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ЧАСОПИС СРПСКОГ ЛЕКАРСКОГ ДРУШТВА ОСНОВАН 1872. ГОДИНЕ

# ЗА ЦЕЛОКУПНО ЛЕКАРСТВО

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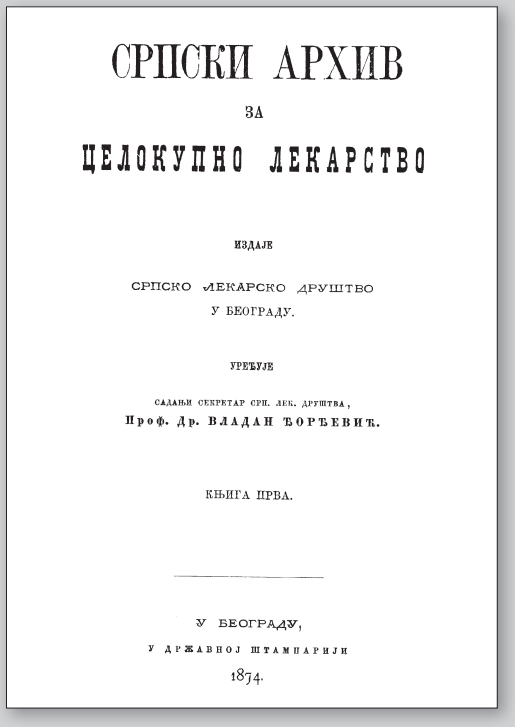

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ЗА

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У БЕОГРАДУ.

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1874.

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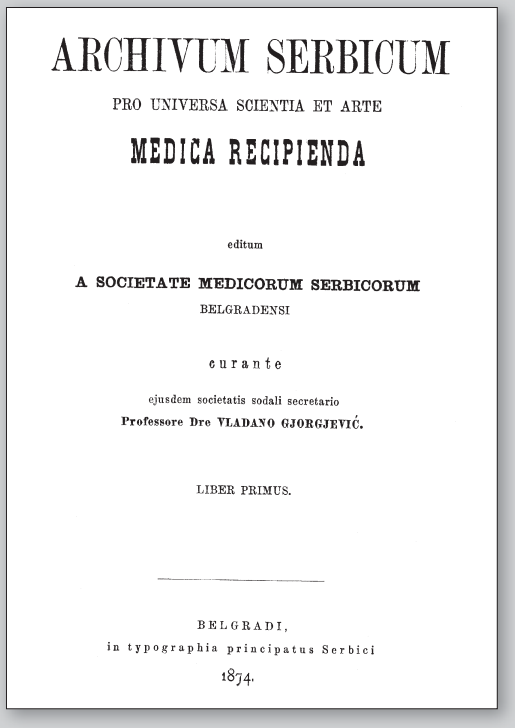

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# The effect of recasting on biological properties of Ni-Cr dental alloy

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**SUMMARY**

**Introduction** Increases in market prices of gold over the last 20 years have led to expansion of basic dental alloys, which, primarily due to their good mechanical properties and acceptable prices, have found their place in everyday dental practice. However, within the procedure of making dental prosthetic restorations, the alloys are melted and cast, which leads to changes in their physical, mechanical and biological properties.

**Objective** The objective of the study was to test biocompatibility of a Ni-Cr dental alloy (WIRON 99) depending on the number of melting and casting processes.

**Methods** The working method included the testing of cytotoxicity of the alloy obtained by casting after one, after four, and after eight successive processes of melting. Cytotoxicity of samples was tested by means of a 24-hour and a three-day cytotoxicity test, done on L929 fibroblasts.

**Results** A repeatedly melted and cast alloy shows a reduced biocompatibility and causes specific responses of the tissues in the surrounding area. Since the cytotoxic effect is more significant in the extended contact with the culture cells, a three-day cytotoxicity test showed discrete changes which were the indicator of cell growth inhibition in the cell culture.

**Conclusion** The obtained results confirm the working hypothesis that repeated alloy melting and casting will decrease biocompatibility of dental alloys and will lead to specific responses of the tissue in the surrounding area.

**Keywords:** biocompatibility; dental alloys; cytotoxicity

## INTRODUCTION

Despite many weaknesses, metals are irreplaceable constructive materials in everyday dental practice. Metals are also present in all living organisms and have significant roles – they are structural elements, stabilizers of biological structures, components of control mechanisms in nerves and muscles, and above all they are enzyme activators and redox-system components. The essential metals are those metals that are necessary for human life, and without which there is no normal cell functioning. Metal cations, with their electrophilicity, stabilize all electronrich functional groups thus maintaining the stability of molecules (DNA, RNA, structural proteins, enzymes, hormones, receptors), and due to their chemical potency they are often in the center of activities of cell processes. Other metals, which are not essential, can cause an increase in toxic manifestations even in case their intake is only slightly higher than that in the natural surroundings. A cation is a toxicity carrier and a compound or an element in the given environment will be as much toxic as the amount of cations chemically released. A cation reacts with molecules in the bio-environment, i.e. with certain functional groups towards which it shows affinity (carboxylic, amino, hydroxyl, sulphhydryl, imidazole functional group, etc.) [1, 2].

One of the mechanisms for revealing toxicity of metals is by explaining their interaction with bioelements. Cations that enter this interaction are similar enough to a certain bioelement, with regard to their physical and chemical properties, and therefore can come into reaction with a bio-ligand instead of it, and yet they are different enough to lead to weakening or ending the function of the cell process in which they are involved. However, cations can react even in situations where metals, i.e. bioelements do not have a specific role, if the appropriate chemical conditions have been provided. These reactions are not usually in function of the cell system and they cause disorders [3].

Once absorbed, the metal remains in the organism until being excreted. In contrast to the majority of organic compounds which are eliminated from the tissue by means of metabolic degradation, metals are indestructible as elements and therefore they have the power of cumulation, which leads to the occurrence of chronic effects in the organism. Excretion is the only way of elimination of metals from the organism. Accumulation in tissues does not always necessarily result in a toxic effect (e.g. lead is deposited in bones in an inert form).

Microscopic observations of thus formed metal materials reveal their microstructural texture. Microstructure determines mechanical, chemical and other properties of dental materials. Microstructure, which is seen through the microscope on the polished and etched surface, is composed of elements. When

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making castings of precious dental alloys, the aim is to achieve a fine grained microstructure [4].

Ageing is a universal process. Ageing of materials in dentistry can be defined as fatigue of materials, hardening, corrosion, degradation, sorption, and deformation. During the process of definite formation, constructive elements are exposed to some changes, either in the laboratory (*in vitro*) or in the mouth (*in vivo*). Those changes can be spontaneous or deliberately caused in order to improve physical and chemical properties or to passivate the surfaces. Over time, in the mouth, changes in temperature or the pH value, the effect of mastication and other factors may result in changes in constructive materials. Understanding the ageing of materials requires knowledge of the theory of ageing, which takes place in multi-phase solutions. The consequence of changes in the constructive material itself can be dissolution of the material, release of ions or another way in which the material influences the surrounding tissue, either causing or not causing the reaction [5].

Metal alloys are used in fabrication of numerous fixed restorations and orthodontic appliances. Each alloy is accompanied by the information on its exact composition provided by the manufacturer. In everyday dental practice, it is usual that, due to economic reasons, a previously recast alloy is not thrown away, but 50% of the new (not recast) alloy is added to it. This can lead to serious changes in microstructure and consequently to changes of the biological values of the dental alloy. The matter of the precise chemical composition of castings obtained in this way still remains unclear. This generates another, more important issue – what are the biological properties of castings obtained in this manner? An important fact is that prosthetic restorations remain in the patient's mouth for many years and therefore it is crucial to examine the biocompatibility of dental alloys.

## OBJECTIVE

The aim of this study was to examine biocompatibility of one commonly used Ni-Cr dental alloy depending on the number of melting and casting processes. The starting point in this study was the working hypothesis that repeatedly melted and cast alloy changes its chemical composition, which affects physical and mechanical properties of the alloy. Modified physical and mechanical properties have an adverse effect on the surrounding area where the dental restoration is placed and this further leads to unfavorable reactions and brings into question the biological quality of such a restoration.

## METHODS

A dental alloy used in this research has a wide variety of applications in dental practice. The experiment included several *in vitro* tests.

Test samples of dental Ni-Cr alloy were prepared in the dental-technical laboratory of the Clinic for Dental Prosthetics, Faculty of Dental Medicine in Belgrade. Composition of the alloy was as follows: Ni – 65%; Cr – 22.5%; Mo – 9.5%; Nb, Si, Fe, Cr, C. Alloy samples were shaped as discs, 5 mm in diameter and 1 mm thick, and 10 mm in diameter and 1 mm thick. All samples were cast in the induction appliance for dental alloy casting. The number of alloy samples and controls was six.

The samples of the tested dental alloy were marked as follows: W1 – the alloy melted once in the induction appliance and cast in the dental-technical laboratory; W4 – the alloy melted four times in the induction appliance and cast in the dental-technical laboratory; W8 – the alloy melted eight times in the induction appliance and cast in the dental-technical laboratory.

For the cell culture, a complete culture medium was used, which contained RPMI 1640 medium (Sigma-Aldrich, Hamburg, Germany), 10% fetal veal serum (Galenika a.d., Belgrade, Serbia), 2 mM glutamine (Sigma-Aldrich), 100 IU/ml penicillin (Galenika a.d.) and 0.5% streptomycin (Galenika a.d.). Fibroblast cells (NCTC, clone L929) originating from the adipose mouse tissue were used. The cells were obtained from Hammersmith Hospital (London, U.K., Department of Immunology).

As a negative control, a material that does not cause a cytotoxic reaction was used – glass slides for histological preparations, cut with a diamond needle, 5 mm in diameter and 1 mm thick.

As a positive control, a preparation confirmed to lead to cytotoxic changes was applied – a sterile water solution of phenol in 4% concentration, which was applied on a sterile filter of 5 mm in diameter.

As another negative control in the experiment, a culture of fibroblast cells was used without any test material.

Cytotoxicity tests were done in a standard way, and thereafter the qualitative and quantitative test results were interpreted after a 24-hour and a three-day cytotoxicity test.

### Qualitative test for estimation of cytotoxicity

A cytotoxic effect was estimated on the basis of morphological characteristics of cell cultures, using the ISO 10993-5:1992 qualitative test [6]. Degenerative changes, such as cell vacuolization, cell fragmentation and lysis, detaching of cells from the base, changes in the cell volume, were analyzed and recorded. Cells around the placed implant were separately analyzed (sample test). The results were interpreted in such a way that the changes in normal morphological characteristics of cells were numerically expressed with the following index of changes: 0 – no changes; 1 – mild changes; 2 – moderate changes; 3 – serious changes.

### Quantitative test for estimation of cytotoxicity

The percentage of necrotic cells in the culture (percentage of cytotoxicity) was used to define the cytotoxicity ISO 7405:1997 [7]. Corresponding indexes were calculated by means of numerical indicators and on the basis of this calculation the results were interpreted as follows: index

0 – no cytotoxic effect; index 1 – mild cytotoxicity; index 2 and 3 – moderate cytotoxicity; index 4 and 5 – serious cytotoxicity.

## RESULTS

### Effect of dental alloy samples on the fibroblast L929 cell culture in a 24-hour test

The first aim of the *in vitro* testing was to check a possible cytotoxic effect of dental alloys on the fibroblast cell line (L929) in the culture. The experiments were done observing corresponding ISO standards for testing cytotoxicity.

### Results of the qualitative test

The results showed that none of the tested samples of the dental alloy, either once or multiple times cast, demonstrated a detectable cytotoxic effect. The index of changes in the cell cultures in the presence of dental alloys and negative controls (glass or the culture of L929 cells which was cultivated only in presence of the medium) was marked as 0. As it was expected, a positive control (a filter paper sample soaked in phenol) exhibited the full cytotoxicity (cytotoxicity index 3).

Results of testing negative control cultures showed a normal morphology of L929 cells, which in a confluent stage of growth exhibited a typical mosaic or cobble pattern. The cells were in close contact with each other, often having a polygonal form or, less frequently, a slightly longitudinal racket-like form. Around the control glass sample (Figure 1), no detectable changes were seen in the morphology of cells in comparison with the cells which were not in immediate contact with the sample.

Figure 2 displays a typical appearance of positive control cultures, which exhibited a maximum cytotoxic effect. The cells within the whole basin area of the cultivation plate demonstrated an altered appearance, such as reduced volume, detachment from the base, fragmentation of cells or their nuclei, and presence of distinct degenerative changes.

In all cultures with dental alloys, either in immediate contact or in zones away from the placed samples, no changes which could differ from the negative controls were noticed.

