tibiofibular syndesmosis. Blood vessels are lateral penetrating branches, a. peronealis and a. tibialis anterior (2, 9, 30, 31). The current recommendation to hold the foot at an angle of 90 degrees when operating syndesmosis transfixation is not justified. The same goes for excessive tightening of the screw-i.e., syndesmosis, because high compression has no effect on the range of motion in the ankle joint or on the course of healing of ligaments (18, 20, 32). During the operational work, we adhered to this rule as well-i.e., we did not strive towards the tightening of the screws and dorsal flexion of the foot.

In our series we had 34 (73.9%) test subjects with excellent and good results and 12 (26.1%) with satisfactory and poor results. The movement circumference in an operated ankle joint is on average 89% of the healthy movement. Other authors have published similar results. Robert et al. (19) in the study of 24 patients had 19 (79.2%) with excellent and good results, and 5 (20.8%) with satisfactory and poor results. The movements in operated ankle joint on average are 87% of the movements of non-operated ankle joint. David et al. (20) in the series of 33 patients had excellent and good results in 23 (69.7%), and satisfactory and poor results in 10 (33.3%) patients. The average dorsiflexion value was 17 degrees, plantar flexion 40 degrees. Yablon et al. (24) have shown the series of 53 patients, there were 14 (26.4%) patients with poor results - i.e., there was a talar tilt. Dorsiflexion in the ankle joint has been reduced on average by 6 degrees and plantar flexion by 10 degrees.

As to the question of when do we need to remove the transfixation screw, opinions are divided. One group of authors believes that it should be removed as soon as ligament structures coalesce – after 6 weeks, while others believe that the screw does not affect the function of the ankle and it can be removed after six months (7, 16, 20, 24, 29, 30). We removed the entire osteosynthetic material at once, six months after the operation.

After the surgery, we placed plaster immobilization and we ordered the exemption from loading for up to 6 weeks, which is the attitude of other authors as well (19, 20, 21).

Complications in treatment of the ankle joint fracture are possible, and they depend on the range of soft tissue injuries, the type of fracture and the degree of anatomical reduction. In our series we have had the following complications: synostasis, talar instability, screw rupture, postoperative wound moisturizing, displacement of the fibula fracture and medial malleolus and posttraumatic arthrosis. Other authors have listed complications as well. Bosman (29) lists postoperative displacement of the fibula fracture in 9 (8.8%) and postoperative wound moisturizing in 6 (5.9%) out of 102 patients. Yablon et al. (24) in a study of 53 patients list infection in 2, and painful contact between talus and lateral malleous, the so-called impingement, in 3 patients. They also list talar instability which is a predisposition for degenerative arthritis. Robert and Scott (19) list that post traumatic arthrosis of the ankle becomes clinically evident within one year of the injury.

Phillips et al. (21) list that synostosis of distal tibiofibular syndesmosis was detected on the follow-up radiographs of 7 (5.1%) and degenerative changes after one year follow-up of 27 (19.6%) of the 138 patients.

Conclusion

We conclude that the basis of these studies is that the lateral malleolus and tibiofibular syndesmosis is the key to the anatomical reduction of displaced fractures, and that restoring the integrity of the lateral malleolus establishes stability of the ankle.

Acknowledgment

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PRELOMI FIBULE IZNAD DONJE TIBIOFIBULARNE SINDESMOZE

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Prelomi skočnog zgloba su česti u svakodnevnom životu. Prema Lauge-Hansen klasifikaciji, postoji pet tipova preloma skočnog zgloba. Kod određenih tipova preloma nastaju povreda tibiofibularne sindesmoze, prelom fibule iznad sindesmoze i prelom medijalnog maleolusa ili kidanje deltoidnog ligamenta i to: supinaciono-everzioni (SE), pronaciono-everzioni (PE) i pronaciono-abdukcioni (PA) tip. Povreda sindesmoze dovodi do nestabilnosti skočnog zgloba, jer je ona, sa lateralnim maleolusom i zadnjim rubom tibije, njegov ključ stabilnosti.

Prikazujemo grupu od 46 ispitanika, koja je lečena na Ortopetskoj klinici u Nišu, zbog preloma skočnog zgloba i kidanja distalne tibiofibularne sindesmoze. Svi su operisani neposredno posle povređivanja, u proseku 28 časova nakon povrede, kada je urađena osteosinteza maleolusa i transfiksacija sindesmoze spongioznim šrafom koji se postavlja iznad nje.

Postoperativno smo postavljali gipsanu longetu koja je nošena 3 nedelje, a zabrana oslonca na operisanu nogu traje do 6 nedelja. Posle skidanja gipsa, svi ispitanici bili su uključeni u fizikalni tretman. Osteosintetski materijal vađen je posle 6 meseci.

Rezultate lečenja procenjivali smo prema Olerud-Molander klasifikaciji. Praćeni su subjektivni i objektivni znaci i dobili smo sledeće rezultate: u grupi odličnih i dobrih rezultata bilo je 34 ispitanika (73,9%), u grupi zadovoljavajućih rezultata bilo je 7 ispitanika (15,2%) i u grupi loših rezultata bilo je 5 ispitanika (10,9%).

Zaključak je da su lateralni maleolus i tibiofibularna sindesmoza ključ stabilnosti skočnog zgloba.

Acta Medica Medianae 2019;58(4):165-171.

Ključne reči: *tibiofibularna sindesmoza, prelom fibule, osteosinteza, transfiksacija sindesmoze*

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