### Results of the quantitative test

Since in cell cultures growing confluently a certain number of cells die spontaneously and nonviable cells can be present, it was necessary for the results of the qualitative analysis to be confirmed by quantitative tests. The results shown in Table 1 indicate that the percentage of nonviable (necrotic) cells in the cultures with dental alloys was very low and ranged within 2.5 ± 0.5% (sample W1). These values were not statistically significantly different from those of the negative control (the cells in the medium: 2 ± 0.6%; control glass sample: 2.7 ± 0.5%). As expected, cytotoxicity in the cultures of the positive control was 100% (all cells were necrotic).

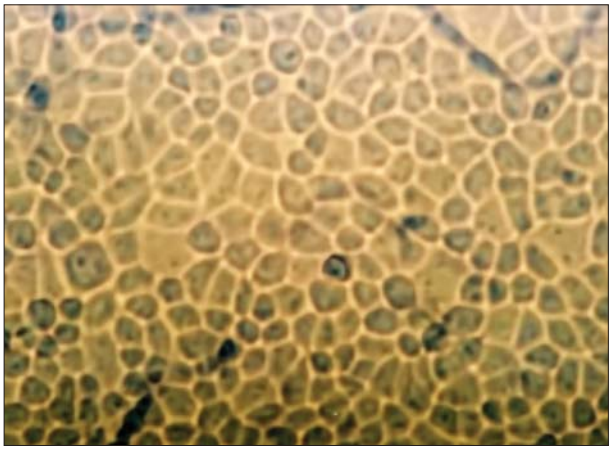

**Figure 1.** Appearance of L929 cells cultivated for 24 hours in the presence of the control sample (glass); normal morphology of the cells can be noticed (×640)

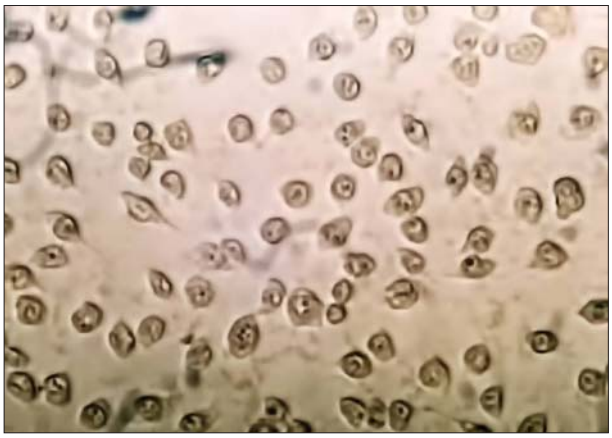

**Figure 2.** Appearance of L929 cells cultivated for 24 hours in the presence of the positive control; remarkable cytotoxicity is noticed; the index of changes is marked as 3 (×640)

**Table 1.** Effect of dental alloys on fibroblast L929 cells in the 24-hour culture (quantitative test)

| Type of sample | Percentage of necrotic cells ± SD |
|---|---|
| Negative control (medium) | 2.0 ± 0.6 |
| Negative control (glass) | 2.7 ± 0.5 |
| Positive control | 100 ± 0 |
| W1 | 2.5 ± 0.5 |
| W4 | 3 ± 0.9 |
| W8 | 3.3 ± 0.9 |

Degree of cytotoxicity (cell necrosis) was determined on the basis of ISO-7405:1997.

### Effect of dental alloy samples on L929 cell culture in the three-day test

Since cytotoxic effect of dental alloys is more expressed in extended contact with the tested cells, in the experiments which followed we modified the previous two tests by placing the samples of dental alloys on semi-confluent layers of fibroblast L929, after which the cultivation of cells was performed for three days.

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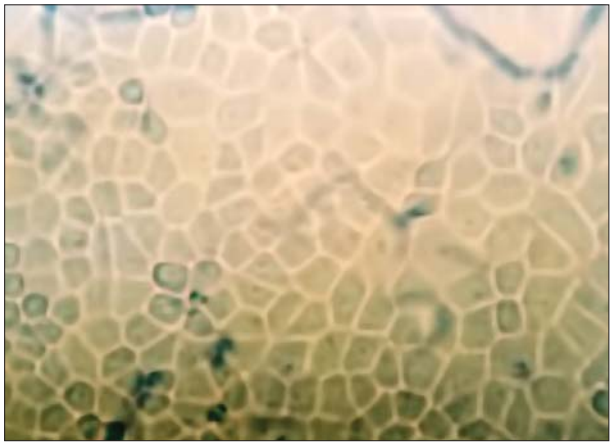

**Figure 3.** Appearance of L929 cells cultivated for three days in the presence of the Wiron 99 alloy (W4); normal cell morphology is noticed, including a confluent growth as well (×640)

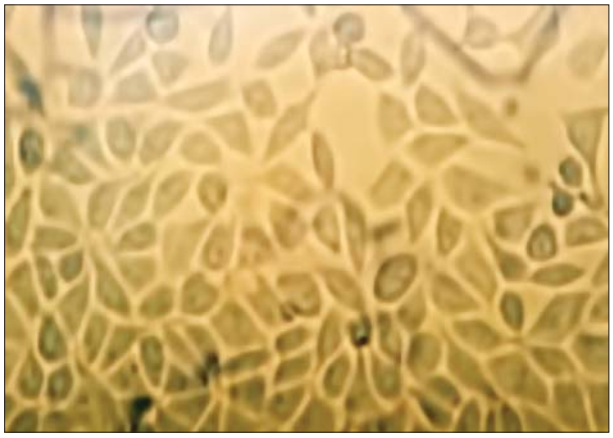

**Figure 4.** Appearance of L929 cells cultivated for three days in the presence of the Wiron 99 alloy; cell morphology is noticed as normal, but in certain smaller zones next to the explant the cells are of an elongated, racket-like and fibroblastoid form (×640)

**Table 2.** Effect of dental alloys on fibroblast L929 cells in the three-day culture (quantitative test)

| Type of sample | Percentage of necrotic cells ± SD |
|---|---|
| Negative control (medium) | 1.8 ± 1.0 |
| Negative control (glass) | 8.2 ± 2.3 |
| Positive control | 100 ± 0 |
| W1 | 8.3 ± 2.6 |
| W4 | 9.5 ± 1.6 |
| W8 | 10 ± 1.7 |

Degree of cytotoxicity (cell necrosis) was determined by means of trypan blue dye

### Results of the qualitative test

In the negative control (glass) cultures, no morphological changes in L929 cells were noticed, either along the edge of the preparation or along the whole basin surface of the cultivation plate. The appearance of the cells was very similar to the control cultures (medium control). The cell morphology was similar to that of cells in the negative control of 24-hour cultures (Figures 1 and 2), except for periodically present oval-shaped cells which were leaning on the adherent cells. This finding was typical for the long-therm tests. Only individual cells in these cultures exhibited degenerative changes in a sense of cytoplasmic vacuolization or volume reduction.

The cultures of Wiron 99 (BEGO GmbH & Co. KG, Bremen, Germany) (W1, W4, W8) exhibited changes which were either discrete (mildly inhibited growth around the alloy samples) or they did not differ from the negative controls (Figures 3 and 4). However, the index of changes for all cultures with dental alloys and for negative controls was marked 0.

### Results of the quantitative test

The quantitative test results are given in Table 2. The percentage of necrotic cells (8.2 ± 2.3%) was significantly higher in the control sample (glass) than in the control without a sample (medium control), where the basal cytotoxicity was 1.8 ± 1.0%. In the cultures with the Wiron 99 samples, no statistically significant changes in percentage of necrotic cells were noticed in comparison to the control (glass). Although the percentage of necrotic cells in the cultures with multiple recasting (W4 and W8) was slightly higher than the one relating to once-cast alloy samples (W1), the differences among them were not statistically significant.

## DISCUSSION

The oral cavity is a complex and dynamic environment where constructive dental materials should endure prolonged contact with the saliva (electrolyte), extended pressure of chewing, and contact with various chemical agents originating from food. Normally, every material in such conditions has to undergo some changes in its chemical composition and structure.

During fabrication, dental alloys are thermally treated and enriched with various elements that improve their mechanical properties but also significantly influence the reduction in biocompatibility. As indicators of biocompatibility, the responses of the surrounding tissues (cell viability, changes in cell morphology, changes in cell metabolic activities, etc.) were observed. The cytotoxicity tests which were performed in this study are very reliable [8–11].

In this research, the cytotoxicity of a Ni-Cr dental alloy was tested. Reference data indicate the disagreement in attitudes among different authors concerning the issue of possible repeated casting of these alloys [12–17]. Knowing the procedure of fabrication of dental restorations, the question which arises is whether the use of previous casting residues is harmful. In practice, up to 50% of an alloy which has not been recast is added to such residues and this mixture is used again in fabrication of dental restorations. It is obvious how important it is to find out whether recasting of the alloy will change its biocompatibility [18–23]. In this research, cytotoxicity was observed after the first, fourth, and eighth recasting of the alloy.

Degradation of the material is the main limiting factor in selection and use of a certain dental alloy and it directly influences the biocompatibility of the alloy. Corrosion is the most common cause of degradation of dental alloys.

One of the signs of corrosion can be discoloration if the outer layer of oxide, sulphide, or chloride is porous.

Many published studies describe the microstructure and properties of precious and basal alloys in their cast form and after a thermal treatment [24–28]. By artificial ageing, i.e. exposing the constructive dental alloys to alternate influences of high and low temperatures and pressures, the conditions close to clinical conditions in the mouth can be created. In this way, changes being the consequence of an ageing process are noticed faster than it is possible in the clinical conditions. A multiple remelted and recast alloy used in fabrication of fixed dental restorations and metal skeletons for partial prosthesis has accelerated ageing of the alloy as a consequence. Alterations which occur due to burning of micro constituents (deoxidants) and binding of some elements affect the alloy microstructure and are responsible for changes in mechanical and other properties of the alloy. Modified physical and mechanical properties of the alloy have an adverse effect on the environment where the dental restoration has been placed, which can cause unfavorable reactions that may affect the response of the surrounding tissue.

Results of this research concerning specific responses of the surrounding tissue, reference data, and known phenomena which are difficult to be controlled (metabolic products of bacteria, enzymes, water, dissolvents of either endogenic or exogenic origin on the one hand, and wearing, dissolving, disintegration, ageing, and degradation of constructive materials on the other hand) explicitly point out that it is best to use the alloy in its original form. This is not the cheapest, but it is the safest method of working with dental alloys.

## CONCLUSION

The obtained results confirm the working hypothesis that repeated melting and casting of alloys decreases the biocompatibility of dental alloys. However, reduced biocompatibility is not within the limits of statistical significance and thus all the changes do not have a cytotoxic effect.

Correlation between the number of melting and casting processes and the degree of cytotoxicity was proved. There were some degenerative changes in cell morphology, modified shape of cells and nonconfluent cell growth. Those changes were in direct proportion to the number of recastings, and were more pronounced at the three-day cytotoxicity test due to the prolonged contact between the examined samples and cells in the media.

It is well known from daily practice, as well as from literature, that dental alloys containing nickel represent potential threat to the health of patients and dental technicians. Recommendation of the authors of this paper is to use base dental alloys that do not contain nickel.

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# Ефекат претапања на биолошка својства *Ni-Cr* денталне легуре

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**КРАТАК САДРЖАЈ**

**Увод** Пораст цене злата на светском тржишту у последњих 20 година довео је до експанзије базних денталних легура, које су пре свега због добрих механичких својстава и приступачних цена нашле примену у свакодневној стоматолошкој пракси. У процедури израде протетских радова легуре се топе и изливају, што доводи до промена њихових физичко-механичких и биолошких својстава.

**Циљ рада** Циљ рада био је испитивање биокомпатибилности једне *Ni-Cr* легуре (*WIRON* 99) у зависности од броја топљења и ливења.

**Методе рада** У раду је испитивана цитотоксичност легуре добијене изливањем после једног топљења, после четири и после осам узастопних топљења. Цитотоксичност узорака је испитивана помоћу 24-часовног и тродневног теста цитотоксичности на *L*929 фибробластима.

**Резултати** Више пута топљена и ливена легура показала је смањену биокомпатибилност, доводећи до специфичних одговора ткива у непосредном окружењу. Будући да је цитотоксични ефекат израженији у продуженом контакту са ћелијама из културе, тродневни тест цитотоксичности је показао дискретне промене које су показатељ инхибиције раста ћелија у култури.

**Закључак** Добијени резултати потврђују радну хипотезу да више пута понављана топљења и ливења легура смањују биокомпатибилност денталних легура и доводе до специфичних одговора ткива у непосредном окружењу.

**Кључне речи:** биокомпатибилност; денталне легуре; цитотоксичност

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# Self-perception and satisfaction with dental appearance and aesthetics with respect to patients' age, gender, and level of education

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**SUMMARY**

**Introduction** Patient's subjective evaluation of dental appearance and aesthetics is becoming an increasingly important factor in aesthetic treatments and prosthetic therapy.

**Objective** The aim of this study was to investigate the influence of age, education level, gender, and different dental status and the appearance of the upper anterior teeth (color, size, shape, position and alignment of the anterior teeth) on the satisfaction of the respondents with dental appearance and aesthetics of their upper anterior teeth and their desire for improvement.

**Methods** The study encompassed 480 people aged 20 to 50 years with an average age of 30.84 years. There were 236 male and 244 female subjects. The respondents were interviewed using a questionnaire specially designed for the purpose of this research. For the study, the subjects were divided into the following three age groups: the younger age group (20–30 years of age), the middle age group (31–40 years of age), and the older age group (41–50 years of age).

**Results** The conducted study did not reveal statistical significance with respect to gender in any of the examined parameters ($p > 0.05$). A little more than one half of the respondents in each age group were satisfied with their dental appearance and aesthetics (60.3% of the respondents in the age group of 20–30 years, 55.7% in the age group of 31–40, and 53.7% in the age group of 41–50 years of age). Satisfaction with dental appearance and aesthetics increases linearly with the increase in the level of education and was the highest among the respondents with university degree (33.3%).

**Conclusion** Female respondents were more dissatisfied with their dental appearance and aesthetics as compared with male respondents, but the difference was found to be non-significant. Patients with higher education level were more satisfied with their dental appearance and aesthetics than those with lower education.

**Keywords:** dental aesthetics; dental appearance; anterior teeth; self-perception; tooth color

## INTRODUCTION

The ultimate objective of aesthetics in dentistry is the creation of a beautiful smile with teeth of pleasing proportions and pleasant mutual relations of teeth in harmony with the gingiva and the patient's face. The difference between the subjective (patient) and objective (dental) assessment of aesthetic appearance of the teeth and the degree of satisfaction represents a very important aspect of aesthetic dental medicine [1]. In everyday practice, it is of utmost importance for the dentist to obtain confirmatory information from patients in order to avoid failures of aesthetic treatments [2]. The appearance of the teeth can play a key role in developing the first impression about another person [3]. It was established that the judgment of some personal characteristics of other people is affected by dental appearance [4, 5]. Physical appearance plays a key role in social interaction and smile and teeth have an important function in determining the level of attractiveness of the face. Among other things, the mouth is considered extremely important in social interaction. Tooth color influences social perception. The results can be explained by negative beliefs about tooth decay as well as their relationship with poor oral hygiene [6]. Tooth color is the main factor associated with the satisfaction with the aesthetics of teeth. Most patients are interested in teeth whitening to improve their looks [7]. Prior to performing aesthetic treatment for tooth color, the dentist must ascertain and plan treatment to meet the expectations of the patient [8]. The use of questionnaires and written documents for ascertaining patients' expectations has been proposed. These forms should enquire about the aspect of aesthetic treatment that is important to the patient, e.g. the color, shape, alignment of the teeth, etc. [9]. Some studies indicated the correlation between dental appearance and quality of life and general health [10]. Aging is not necessarily associated with negative self-perception of dental appearance or tooth color. Greater dissatisfaction with dental appearance or color in younger ages may suggest that perceived appearance is linked to cognitive factors other than social and cultural ones [11]. The interest in dental aesthetics has increased highly over the past few decades, in both patients and dentists, and natural-looking teeth have become an important task in dentistry, especially in prosthodontics and restorative

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dentistry. In females, psychological elements are the main predictors that influence subjecting to dental treatments. Understanding the prevalence of dissatisfaction with current dental appearance and desire for treatment to improve the aesthetics can be a guide for an intervention strategy to improve the aesthetics [12]. Dental appearance satisfaction is important among young adults because judgment concerning the personal characteristics of individuals is influenced by their dental appearance in the absence of other information. It has been reported that individuals with less dental disease are judged to be more socially competent, show greater intellectual achievement and have better psychologic adjustment [4]. This is further supported by the fact that adults with visible dental problems are reluctant to seek employment because of their looks or damaged speech [3]. The knowledge and understanding of a patient's perception of dental appearance is an important aspect of patient management that can help dentists in the planning of treatment that is acceptable to the patient and achieving the highest level of patient satisfaction [13].

## OBJECTIVE

The aim of this study was to investigate the influence of age, education level, gender, and different dental status and the appearance of the upper anterior teeth (color, size, shape, the position and alignment of the front teeth) to the satisfaction of the respondents with their dental appearance and aesthetics of the upper anterior teeth and their desire for improvement.

## METHODS

The study included 480 people aged 20 to 50 years. Respondents were interviewed in an urban area – city of Novi Sad, Serbia. There were 236 male and 244 female subjects. Criteria for the selection of the sample were as follows: age (the period after completion of growth and development), eugnathic skeletal jaw relationship, harmony in the area of the face and jaw, the presence of all six anterior upper teeth, lack of temporary prosthetic restorations on anterior teeth, the absence of large abrasion on anterior teeth, the absence of diastema, the absence of fixed orthodontic appliances, unbleached teeth, non-wearing of splint for craniomandibular disorders. The respondents were interviewed using a questionnaire (Table 1) specially made for the purpose of this research. The questionnaire was divided into social part (name, gender, age, level of education, place of birth, and place of residence) and the part related to the satisfaction with dental appearance and aesthetics containing 15 questions related to the satisfaction with the color, shape, size, position and alignment of the teeth, presence of dentures, conservative restoration, orthodontic appliances, desire for a change of dental aesthetics, desire for correction of teeth alignment, and desire for artificial crown. For the study, the subjects were divided into the following three age groups: younger age group (20–30 years of age), middle age group (31–40 years of age), and older age group (41–50 years of age). The Ethics Committee of the Clinic for Dentistry of Vojvodina approved the implementation of this research. Before interviewing, each participant received information for respondents and signed a written consent. Statistical analysis was performed using statistical analysis software SPSS 22.0 (IBM Corp., Armonk, NY, USA). Statistical methods included ANOVA, LSD, and t-test.

## RESULTS

Descriptive statistics of demographic variables are shown in Table 2. Of the total of 480 respondents, 50.8% were women and 49.2% men; 60.8% of the respondents belonged to the 20–30 years age group, whereas 22.1% and 17.1% of the respondents were from the 31–40 and 41–50 years age groups, respectively. In regard to the level of education, 1% of the respondents were persons with primary school degree, 31.7% of the respondents had a secondary education degree, 26.3% were university students, 35.2%

**Table 1.** Questionnaire pertaining to self-perception and satisfaction with dental appearance and aesthetics

| | | | | | |
|---|---|---|---|---|---|
| Name and surname: | | | | | |
| Age: | | | | | |
| Gender: | | | | M | F |
| Place of birth: | | | | | |
| Place of residence: | | | | | |
| Level of education: | primary school | secondary school | student | university | master |
| Remark: the questionnaire refers to the upper anterior teeth | | | | | |
| 1. Are you satisfied with the color of your natural teeth if you have not bleached them? | | | | YES | NO |
| 2. Do you want whiter natural teeth? | | | | YES | NO |
| 3. Are you satisfied with the teeth position and alignment? | | | | YES | NO |
| 4. Are you wearing an orthodontic appliance for correcting the position of teeth? | | | | YES | NO |
| 5. Do you have a desire to correct the position of your teeth? | | | | YES | NO |
| 6. Are you satisfied with the shape of your natural teeth? | | | | YES | NO |
| 7. Are you satisfied with the size of your natural teeth? | | | | YES | NO |
| 8. Do you have the desire to change the size of your natural teeth? | | | | YES | NO |
| 9. Do you have a completely healthy upper anterior teeth without the presence of fillings or artificial dental crowns? | | | | YES | NO |
| 10. Do you have fillings in the front anterior teeth? | | | | YES | NO |
| 11. Do you have an artificial crown on the anterior upper teeth? | | | | YES | NO |
| 12. Do you have a desire for artificial dental restorations? | | | | YES | NO |
| 13. Are you satisfied with the appearance and aesthetics of your teeth? | | | | YES | NO |
| 14. Do you have a desire for aesthetic dental treatment? | | | | YES | NO |
| 15. Are you satisfied with the aesthetics of your smile? | | | | YES | NO |

**Table 2.** Distribution of the respondents according to age, gender, and level of education

| Sample | | Number of participants | Percentage of participants (%) |
|---|---|---|---|
| Total | | 480 | 100 |
| Gender | women | 244 | 50.8 |
| | men | 236 | 49.2 |
| Level of education | primary school | 5 | 1 |
| | secondary school | 152 | 31.7 |
| | student | 126 | 26.3 |
| | university | 169 | 35.2 |
| | master | 28 | 5.8 |
| Age group | 20–30 | 292 | 60.8 |
| | 31–40 | 106 | 22.1 |
| | 41–50 | 82 | 17.1 |

of the respondents had a university degree, and 5.8% were respondents with a master's degree.

The frequency of each test parameter and crosstabulations by sex is shown in Table 3. Crosstabulations related to gender show very uniform distribution among both sexes. The desire for teeth alignment was higher in women (57.3%) than in men (42.7%) as well as the desire for aesthetic dental treatment, which is also higher among women (54.8%) than in men (45.2%); however, the differences between the sexes were not statistically significant – $p > 0.05$ (Table 3).

Table 4 shows the frequency of each test parameter and crosstabulations with age. Satisfaction with tooth color was expressed by 51.7% of the respondents from the 20–30 age group, 51.9% from the 31–40 age group, and 50% from the 41–50 age group. The desire for whiter teeth was expressed by 48.3% of the respondents from the 20–30 age group, 48.1% from the age group 31–40, and 50% from the 41–50 age group. Satisfaction with dental appearance and aesthetics was recorded in 60.3% of the respondents from the 20–30 age group, 55.7% from the 31–40 age group, and 53.7% from 41–50 age group. Satisfaction with teeth position and alignment was expressed by 65.1% of the respondents from the 20–30 age group, 65.1% from the age group 31–40, and 72.2% from the 41–50 age group. High percentage of satisfaction with the shape of natural teeth was observed in all groups, with 84.6% in the 20–30 age group, 83% in the age group 31–40, and 85.4% in the 41–50 age group. High rate of satisfaction with size of natural teeth was observed in respondents from all the groups, being 86.6% in the 20–30 age group, 84% in the age group 31–40, and 92.7% in the 41–50 age group. High percentage of 'no' as an answer to the question on the desire to change the size of natural anterior teeth was recorded in all the groups, being 86.6%, 84%, and 92.7% in the 20–30, 31–40, and 41–50 age groups, respectively. The presence of artificial crowns on the anterior teeth linearly increases with the age of respondents. In the age group of 20–30, the percent-

**Table 3.** Results of frequency, crosstabulation, and significance of differences between the sexes

| Dependent variable | Sex | Frequency and crosstabulation (%) | | t-test | | | |
|---|---|---|---|---|---|---|---|
| | | | | X | Mean difference | SE | p |
| 1. Satisfaction with the color of teeth | F | Yes | 50.6 | 1.49 | 0.005 | 0.46 | 0.919 |
| | | No | 51.5 | | | | |
| | M | Yes | 49.4 | 1.48 | | | |
| | | No | 48.9 | | | | |
| 2. Desire for whiter teeth | F | Yes | 51.1 | 1.51 | -0.005 | 0.46 | 0.919 |
| | | No | 50.6 | | | | |
| | M | Yes | 48.9 | 1.52 | | | |
| | | No | 49.4 | | | | |
| 3. Satisfaction with the position and alignment of teeth | F | Yes | 49.7 | 1.35 | 0.030 | 0.43 | 0.482 |
| | | No | 53.1 | | | | |
| | M | Yes | 50.3 | 1.32 | | | |
| | | No | 46.9 | | | | |
| 4. Previous orthodontic tretment | F | Yes | 55.2 | 1.63 | -0.060 | 0.43 | 0.169 |
| | | No | 48.6 | | | | |
| | M | Yes | 44.8 | 1.69 | | | |
| | | No | 51.4 | | | | |
| 5. Desire for teeth alignment | F | Yes | 57.3 | 1.73 | -0.063 | 0.39 | 0.110 |
| | | No | 48.8 | | | | |
| | M | Yes | 42.7 | 1.79 | | | |
| | | No | 51.2 | | | | |
| 6. Intact natural upper anterior teeth | F | Yes | 51.8 | 1.45 | -0.020 | 0.46 | 0.667 |
| | | No | 49.8 | | | | |
| | M | Yes | 48.2 | 1.47 | | | |
| | | No | 50.2 | | | | |
| 7. Presence of fillings on the upper anterior teeth | F | Yes | 52.3 | 1.58 | -0.024 | 0.45 | 0.597 |
| | | No | 49.8 | | | | |
| | M | Yes | 47.7 | 1.60 | | | |
| | | No | 50.2 | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8. Presence of artificial crowns on the upper anterior teeth | F | Yes | 48.7 | 1.84 | -0.014 | 0.34 | 0.684 |
| | | No | 51.2 | | | | |
| | M | Yes | 51.3 | 1.83 | | | |
| | | No | 48.8 | | | | |
| 9. Desire for artificial crown on the upper anterior teeth | F | Yes | 50.0 | 1.75 | 0.008 | 0.40 | 0.832 |
| | | No | 51.1 | | | | |
| | M | Yes | 50.0 | 1.74 | | | |
| | | No | 48.9 | | | | |
| 10. Satisfaction with dental appearance and aesthetics | F | Yes | 49.1 | 1.44 | 0.40 | 0.45 | 0.373 |
| | | No | 53.2 | | | | |
| | M | Yes | 50.9 | 1.40 | | | |
| | | No | 46.8 | | | | |
| 11. Desire for aesthetic dental treatment | F | Yes | 54.8 | 1.49 | -0.76 | 0.46 | 0.097 |
| | | No | 47.2 | | | | |
| | M | Yes | 45.2 | 1.56 | | | |
| | | No | 52.8 | | | | |
| 12. Satisfaction with the shape of the teeth | F | Yes | 49.9 | 1.17 | 0.32 | 0.33 | 0.331 |
| | | No | 56.0 | | | | |
| | M | Yes | 50.1 | 1.14 | | | |
| | | No | 44.0 | | | | |
| 13. Satisfaction with the size of teeth | F | Yes | 50.5 | 1.14 | 0.12 | 0.31 | 0.687 |
| | | No | 53.2 | | | | |
| | M | Yes | 49.5 | 1.12 | | | |
| | | No | 46.8 | | | | |
| 14. Desire to change the size of teeth | F | Yes | 53.2 | 1.86 | -0.12 | 0.31 | 0.687 |
| | | No | 55.0 | | | | |
| | M | Yes | 46.8 | 1.88 | | | |
| | | No | 49.5 | | | | |
| 15. Satisfaction with the aesthetics of smile | F | Yes | 49.1 | 1.67 | 0.24 | 0.43 | 0.581 |
| | | No | 51.7 | | | | |
| | M | Yes | 50.9 | 1.64 | | | |
| | | No | 48.3 | | | | |

X – mean; SE – standard error; p – value

**Table 4.** Results of frequency, crosstabulation, ANOVA test, and LSD test in relation to the age group

| Dependent variable | Frequency and Crosstabulation | | | | | ANOVA test between groups | | LSD test multiple comparation | |
|---|---|---|---|---|---|---|---|---|---|
| | (I) Age group | Yes (%) | | No (%) | | F | Sig. | (J) Age group | Sig. |
| 1. Satisfaction with the colour of teeth | 20–30 | 51.7 | 51.5 | 48.3 | 48.5 | 0.042 | 0.959 | 31–40 | 0.976 |
| | | | | | | | | 41–50 | 0.785 |
| | 31–40 | 51.9 | | 48.1 | | | | 20–30 | 0.976 |
| | | | | | | | | 41–50 | 0.798 |
| | 41–50 | 50.0 | | 50.0 | | | | 20–30 | 0.785 |
| | | | | | | | | 31–40 | 0.798 |
| 2. Desire for whiter teeth | 20–30 | 48.3 | 48.5 | 51.7 | 51.5 | 0.042 | 0.959 | 31–40 | 0.976 |
| | | | | | | | | 41–50 | 0.785 |
| | 31–40 | 48.1 | | 51.9 | | | | 20–30 | 0.976 |
| | | | | | | | | 41–50 | 0.798 |
| | 41–50 | 50.0 | | 50.0 | | | | 20–30 | 0.785 |
| | | | | | | | | 31–40 | 0.798 |
| 3. Satisfaction with the position and alignment of teeth | 20–30 | 65.1 | 66.2 | 34.9 | 33.8 | 0.716 | 0.489 | 31–40 | 0.996 |
| | | | | | | | | 41–50 | 0.246 |
| | 31–40 | 65.1 | | 34.9 | | | | 20–30 | 0.996 |
| | | | | | | | | 41–50 | 0.325 |
| | 41–50 | 72.0 | | 28.0 | | | | 20–30 | 0.246 |
| | | | | | | | | 31–40 | 0.325 |
| 4. Previous orthodontic tretment | 20–30 | 34.6 | 34.0 | 65.4 | 66.0 | 0.510 | 0.601 | 31–40 | 0.815 |
| | | | | | | | | 41–50 | 0.370 |
| | 31–40 | 35.8 | | 64.2 | | | | 20–30 | 0.815 |
| | | | | | | | | 41–50 | 0.346 |
| | 41–50 | 29.3 | | 70.7 | | | | 20–30 | 0.370 |
| | | | | | | | | 31–40 | 0.346 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5. Desire for teeth alignment | 20–30 | 27.4 | 24.4 | 72.6 | 75.6 | 2.434 | 0.089 | 31–40 | 0.328 |
| | | | | | | | | 41–50 | 0.032* |
| | 31–40 | 22.6 | | 77.4 | | | | 20–30 | 0.328 |
| | | | | | | | | 41–50 | 0.282 |
| | 41–50 | 15.9 | | 84.1 | | | | 20–30 | 0.032* |
| | | | | | | | | 31–40 | 0.282 |
| 6. Intact natural upper anterior teeth | 20–30 | 56.8 | 53.5 | 43.2 | 46.5 | 1.661 | 0.191 | 31–40 | 0.169 |
| | | | | | | | | 41–50 | 0.137 |
| | 31–40 | 49.1 | | 50.9 | | | | 20–30 | 0.169 |
| | | | | | | | | 41–50 | 0.838 |
| | 41–50 | 47.6 | | 52.4 | | | | 20–30 | 0.137 |
| | | | | | | | | 31–40 | 0.838 |
| 7. Presence of fillings on the upper anterior teeth | 20–30 | 38.0 | 41.0 | 62.0 | 59.0 | 1.691 | 0.185 | 31–40 | 0.335 |
| | | | | | | | | 41–50 | 0.080 |
| | 31–40 | 43.4 | | 56.6 | | | | 20–30 | 0.335 |
| | | | | | | | | 41–50 | 0.457 |
| | 41–50 | 48.8 | | 51.2 | | | | 20–30 | 0.080 |
| | | | | | | | | 31–40 | 0.457 |
| 8. Presence of artificial crowns on the anterior frontal teeth | 20–30 | 9.9 | 16.2 | 90.1 | 83.8 | 14.161 | 0.000* | 31–40 | 0.008** |
| | | | | | | | | 41–50 | 0.000*** |
| | 31–40 | 20.8 | | 79.2 | | | | 20–30 | 0.008** |
| | | | | | | | | 41–50 | 0.022* |
| | 41–50 | 32.9 | | 67.1 | | | | 20–30 | 0.000*** |
| | | | | | | | | 31–40 | 0.022* |
| 9. Desire for artificial crown on the upper anterior teeth | 20–30 | 22.6 | 25.4 | 77.4 | 74.6 | 1.576 | 0.208 | 31–40 | 0.179 |
| | | | | | | | | 41–50 | 0.148 |
| | 31–40 | 29.2 | | 70.8 | | | | 20–30 | 0.179 |
| | | | | | | | | 41–50 | 0.846 |
| | 41–50 | 30.5 | | 69.5 | | | | 20–30 | 0.148 |
| | | | | | | | | 31–40 | 0.846 |
| 10. Satisfaction with dental appearance and aesthetics | 20–30 | 60.3 | 58.1 | 39.7 | 41.9 | 0.743 | 0.476 | 31–40 | 0.411 |
| | | | | | | | | 41–50 | 0.285 |
| | 31–40 | 55.7 | | 44.3 | | | | 20–30 | 0.411 |
| | | | | | | | | 41–50 | 0.783 |
| | 41–50 | 53.7 | | 46.3 | | | | 20–30 | 0.285 |
| | | | | | | | | 31–40 | 0.783 |
| 11. Desire for aestetic dental treatment | 20–30 | 45.5 | 47.5 | 54.5 | 52.5 | 1.119 | 0.328 | 31–40 | 0.775 |
| | | | | | | | | 41–50 | 0.136 |
| | 31–40 | 47.2 | | 52.8 | | | | 20–30 | 0.775 |
| | | | | | | | | 41–50 | 0.295 |
| | 41–50 | 54.9 | | 45.1 | | | | 20–30 | 0.136 |
| | | | | | | | | 31–40 | 0.295 |
| 12. Satisfaction with the shape of teeth | 20–30 | 54.9 | 84.4 | 45.1 | 15.6 | 0.109 | 0.897 | 31–40 | 0.704 |
| | | | | | | | | 41–50 | 0.865 |
| | 31–40 | 83.0 | | 17.0 | | | | 20–30 | 0.704 |
| | | | | | | | | 41–50 | 0.661 |
| | 41–50 | 85.4 | | 14.6 | | | | 20–30 | 0.865 |
| | | | | | | | | 31–40 | 0.661 |
| 13. Satisfaction with the size of teeth | 20–30 | 86.6 | 87.1 | 13.4 | 12.9 | 1.628 | 0.197 | 31–40 | 0.481 |
| | | | | | | | | 41–50 | 0.150 |
| | 31–40 | 84.0 | | 16.0 | | | | 20–30 | 0.481 |
| | | | | | | | | 41–50 | 0.078 |
| | 41–50 | 92.7 | | 7.3 | | | | 20–30 | 0.150 |
| | | | | | | | | 31–40 | 0.078 |
| 14. Desire to change the size of teeth | 20–30 | 13.4 | 12.9 | 86.6 | 87.1 | 1.628 | 0.197 | 31–40 | 0.481 |
| | | | | | | | | 41–50 | 0.150 |
| | 31–40 | 16.0 | | 84.0 | | | | 20–30 | 0.481 |
| | | | | | | | | 41–50 | 0.078 |
| | 41–50 | 7.3 | | 92.7 | | | | 20–30 | 0.150 |
| | | | | | | | | 31–40 | 0.078 |
| 15. Satisfaction with the aesthetics of smile | 20–30 | 35.3 | 34.4 | 64.7 | 65.6 | 0.551 | 0.577 | 31–40 | 0.347 |
| | | | | | | | | 41–50 | 0.826 |
| | 31–40 | 30.2 | | 69.8 | | | | 20–30 | 0.347 |
| | | | | | | | | 41–50 | 0.361 |
| | 41–50 | 36.6 | | 63.4 | | | | 20–30 | 0.826 |
| | | | | | | | | 31–40 | 0.361 |

I – age group samples; J – age group samples; Sig. – significance; F – value; LSD test – Fisher's least significant difference
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;

age was 9.9%, in the 31–40 age group it was 20.8%, and in the 41-50 age group it reached 32.9%, and the differences between the age groups are statistically significant at the level of $p < 0.05$ (Table 4). The desire for artificial crowns also linearly increases with the age of respondents, ranging from 22.6% in the 20–30 age group, to 29.2% in the 31–40 age group, to 30.5% in the 41–50 age group, but the differences between the age groups were not statistically significant – $p > 0.05$ (Table 4). Desire for teeth alignment linearly decreases with the age of respondents from 27.4% (age group 20–30), to 22.6% (age group 31–40) to 15.9% (age group 31–40). The difference between the age groups 20–30 and 41–50 was statistically significant at the significance level $p<0.05$ (Table 4). Satisfaction with aesthetics of the smile was reported by 35.3% of respondents from the age group 20–30, 30.2% of respondents from the age group 31–40 and 36.6% of respondents from the age group 41–50. The desire for aesthetic dental treatment was recorded in 45.5% patients from the age group 21–30, 47.2% from the age group 31–40, and 54.9% from the age group 41–50. The presence of fillings linearly increases with age, being 38% in the age group 20–30, 43.4% in the age group 31–40, and 48.8% in the age group 41–50. Intact teeth linearly decrease with the age of respondents, dropping from 56.8% (age group 20–30) to 49.1% (age group 31–40) and 47.6% (age group 41–50); however, the differences were not statistically significant – $p > 0.05$ (Table 4).

Satisfaction with dental appearance and aesthetics, satisfaction with teeth position and alignment, satisfaction with the shape of natural tooth, satisfaction with the size of natural teeth, satisfaction with aesthetics of a smile (Table 5) have a substantially linear rule of increasing satisfaction with the increase the level of education of responders. With respect to the level of education (Table 5), respon-

**Table 5.** Results of frequency, crosstabulation, ANOVA test, and LSD test in relation to the education level

| Dependent variable | Frequency and crosstabulation | | | ANOVA test between groups | | LSD test multiple comparation | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Education level | Yes (%) | No (%) | F | Sig. | Primary | Secondary | Student | University | Master |
| | | | | | | Sig. | | | | |
| 1. Satisfaction with the colour of teeth | primary | 0.4 | 1.7 | 1.297 | 0.270 | | 0.229 | 0.111 | 0.174 | 0.094 |
| | secondary | 29.1 | 34.3 | | | | | 0.136 | 0.529 | 0.195 |
| | student | 28.7 | 23.6 | | | | | | 0.354 | 0.676 |
| | university | 34.8 | 35.6 | | | | | | | 0.336 |
| | master | 6.9 | 4.7 | | | | | | | |
| 2. Desire for whiter teeth | primary | 1.7 | 0.4 | 1.297 | 0.270 | | 0.229 | 0.111 | 0.174 | 0.094 |
| | secondary | 34.3 | 29.1 | | | | | 0.136 | 0.529 | 0.195 |
| | student | 23.6 | 28.7 | | | | | | 0.354 | 0.676 |
| | university | 35.6 | 34.8 | | | | | | | 0.336 |
| | master | 4.7 | 6.9 | | | | | | | |
| 3. Satisfaction with the position and alignment of teeth | primary | 0.3 | 2.5 | 2.567 | 0.037* | | 0.051 | 0.043* | 0.015* | 0.016* |
| | secondary | 29.6 | 35.8 | | | | | 0.771 | 0.050 | 0.174 |
| | student | 25.2 | 28.4 | | | | | | 0.117 | 0.242 |
| | university | 38.4 | 29.0 | | | | | | | 0.770 |
| | master | 6.6 | 4.3 | | | | | | | |
| 4. Conducted orthodontic tretment | primary | 1.8 | 0.6 | 2.487 | 0.043* | | 0.131 | 0.447 | 0.190 | 0.224 |
| | secondary | 25.8 | 34.7 | | | | | 0.005** | 0.412 | 0.642 |
| | student | 33.7 | 22.4 | | | | | | 0.035* | 0.243 |
| | university | 33.1 | 36.3 | | | | | | | 0.984 |
| | master | 5.5 | 6.9 | | | | | | | |
| 5. Desire for teeth alignment | primary | 2.6 | 0.6 | 2.916 | 0.021* | | 0.110 | 0.098 | 0.034* | 0.018* |
| | secondary | 37.6 | 29.8 | | | | | 0.820 | 0.036* | 0.038* |
| | student | 29.9 | 25.1 | | | | | | 0.079 | 0.056 |
| | university | 27.4 | 37.7 | | | | | | | 0.345 |
| | master | 2.6 | 6.9 | | | | | | | |
| 6. Intact natural upper anterior teeth | primary | 0.8 | 1.3 | 1.878 | 0.113 | | 0.767 | 0.318 | 0.557 | 0.574 |
| | secondary | 27.6 | 36.3 | | | | | 0.008* | 0.240 | 0.503 |
| | student | 30.7 | 21.1 | | | | | | 0.107 | 0.380 |
| | university | 35.0 | 35.4 | | | | | | | 0.380 |
| | master | 5.8 | 5.8 | | | | | | | |
| 7. Presence of fillings on the upper anterior teeth | primary | 1.5 | 0.7 | 3.401 | 0.009** | | 0.610 | 0.158 | 0.464 | 0.305 |
| | secondary | 37.6 | 27.6 | | | | | 0.001** | 0.369 | 0.196 |
| | student | 18.3 | 31.8 | | | | | | 0.008** | 0.484 |
| | university | 37.6 | 33.6 | | | | | | | 0.417 |
| | master | 5.1 | 6.4 | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 8. Presence of artificial crowns on the upper anterior teeth | primary | 1.3 | 1.0 | 0.458 | 0.767 | | 0.772 | 0.771 | 0.949 | 0.606 |
| | secondary | 29.5 | 32.1 | | | | | 0.991 | 0.358 | 0.562 |
| | student | 24.4 | 26.6 | | | | | | 0.377 | 0.573 |
| | university | 41.0 | 34.1 | | | | | | | 0.277 |
| | master | 3.8 | 6.2 | | | | | | | |
| 9. Desire for artificial crown on the upper anterior teeth | primary | 3.3 | 0.3 | 4.145 | 0.003** | | 0.014* | 0.002** | 0.005** | 0.001** |
| | secondary | 39.3 | 29.1 | | | | | 0.024 | 0.163 | 0.019* |
| | student | 20.5 | 28.2 | | | | | | 0.323 | 0.310 |
| | university | 34.4 | 35.5 | | | | | | | 0.108 |
| | master | 2.5 | 7.0 | | | | | | | |
| 10. Satisfaction with dental appearance and aesthetics | primary | 0.4 | 2.0 | 1.760 | 0.136 | | 0.109 | 0.049* | 0.118 | 0.046* |
| | secondary | 30.5 | 33.3 | | | | | 0.159 | 0.871 | 0.239 |
| | student | 29.0 | 22.4 | | | | | | 0.111 | 0.729 |
| | university | 33.3 | 37.8 | | | | | | | 0.202 |
| | master | 6.8 | 4.5 | | | | | | | |
| 11. Desire for aestetic dental treatment | primary | 1.8 | 0.4 | 1.943 | 0.102 | | 0.176 | 0.071 | 0.217 | 0.125 |
| | secondary | 32.9 | 30.6 | | | | | 0.082 | 0.624 | 0.527 |
| | student | 21.5 | 30.6 | | | | | | 0.025* | 0.703 |
| | university | 38.6 | 32.1 | | | | | | | 0.365 |
| | master | 5.3 | 6.3 | | | | | | | |
| 12. Satisfaction with the shape of teeth | primary | 0.2 | 5.3 | 5.215 | 0.000*** | | 0.000*** | 0.000*** | 0.000*** | 0.000*** |
| | secondary | 30.1 | 40.0 | | | | | 0.206 | 0.048* | 0.220 |
| | student | 26.7 | 24.0 | | | | | | 0.560 | 0.633 |
| | university | 36.8 | 26.7 | | | | | | | 0.878 |
| | master | 6.2 | 4.0 | | | | | | | |
| 13. Satisfaction with the size of teeth | primary | 0.2 | 6.5 | 9.318 | 0.000*** | | 0.000*** | 0.000*** | 0.000*** | 0.000*** |
| | secondary | 28.9 | 50.0 | | | | | 0.018* | 0.000*** | 0.048* |
| | student | 26.8 | 22.6 | | | | | | 0.229 | 0.559 |
| | university | 37.8 | 17.7 | | | | | | | 0.924 |
| | master | 6.2 | 3.2 | | | | | | | |
| 14. Desire to change the size of teeth | primary | 6.5 | 0.2 | 9.318 | 0.000*** | | 0.000*** | 0.000*** | 0.000*** | 0.000*** |
| | secondary | 50.0 | 28.9 | | | | | 0.018* | 0.000*** | 0.048* |
| | student | 22.6 | 26.8 | | | | | | 0.229 | 0.559 |
| | university | 17.7 | 37.8 | | | | | | | 0.924 |
| | master | 3.2 | 6.2 | | | | | | | |
| 15. Satisfaction with the aesthetics of smile | primary | 0.0 | 1.6 | 1.985 | 0.096 | | 0.160 | 0.061 | 0.131 | 0.044* |
| | secondary | 27.9 | 33.7 | | | | | 0.074 | 0.667 | 0.098 |
| | student | 30.9 | 23.8 | | | | | | 0.155 | 0.548 |
| | university | 33.3 | 36.2 | | | | | | | 0.151 |
| | master | 7.9 | 4.8 | | | | | | | |

Sig. – significance; F – value; LSD test – Fisher's least significant difference
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

dents with university degree reported greatest satisfaction with their dental appearance and aesthetics (33.3%), satisfaction with teeth position and alignment (38.4%), satisfaction with the shape of natural teeth (36.8%), satisfaction with the size of natural teeth (37.8%), satisfaction with the aesthetics of smile (33.3%), and satisfaction with tooth color (34.8%). The intact anterior teeth also showed significant increasing pattern with the increase in the level of education (Table 5), with the highest percentage in respondents with a university degree (35%). University students (Table 5) had the lowest percentage of fillings on anterior teeth (18.3%). As compared with other groups, the respondents with a university degree (Table 5) had the most 'no' answers related to the desire for teeth alignment (37.7%), desire for teeth size change (37.8%), desire for whiter teeth (34.8%), desire for artificial crown (35.5%). The greatest desire for artificial crowns (Table 5) was expressed by respondents with secondary education degree (39.3%). Previous orthodontic treatment (Table 5) was reported by only one quarter of the total number of respondents, mostly students (33.7%), as compared with all the other groups.

## DISCUSSION

Attitudes toward the importance of our dental appearance and aesthetics have shown rapid changes over the past decades. Patient's subjective evaluation and satisfaction with dental appearance and aesthetics is becoming more important factor in aesthetic treatments, restorative procedures, and prosthetic therapy. This is of great importance for a

predictable transition between initial contact insertion to definitive restoration in the therapeutic procedure.

In this study, satisfaction with dental appearance and aesthetics was expressed by 58.1% of respondents from Novi Sad, Serbia (Table 4). This result is similar to data reported by Samorodnitzky-Naveh et al. [7] in Israel (62.7%), Tin-Oo et al. [13] in Malaysia (47.2%), Akarslan et al. [14] in Turkey (57.3%), Lajnert et al. [15] in Croatia (43%), but this percentage is lower than that obtained by Azodo et al. [16] in the study of young adults in Nigeria (79.4%), Alkhatib et al. [11, 17] in the United Kingdom (75%), and Meng et al. [18] in Florida (76%).

Many factors are important for subjective evaluation of dental appearance [5]. Individuals exhibit varying degrees of sensitivity to certain esthetic issues [19]. The results of this study can be explained by the fact that the standard of beauty differs between people of different race, place of residence and period in which the research is being conducted. All this can result in variations in the self-perception and subjective evaluation of dental appearance and aesthetics.

According to the results of Akarslan et al. [14], 55.1% of respondents in Turkey were dissatisfied with the color of their teeth. Study from the United States showed that 34% of adults were dissatisfied with their tooth color [20]. It was reported that 31.6% of the participants in a study conducted in North America and 52.6% in China were dissatisfied with their tooth color [21, 22]. Samorodnitzky-Naveh et al. [7] concluded that 37.3% of respondents in Israel were dissatisfied with their dental appearance and the color of the teeth was the main reason for dissatisfaction in 89.3% of participants. Of the total number of respondents dissatisfied with their tooth color, 88.2% of participants said that they would undergo the procedure of teeth whitening. Similar to the results of previous authors and according to our results, 48.5% of participants were dissatisfied with the color of their teeth (51.1% female and 48.9% male respondents), whereas 48.3% of respondents from the age group 20–30, 48.1% of respondents from the age group 31–40, and 50.1% of respondents from the age group 41–50 desired whiter teeth.

It is commonly considered that women are more interested in their appearance than men. This agrees well with the idea that physical injury affects women's self-esteem more than men's. The conducted study did not reveal statistical significance with respect to gender in any of the examined parameters but female participants were more dissatisfied with their dental appearance and aesthetics (53.2%) as compared with male ones (46.8%). The desire for teeth alignment is higher among women, being 57.3%, compared to 42.7% in men. Desire for aesthetic dental treatment is also higher in women with 54.8%, compared to 45.2% in men. The results of our research are similar to study of Akarslan et al. [14] from Turkey, who established that females were more dissatisfied with the general appearance of their teeth (43%) as compared with males (41.7%), but the difference was found to be non-significant. Tin-Oo et al. [13] reported that dissatisfaction with general dental appearance was more common in females (79.8%) than in males (20.2%) and differed significantly. Vallittu et al. [23] reported similar results from Eastern Finland. Samorodnitzky-Naveh et al. [7] reported that females (65.4%) were more satisfied with the general appearance of their teeth than males (59.8%) in Israel; however, the sample consisted of more males than females as the participants were selected from patients attending a military clinic. According to Wolfart et al. [24], the degree of satisfaction concerning appearance of anterior incisors in accordance with golden standard values is higher for men than for woman.

According to Vallittu et al. [23], the perception that very white teeth are beautiful decreased with age and young patients expressed greater preference for white teeth than older ones [23]. In the study of Meng et al. [18], 75% of older respondents were satisfied with their dental appearance. Satisfaction with dental appearance and color of teeth was established by Lajnert et al. [15] in 80% of Croatian population, as well as by Alkhatib at al. [11] in 80.3% of the respondents from the 55+ age group in the United Kingdom. According to Alkhatib et al. [11], age had an impact on dissatisfaction with dental aesthetics; they also showed that older people in the United Kingdom were more satisfied with their dental appearance. These findings show a certain degree of agreement with the descriptive outcome of the study of Akarslan et al. [14]. In the present study, satisfaction with dental appearance and aesthetics was expressed by 60.3% of respondents from the 20–30 age group, 55.7% from the age group 31–40, and 53.7% from the 41–50 years of age group. According to our results and the results of the mentioned authors, the age is not necessarily associated with dissatisfaction with dental appearance and aesthetics. Although the dental aesthetic appearance gets worse with age, the level of acceptability of such changes by the elderly is significantly higher than in younger patients. For older patients, the appearance of teeth was not as important as for younger patients. This finding may be due to more advanced cognition in older age which may override effects of cultural or behavioural factors thought to influence self-perceived appearance.

In the present study, respondents with high education levels were more satisfied with their dental appearance and aesthetics than those with lower levels of education. The intact anterior teeth showed significant increasing pattern with the increase in education level. Respondents with a university degree had the most 'no' answers (compared to other groups) related to the desire for teeth alignment, desire for resizing of teeth, desire for whiter teeth, and desire for artificial crown. Respondents with high education levels were more satisfied with the color of the teeth and had no desire for whiter teeth than those with lower levels of education according to studies of Xiao et al. [22] and Akarslan et al. [14]. These findings suggest that higher self-satisfaction with the aesthetics of teeth observed in respondents with higher academic titles may reflect more self-esteem of these respondents. The study of Tin-Oo et al. [13] revealed that satisfaction with tooth shade or general dental aesthetic was not related with educational level of the respondents.

## CONCLUSION

Dentists can expect differences in satisfaction with dental appearance and aesthetics depending on the age, gender, and level of education of the patients. The results of this study suggest that dental appearance and aesthetics might be more important for women than for men, with the difference being minor. The age is not necessarily associated with dissatisfaction with dental appearance and aesthetics even though dental appearance deteriorates with age. Respondents with high education levels were more satisfied with their dental appearance and aesthetics than those with lower levels of education. The varying attitudes toward dental appearance and aesthetics must be acknowledged in treatment decisions. Understanding the prevalence of satisfaction with present dental appearance and desired treatments for the improvement of their aesthetics can guide the dentists in planning intervention strategies to improve esthetics. Close communication between the patient and dentist is required when aesthetic restorative procedures of upper front teeth are planned. When planning aesthetic treatments and therapies, the dentist should take into consideration patients' subjective evaluation of the aesthetics to harmonize the function, structure and biology in order to achieve the highest level of patient satisfaction. Improvement in esthetic satisfaction improved the quality of life related to oral health and its dimensions of psychological discomfort and psychological disability.

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# Субјективно вредновање и однос према денталном изгледу и естетици у односу на године старости, пол и степен образовања

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**КРАТАК САДРЖАЈ**

**Увод** Пацијентово субјективно вредновање денталног изгледа и естетике постаје све важнији фактор приликом естетских третмана и протетских терапија.

**Циљ рада** Циљ истраживања био је да истражи утицај година старости, нивоа образовања, пола испитаника и различити зубни статус и изглед горњих предњих зуба (боја, величина, облик, поредак предњих зуба) на задовољство испитаника денталним изгледом и естетиком горњих предњих зуба и постојање жеље за унапређењем денталне естетике.

**Методе рада** Истраживање је спроведено на 480 особа од 20 до 50 година, просечне старости 30,84 година. Било је 236 испитаника мушког пола и 244 испитаника женског пола. Испитаници су интервјуисани путем упитника специјално направљеног за потребе овог истраживања. За потребе истраживања испитаници су подељени према годинама старости у три старосне групе: млађа старосна група (20–30 година), средња старосна група (31–40 година), старија старосна група (41–50 година).

**Резултати** Спроведена студија није утврдила статистичку сигнификантност у односу на пол ни у једном од испитиваних параметара ($p > 0,05$). Половина испитаника у свакој старосној групи била је задовољна денталним изгледом и естетиком: 60,3% испитаника у старосној групи 20–30 година, 55,7% у старосној групи 31–40 година и 53,7% у старосној групи 41–50 година. Задовољство денталним изгледом и естетиком има линеарно правило пораста задовољства са порастом степена образовања и највеће је код испитаника са завршеним факултетом (33,3%).

**Закључак** Жене су биле незадовољније својим денталним изгледом и естетиком у односу са мушкарце, али разлика није статистички значајна. Пацијенти са високим степеном образовања били су задовољнији денталним изгледом и естетиком него испитаници са нижим степеном образовања.

**Кључне речи:** дентална естетика; дентални изглед; предњи зуби; субјективно вредновање; боја зуба

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# The effects of implementation of guideline-directed medical therapy on relief of angina in patients with stable coronary artery disease in Serbia

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**SUMMARY**

**Introduction** Adherence to proposed lifestyle changes and prescribed medication in patients with stable coronary artery disease (SCAD) is poor.

**Objective** We sought to investigate the influence of adjusting guideline proposed medications on relief of angina in a large group of patients with SCAD in Serbia.

**Methods** The study included a total of 3,490 patients from 15 cardiology clinics with symptoms of stable angina and at least one of the following criteria: abnormal electrocardiogram (ECG), history of myocardial infarction (MI), positive stress test, significant coronary artery disease on coronary angiogram or previous revascularization. All the patients underwent comprehensive evaluation at initial visit and after two months. The relief of angina was study end-point defined as any reduction in Canadian Cardiology Society (CCS) class, number of angina attacks per week and/or number of tablets of short-acting nitrates per week.

**Results** Most patients were included based on abnormal ECG (48.4%). At Visit 1, the average number of prescribed classes of medications to a single patient increased from 4.16 ± 1.29 to 4.63 ± 1.57 ($p < 0.001$). At the follow-up, the patients had significantly lower blood pressure (141 ± 19 / 85 ± 11 vs. 130 ± 12 / 80 ± 8 mmHg; $p < 0.001$) and most of them reported CCS class I (63.3%). The average weekly number of angina attacks was reduced from 2.82 ± 2.50 at Visit 1 to 1.72 0 ± 1.66 at Visit 2, as well as average weekly use of short-acting nitrates to treat these attacks (2.69 ± 2.53 to 1.74 ± 1.47 tablets; $p < 0.001$ for all).

**Conclusion** Adjustment of prescribed medications to guideline recommendations in a large Serbian patient population with prevalent risk factors led to significant relief of angina.

**Keywords:** stable coronary artery disease; guidelines; medical therapy; trimetazidine

## INTRODUCTION

As a consequence of high prevalence of risk factors for atherosclerosis, low income, and insufficient level of health education, ischemic heart disease is the leading cause of death in Serbian population [1]. The latest guidelines on stable coronary artery disease (SCAD) have been implemented for several years, but patients' adherence to proposed lifestyle changes, prescribed medication, and cardiovascular rehabilitation remains poor [2, 3]. Although improvement in medical treatment has been noted in large registries of patients with SCAD, there is room for further step-up [4].

Traditionally, the treatment of SCAD is based on hemodynamic agents that reduce the energy requirements of myocardial cells by lowering blood pressure and heart rate (beta blockers, calcium antagonists) or through vasodilation enhance coronary blood flow by systemic and coronary arteriolar and venous vasodilatation with consequent preload reduction (nitrates), leading to symptom relief. On the other hand, lately, along with these well established drugs, new classes of treatments with entirely (trimetazidine, ivabradine) or partly (nicorandil) different mechanisms of action have been introduced in the treatment of SCAD and supported by current guidelines

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[2]. Metabolic agents, like trimetazidine (TMZ), act as anti-ischemic medications by metabolic modulation. Angina patients accumulate free fatty acids (FFAs), which the cardiac muscle oxidizes for its energy requirements. FFAs oxidation demands more ATP to breakdown FFAs than glucose oxidation, requiring more oxygen to be supplied to the ischemic myocardium. Trimetazidine inhibits the use of FFAs as an energy source, shifting the myocardial metabolism to glucose utilization (glycolysis), which requires less oxygen than FFAs.

The effect of TMZ has been extensively studied in patients with SCAD. It has been shown that adding TMZ to standard therapy in patients with CAD, especially beta-blocking agents, confers clinical benefit of reduction of number of angina attacks, increasing exercise capacity, and prolonging exercise period before occurrence of ischemia (time to 1 mm ST segment depression). These benefits have been proven in different subsets of patients with coronary artery disease such as diabetics, patients with previous myocardial infarction or acute myocardial infarction undergoing primary percutaneous coronary intervention (PCI) or thrombolysis, undergoing coronary artery bypass grafting (CABG), as well as those not suitable for any kind of revascularization [4–10].

The reports dealing with management of SCAD in Serbia are scarce. Furthermore, there are no data regarding the current use of these "new treatments" in Serbian population of patients with SCAD.

## OBJECTIVE

We sought to investigate the influence of strict implementation of current guideline-proposed medications on relief of angina assessed by the reduction of the functional class of angina pectoris, weekly occurrence of the angina, and the weekly use of short acting nitrates in a large cohort of Serbian patients with SCAD.

## METHODS

In this multicenter, longitudinal study, we prospectively enrolled 3,490 consecutive patients from 15 outpatient cardiology clinics with symptoms of stable angina in an open design with repeated measurements of the patients' characteristics at two office visits. Patients were enrolled during February and March of 2014. Fifty cardiologists in those clinics evaluated the patients and included them in the study.

Before inclusion in the study, the patients were fully informed about the aims of the study and accepted correction of their prescribed therapy to meet the guideline-proposed goals. The patients were asked to consent to collecting data from their medical records on their condition, and were explained that their treatment will be corrected based on their clinical status and following the current recommendations for the condition. They had an opportunity to refuse institution of new medicines and use of their medical records for study purposes. The participating physicians were asked to act based on their own clinical judgment and to follow the recommendations given by the current guidelines. This means they had an opportunity to implement second-line drugs for treatment of SCAD if they considered that first-line drugs were not sufficient or if patients experienced any of the undesirable effects of the first-line treatment. Also, physicians had an opportunity not to change treatment of patients for whom they thought were in stable condition with well controlled risk factors for atherosclerosis. The study protocol was approved by the ethics committee of each participating institution. Patients were included in the study if they were older than 18 years of age and had SCAD defined according to clinical symptoms (typical or atypical chest pain, or angina equivalent, related to physical activity, lasting several minutes and ceases after stopping exertion or taking short-acting nitrates) and at least one of the following:

- ECG abnormality (presence of abnormal Q waves in any lead, ST segment depression or elevation equal to or greater than 0.5 mm in any two consecutive leads, negative T waves in any two consecutive leads);
- History of documented myocardial infarction (MI) more than three months previously;
- Positive stress test (exercise stress test, echocardiography stress test or myocardial perfusion scintigraphy);
- Performed coronary angiography with at least one diameter stenosis of epicardial coronary artery of more than 70%;
- History of revascularization either by PCI or CABG.

The patients were seen at the office visit on the day of inclusion in the study. The symptoms of angina were assessed and classified using Canadian Cardiology Society (CCS) classification [11]. The frequency of angina attacks and use of short-acting nitrates were also assessed. A 12-channel ECG was recorded in all the patients. Physical examination of the patients included height, weight and blood pressure measurement, the presence of known risk factors for atherosclerosis (diabetes, smoking, hypertension, heredity), and manifestations of atherosclerotic disease (peripheral arterial disease, cerebrovascular disease, heart failure, erectile dysfunction, renal failure) were also examined.

The patients' prescribed medications were thoroughly assessed including type of agent, daily dose and total duration of therapy. These include antiplatelet agents, beta blockers, calcium antagonists, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARB), long-acting nitrates, lipid lowering agents and metabolic agents to treat the angina (TMZ, ranolazine and nicorandil). According to the current European Society of Cardiology guidelines on stable coronary artery disease, new medications were added or previously prescribed ones were stopped, or their dose was adjusted to meet the guideline-proposed recommendations [2].

After two months, the patients were seen again and underwent physical examination and blood pressure measurement. Weekly number of angina attacks, angina class using CCS classification, and use of antianginal medica-

tions were assessed, as well as the tolerance and adherence to medications prescribed on the previous visit. The prescribed medications and their dosage remained unchanged throughout the follow-up period.

The primary end-point of the study was relief from angina consisting of any reduction in CCS class, any reduction in number of angina attacks per week and any reduction in number of tablets of short-acting nitrates taken per week.

### Statistical analysis

Continuous variables are presented as mean values ± standard deviation (SD). Categorical variables are presented as percentages. Depending on the distribution of the data, t-test or Mann-Whitney test were used to compare continuous variables, whereas $\chi^2$ and Fisher's test were used for categorical variables. P-value of <0.05 was considered significant. Statistical analysis was performed using commercially available software (PASW Statistics, version 18, SPSS Inc., Chicago, IL, USA).

## RESULTS

A total of 3,490 patients, mean age 67 ± 10 years (53.2% males) were enrolled in the study. Most patients fulfilled only the ECG criterion for inclusion in the study (Table 1). Significant proportion of enrolled patients were hypertensive, while other atherosclerotic risk factors were present in expected proportions. The patients' clinical characteristics are given in Table 2. For every class of medications (antiplatelets, long-acting nitrates, beta-blocking agents, Ca-antagonists, ACE-inhibitors, ARBs or statins) there was significant change in number of patients taking them, when their therapy was corrected according to guidelines at Visit 1. Of note, this difference was the greatest regarding trimetazidine and it was instituted for the first time in 3,064 out of 3,490 (87.8%) patients, while use of nicorandil

**Table 1.** Enrolment of patients in the study based on inclusion criteria (n = 3,490)

| Inclusion criterion | | Number of patients (%) |
|---|---|---|
| Abnormal ECG at Visit 1 | | 1,689 (48.4) |
| History of myocardial infarction | | 1,270 (36.4) |
| Positive stress test | Exercise stress test | 997 (28.6) |
| | Echocardiography stress test | 121 (3.5) |
| | Positive coronary angiography | 567 (16.2) |
| History of revascularization | Previous PCI | 833 (23.9) |
| | Previous CABG | 475 (13.6) |

CABG – coronary artery bypass grafting; PCI – percutaneous coronary intervention

**Table 2.** Clinical characteristics of the patients at Visit 1 (n = 3,490)

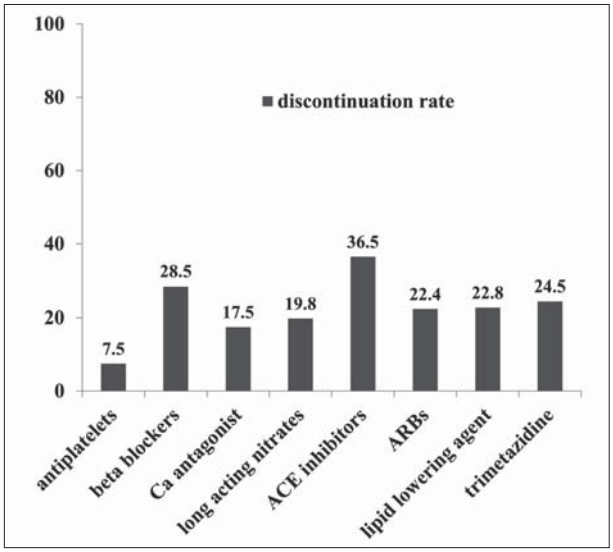
| Clinical characteristics | Values* |
|---|---|
| Age (years) | 66.71 ± 9.75 |
| Male gender (%) | 1,857/3,490 (53.2%) |
| Smoking (%) | 1,361/3,294 (39.0%) |
| Hypertension (%) | 3,162/3,490 (90.6%) |
| DM on OAD (%) | 973/3,490 (27.9%) |
| DM on insulin (%) | 300/3,190 (8.6%) |
| PAD (%) | 475/3,490 (13.6%) |
| Previous CVA (%) | 395/3,490 (11.3%) |
| Chronic renal failure (%) | 211/3,490 (6.0%) |
| BMI (kg/m2) | 27.01 ± 3.58 |
| Systolic BP (mmHg) | 141 ± 19 |
| Diastolic BP (mmHg) | 84 ± 11 |
| Heart failure | 592/3,490 (17.0%) |
| Erectile dysfunction | 126/3,490 (3.6%) |

* The values are presented as mean ± standard deviation and as the number of patients with percentage.
DM – diabetes mellitus; OAD – oral antidiabetics; PAD – peripheral arteria disease; CVA – cerebrovascular accident; BMI – body mass index; BP – blood pressure

**Table 3.** Change in prescribed medication to patients in the study at Visit 1 (n = 3,490)

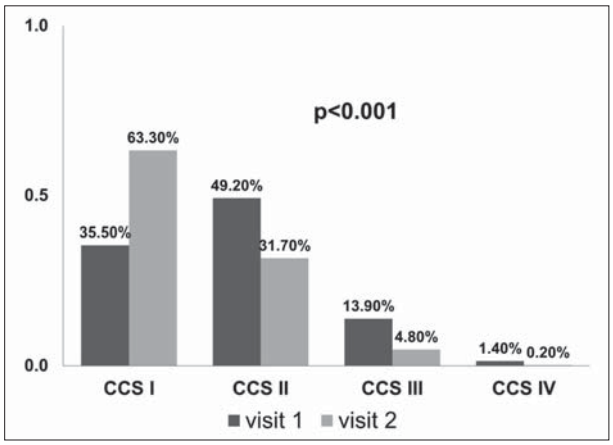
| Drug class | | Number of patients (%) | | p | Duration of therapy before V1 (months) |
|---|---|---|---|---|---|
| | | Prescribed | Not prescribed | | |
| Antiplatelets | Taken before | 2,777 (79.6) | 224 (6.4) | <0.001 | 9.6 ± 6.6 |
| | Not taken before | 239 (6.8) | 250 (7.2) | | |
| Long-acting nitrates | Taken before | 1,070 (30.6) | 264 (7.6) | <0.001 | 36.5 ± 28.6 |
| | Not taken before | 221 (6.4) | 1,935 (55.4) | | |
| Beta-blocking agent | Taken before | 1,890 (54.2) | 753 (21.6) | <0.001 | 52.0 ± 25.7 |
| | Not taken before | 297 (8.5) | 550 (15.7) | | |
| Calcium antagonist | Taken before | 1,200 (34.4) | 254 (7.3) | <0.001 | 48.3 ± 28.2 |
| | Not taken before | 326 (9.3) | 1,710 (49.0) | | |
| ACE inhibitor | Taken before | 1,901 (54.7) | 416 (11.8) | <0.001 | 42.0 ± 30.5 |
| | Not taken before | 316 (9.0) | 857 (24.5) | | |
| Angiotensin receptor blocker | Taken before | 249 (7.1) | 72 (2.1) | <0.001 | 12.8 ± 8.4 |
| | Not taken before | 101 (2.9) | 3,068 (87.9) | | |
| Lipid lowering agent | Taken before | 1,706 (48.9) | 504 (14.4) | <0.001 | 28.4 ± 22.8 |
| | Not taken before | 365 (10.5) | 915 (26.2) | | |
| Trimetazidine | Taken before | 182 (5.2) | 59 (1.7) | <0.001 | 6.2 ± 3.4 |
| | Not taken before | 3,064 (87.8) | 185 (5.3) | | |

ACE – angiotensin-converting enzyme; V1 – Visit 1




**Graph 1.** The rate of discontinuation of prescribed medications at Visit 1; the graph displays high rate of discontinuation of several classes of medications at Visit 1

ACE – angiotensin-converting enzyme; ARB – angiotensin II receptor antagonist




**Graph 2.** Canadian Cardiovascular Society (CCS) angina class in patients at Visit 1 and 2; the graph displays significant reduction in angina severity, expressed as CCS class, in study population at Visit 2, after institution of adjusted medical therapy according to current guidelines

and ranolazine was negligible (0.05% and 0.00%, respectively) (Table 3). There was a high rate of discontinuation of previously prescribed medications, classified in groups, at Visit 1, initiated by treating physician (Graph 1). At Visit 1, number of prescribed medications from previously defined groups of medication increased from 4.16 ± 1.29 to 4.63 ± 1.57 ($p < 0.001$). The median number of prescribed drugs rose from four at Visit 1 (1–8) to five (0–7) at Visit 2.

There were 3,425 patients (98.14%) that were seen at Visit 2 and the data on study end-points were collected for them. At Visit 2, the patients had significantly lower blood pressure (141 ± 19 / 85 ± 11 vs. 130 ± 12 / 80 ± 8 mmHg; $p < 0.001$). The patients' functional class data were available for 3,425 patients (98.14%) and it improved significantly, with most patients reporting CCS class I (Graph 2). While most patients improved their CCS class, several patients deteriorated and some remained in the CCS class III and IV, warranting further investigation and possible need for invasive coronary angiography (Table 4). The average weekly number of angina attacks was reduced from 2.82 ± 2.50 at Visit 1 to 1.72 ± 1.66 at Visit 2, as well as the average weekly use of short-acting nitrates to treat these attacks (2.69 ± 2.53 to 1.74 ± 1.47 tablets; $p < 0.001$ for all).

**Table 4.** Change in CCS class at Visit 2 after adjustment of medical therapy

| CCS class Visit 1 (No. of patients) | CCS class Visit 2 No. of patients (%) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 1 (1,217) | 1,199 (98.5) | 18 (1.5) | 0 (0.0) | 0 (0.0) |
| 2 (1,684) | 923 (54.8) | 749 (44.5) | 12 (0.7) | 0 (0.0) |
| 3 (475) | 46 (9.7) | 295 (62.1) | 134 (28.2) | 0 (0.0) |
| 4 (49) | 6 (12.2) | 21 (42.9) | 15 (30.6) | 7 (14.3) |

CCS – Canadian Cardiovascular Society

## DISCUSSION

To the best of our knowledge, this is the first study evaluating implementation of guideline directed treatment of stable coronary artery disease in Serbian population which is characterized by prevalent atherosclerotic risk factors and low socioeconomic level. Our study demonstrated that strict implementation of guideline-directed medical therapy in patients with SCAD led to significant improvement in patients' status, as indicated by the reduction of number of angina attacks, use of short-acting nitrates, and lower CCS class, but at a cost of increased number of prescribed classes of medications patients are taking. As expected and documented in previous publications, the use of beta-blocking agents and long acting nitrates was independently associated with relief of angina [12, 13, 14]. The use of trimetazidine at the initial study visit was low and substantially increased during the follow-up period. Of note, the existing use of nicorandil and ranolazine in Serbian population with SCAD was negligible.

We also observed a high rate of discontinuation of previously prescribed medication at Visit 1 (Graph 1). It was especially high for ACE inhibitors, but it was also worth of notice for beta blocking agents, ARBs, lipid lowering agents, and trimetazidine. The reason for this could be the presence of adverse events of the drugs, decreased patient compliance, and inefficient treatment for hypertension, which lead to switch from one drug class to another. Unfortunately, adverse events of the medicines and the patients' compliance were not systematically evaluated in the study.

The patients included in the study had a very high prevalence of hypertension, although significant proportion of them had been treated with antihypertensive medications. At Visit 1, besides adding trimetazidine to most patients' treatment, new medications were added and dosage of already instituted antihypertensive medication was corrected to meet the guideline- defined goals. At Visit 2, the patients had significantly lower blood pressure, within treatment goals proposed by guidelines. This might have caused an improvement in angina status and influence the primary end-point of the study *per se* [15]. Also, the increased compliance with already prescribed medication

could cause this effect on blood pressure regulation. Although adjustment of medical therapy has led to improvement in clinical status and CCS class in most of the patients, some still remained in CCS classes III and IV (Table 4). This demonstrates limitations of medical therapy alone in treatment of patients with SCAD, because the patients that haven't improved after correction of therapy may be candidates for invasive coronary angiography and interventional treatment. The important issue is an increase in already high number of classes of medications prescribed to patients at Visit 1. This may be caused by already inefficient treatment for angina, hypertension or dyslipidemia. The patients' blood pressure at Visit 1 was not in the range recommended by the current guidelines [15]. Also, many patients had an already proven CAD, so their treatment must have consisted of antiplatelet agent(s), a lipid lowering drug, and potentially a beta blocker [16], besides treatment for hypertension. It can be argued that already instituted medications were not prescribed in optimal doses, so that participating physicians might have increased the dose of already prescribed medication instead of implementing new ones. Noteworthy, potential interaction between the drugs prescribed affecting their efficacy can also be the issue.

Antiplatelet agents were frequently prescribed to our patients (Table 3), given as secondary prevention, because most patients had a previously proven CAD (history of MI, PCI or CABG) (Table 1) [16]. The same applies for beta-blocking and lipid lowering agents. Increased use of trimetazidine is in line with current guidelines that recommend its use as a second line treatment for patients who are still symptomatic despite use of the first line medications (beta-blocking agents, calcium antagonists and, nitrates) or who cannot tolerate them [2]. However, it should be underlined that indication for its use bears IIB class indication, level of evidence B [2]. The reason to prescribe a second line agent, like TMZ, as a monotherapy should be guided by intolerance of the first line treatment, notably bradycardia induced by beta-blocking agents, headache and hypotension caused by long acting nitrates or bradycardia, hypotension and peripheral edema caused by Ca antagonists. This finding corroborates a large meta-analysis by Peng et al. [17] of randomized trials comparing efficacy of TMZ added to conventional therapy vs. conventional therapy only in patients with stable angina. On the other hand, another meta-analysis, done by Belsey et al. [18], has demonstrated beneficial effects of adding TMZ to beta-blocking agents or Ca antagonists on exercise tolerance and weekly angina frequency, but it was not associated with the decrease in use of short-acting nitrates. The evidence for TMZ as mono-therapy is not so robust and large meta-analysis did not demonstrate clear benefits from TMZ compared to alternative regimen with nitrates. However, relatively few side effects is one of the most important advantages of TMZ that supports its use in stable coronary artery disease [19]. Since the effects of TMZ are related to metabolism of FFA, it also benefited patients with heart failure, improving echocardiographic indices of systolic and diastolic function [20]. It can be argued that the period of two months between the study visits may not be sufficient for instituted therapies to reveal their full effect. However, the studies on effects of TMZ in patients with SCAD evaluated its effects after four to eight weeks of therapy, which makes our study design appropriate [7, 21, 22].

### Study limitations

The study was not randomized and the effects of such design have to be acknowledged. The time interval during which stable angina was present was not assessed at the inclusion in the study, so that complaints may be caused by in-stent restenosis after PCI, especially within six months after intervention, or graft failure after CABG. The effects of prescribed treatment were measured by subjective patient's evaluation of their condition. The design of the study did not allow for a washout period between the discontinuation of the old and the introduction of any new medication. Also, for medical reasons of continuing treatment of angina, the study design did not include a crossover between prescribed medications groups. The patients were interviewed about their compliance and possible undesirable effects of the prescribed treatment both at Visit 1 and Visit 2, but this was not recorded in the study database, which may lead to erroneous conclusions regarding their effectiveness. All this limits the power of the study to assess the effects of individual medication groups on relief of angina as a study end-point.

## CONCLUSION

Adjustment of prescribed medications to meet guideline-proposed goals in a large population of Serbian patients with stable coronary artery disease, characterized by high prevalence of risk factors, led to significant relief of angina, supporting the concept of guideline-directed medical therapy

## NOTE

Epidemiology study database was provided by the Les Laboratoires Servier. Data analysis was performed solely by the authors and the scientific content of the manuscript was not influenced in any way by the Les Laboratoires Servier.

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# Утицај медикаментне терапије усклађене према актуелним терапијским водичима на тежину стабилне коронарне болести код болесника у Србији

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**КРАТАК САДРЖАЈ**

**Увод** Особе које болују од стабилне коронарне болести недовољно се придржавају препорука о промени животних навика и редовном узимању терапије.

**Циљ рада** Циљ студије је испитати утицај медикаментне терапије усклађене према актуелним препорукама на тежину ангине пекторис код пацијената са стабилном коронарном болешћу у Србији.

**Методе рада** У студију је укључено 3.490 пацијената у 15 кардиолошких клиника са симптомима стабилне ангине и/или променама на електрокардиограму (ЕКГ), прележаним инфарктом миокарда (ИМ), позитивним тестом физичког оптерећења, ангиографски доказаном значајном коронарном болешћу или претходном реваскуларизацијом миокарда. Сви болесници су свеобухватно прегледани на првој посети и након два месеца. Циљ истраживања је смањење ангинозних тегоба, дефинисано као било какво смањење класе дефинисане Канадским кардиолошким друштвом (*CCS*), броја ангинозних напада недељно и/или смањење броја узетих таблета краткоделујућих нитрата.

**Резултати** Већина болесника је укључена на основу промена на ЕКГ-у (48,4%). На првом прегледу просечан број класа лекова прописаних пацијенту порастао је са 4,16 ± 1,29 на 4,63 ± 1,57 ($p < 0,001$). На контролном прегледу болесници су имали значајно мање вредности крвног притиска (141 ± 19 / 85 ± 11 вс. 130 ± 12 / 80 ± 8 *mmHg*; $p < 0,001$) и већина је припадала *CCS* класи I (63,3%). Просечан број ангинозних напада недељно смањен је са 2,82 ± 2,50 на првој посети на 1,72 ± 1,66 на другој посети. Такође је смањена недељна употреба краткоделујућих нитрата ради купирања ангинозних напада, са 2,69 ± 2,53 на 1,74 ± 1,47 таблете ($p < 0,001$ за све).

**Закључак** Усклађивање медикаментне терапије према актуелним препорукама доводи до значајног смањења ангинозних тегоба код болесника са присутним факторима ризика у Србији.

**Кључне речи:** стабилна коронарна болест; препоруке; медикаментна терапија; триметазидин

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# Is pacemaker therapy the right key to patients with vasovagal syncope?

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**SUMMARY**

**Introduction** Vasovagal syncope is the most common type of reflex syncope. Efficacy of cardiac pacing in this indication has not been the subject of many studies and pacemaker therapy in patients with vasovagal syncope is still controversial.

**Objective** This study aimed to assess the efficacy and safety of pacing therapy in treatment of patients with vasovagal syncope, to determine contribution of new therapeutic models in increasing its success, and to identify risk factors associated with a higher rate of symptoms after pacemaker implantation.

**Methods** A retrospective study included 30 patients with pacemaker implanted due to vasovagal syncope in the Pacemaker Center, Clinical Center of Serbia, between November 2003 and June 2014. Head-up tilt test was performed to diagnose vasovagal syncope. Patients with cardioinhibitory and mixed type of disease were enrolled in the study.

**Results** Mean age was 48.1 ± 11.1 years and 18 (60%) patients were men. Mean follow-up period was 5.9 ± 3.0 years. Primarily, implantable loop recorder was implanted in 10 (33.3%) patients. Twenty (66.7%) patients presented cardioinhibitory and 10 (33.3%) mixed type of vasovagal syncope. After pacemaker implantation, 11 (36.7%) patients had syncope. In multiple logistic regression analysis we showed that syncope is statistically more likely to occur after pacemaker implantation in patients with mixed type of vasovagal syncope ($p = 0.018$). There were two (6.7%) perioperative surgical complications.

**Conclusion** Pacemaker therapy is a safe treatment for patients with vasovagal syncope, whose efficacy can be improved by strict selection of patients. We showed that symptoms occur statistically more often in patients with mixed type of disease after pacemaker implantation.

**Keywords:** vasovagal syncope; pacemaker therapy; head-up tilt test

## INTRODUCTION

Vasovagal syncope, previously called neurocardiogenic syncope, is the most common type of reflex syncope, usually seen in young patients without cardiovascular history [1]. It is preceded by prodromal symptoms of strong initial sympathetic activation in two thirds of patients. Symptoms such as sweating, pallor, nausea, blurred vision, and confusion are presented for about 60 seconds [2]. Vasovagal syncope is caused by an overemphasized response of autonomic nervous system to various stimuli, such as strong emotions and orthostatic stress [2]. There are different initiators of vasovagal syncope, from extended standing, warm and stifling environment, and showering with hot water, to painful stimulus, fear, or psychological stress [3]. Therefore, peripheral as well as central mechanisms have been included in pathophysiology of vasovagal syncope [1].

After taking history, for the confirmation of diagnosis of vasovagal syncope, the head-up tilt test (HUTT) should be performed. HUTT is a noninvasive orthostatic stress test, and according to guidelines of European Society of Cardiology, it is indicated in patients with suspected vasovagal syncope, based on clinical history and basic diagnostics (class I of recommendations), in the case of an unexplained syncope in high risk settings (for example occupational implications such as pilots or professional drivers), or in situations when we must discriminate reflex syncope and orthostatic hypotension (class IIa of recommendations) [4]. The main dilemma remains whether patients with vasovagal syncope need specific therapy. It is generally accepted that patients with single syncope and without high risk occupations should be educated to recognize and avoid situations that can trigger syncope [1, 2, 4]. Counterpressure maneuvers and orthostatic training may be helpful [1, 2, 4]. According to guidelines of European Society of Cardiology, cardiac pacing is indicated in patients over 40 years of age with recurrent vasovagal syncope, who show prolonged asystole during ECG recording and/or tilt testing, and are informed of the conflicting results of trials (class IIa of recommendations) [4]. Efficacy of cardiac pacing in this indication has not been the subject of many studies and pacemaker therapy is still controversial.

## OBJECTIVE

This study aimed to assess the efficacy and safety of pacing therapy in treatment of patients with vasovagal syncope, to determine

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contribution of new therapeutic models in increasing its success, and to identify risk factors associated with a higher rate of symptoms after pacemaker implantation.

## METHODS

This was a retrospective, observational study, which included patients with pacemaker implanted due to vasovagal syncope, in the Pacemaker Center, Clinical Center of Serbia, between November 2003 and June 2014. The diagnosis of vasovagal syncope was based on clinical history and results of tilt testing. During the testing, we used a protocol divided into three phases:Stabilization phase – the patient is rested supine for five minutes;

- Passive phase – the patient is tilted upright at an angle of 60° for 20 minutes;
- Provocation phase – one dose of 400 μg of sublingual glyceryl trinitrate spray is administered, after which the patient continues the test for 15 minutes.

HUTT was considered positive when asystole longer than three seconds and/or fall in systolic blood pressure higher than 50 mmHg was recorded. All patients were divided into these three hemodynamic types, based on the results of tilt testing:

- Cardioinhibitory type – when bradycardia and asystole longer than three seconds were recorded;
- Vasodepressor type – when fall in systolic blood pressure higher than 50 mmHg was recorded;
- Mixed type – when asystole and hypotension were recorded.

Patients with cardioinhibitory and mixed type of vasovagal syncope were enrolled in the study. Patients who were followed up less than six months were excluded. Pacemakers manufactured by Medtronic (Minneapolis, MN, USA) and St Jude Medical (Saint Paul, MN, USA) were implanted, in VVI and DDD mode of stimulation. Devices with and without special algorithms for treating reflex syncope were implanted. Pacemaker was implanted left or right prepectoral and electrodes were placed endovenously, after cephalic vein cut-down or punction of the subclavian and/or axillary vein. In patients with previously implanted implantable loop recorder (ILR), the device was explanted, after which the pacemaker was implanted during the same intervention. Data were collected from the pacemaker medical records and patients' files from device controls in the Outpatient Department of the Pacemaker Center. All the patients were contacted by phone to check whether there were symptoms after the intervention.

For data processing we used descriptive and analytic statistic methods. From descriptive methods mean and standard deviation were used for continuous variables, and absolute and relative numbers for categorical variables. Multiple binary logistic regression analysis was used to identify the characteristics associated with a higher rate of syncope after pacemaker implantation. A p-value of $<0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 20 software (IBM Corp., Armonk, NY, USA). The efficacy of pacing therapy was determined according to frequency of symptoms recurrence after pacemaker implantation. Therapy safety was assessed based on frequency of perioperative complications in our and other studies, where pacemakers were implanted using standard surgical technique in similar or different indications.

## RESULTS

Thirty patients were included in this study. Mean follow-up period was 5.9 ± 3.0 years. Mean age was 48.1 ± 11.1 years and 18 (60%) patients were man. Patient and procedure characteristics and the incidence of risk factors are presented in Tables 1 and 2. Preoperatively, all the patients had syncope, HUTT was performed in all of them and based on the results, cardioinhibitory type of vasovagal syncope was diagnosed in 20 (66.7%) patients and mixed type in 10 (33.3%). Pacemaker in VVI mode of stimulation was implanted in six (20%) and in DDD mode of stimulation in 24 (80%) patients. Eight (26.6%) patients got device with special algorithm for treating reflex syncope. Primarily, ILR was implanted in 10 (33.3%) patients, after which, based on ILR records, implantation of pacemaker was indicated. After pacemaker implantation, during the follow-up period, 11 (36.7%) patients had syncope and 19 (63.3%) had no symptoms. Mean follow-up period from pacemaker implantation to the first syncope was 1.0 ± 0.4 years. In multiple logistic regression analysis we identified the type of vasovagal syncope as an independent risk factor for the occurrence of syncope after the pacemaker implantation (Table 3). We showed that the occurrence of syncope is statistically more likely after the pacemaker implantation in patients with mixed type of vasovagal syncope ($p = 0.018$). There were two (6.7%) instances of perioperative surgical complications, and a reintervention was required in one patient. We recorded no ventricular arrhythmias, ventricular tachycardia/fibrillation, and one patient died during the follow-up period.

## DISCUSSION

Vasovagal syncope is a rare indication for pacemaker implantation. Medical doctors, even they are aware that according to guidelines there is an indication for pacing therapy, unwillingly make decision to implant the device because patients are usually young persons, who consider themselves healthy. If we look at guidelines, especially at the level of evidence, it will be completely clear why there are doubts about the role of cardiac pacing therapy in management of vasovagal syncope. Efficacy of cardiac pacing in this indication has not been the subject of many studies, and results and findings of those trials are inconsistent [4]. Firstly, efficacy of pacemaker therapy was confirmed in a few small randomized studies, with control group without specific therapy (VPS I, VASIS, SYDIT) [5, 6, 7]. However, the superiority of pacing therapy has not been confirmed in double-blind placebo-controlled trials (VPS II, SYNPACE) [8, 9].

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**Table 1.** Patient and procedure characteristics

| Parameter | | Number of patients (%) |
|---|---|---|
| Male | | 18 (60) |
| Age | | 48.1 ± 11.1 |
| Syncope before PM implantation | | 30 (100) |
| HUTT before PM implantation | | 30 (100) |
| ILR implanted before PM implantation | | 10 (33.3) |
| Hemodynamic type of VVS | Cardioinhibitory | 20 (66.7) |
| | Mixed | 10 (33.3) |
| PM mode stimulation | VVI | 6 (20) |
| | DDD | 24 (80) |
| PM with special algorithm | | 8 (26.6) |
| Syncope during follow-up | | 11 (36.7) |

PM – pacemaker; HUTT – head-up tilt test; ILR – implantable loop recorder; VVS – vasovagal syncope

**Table 2.** Incidence of risk factors

| Parameter | Number of patients (%) |
|---|---|
| Ischemic heart disease | 5 (16.6) |
| Atrial fibrillation before implantation | 5 (16.6) |
| Chronic obstructive pulmonary disease | 2 (6.7) |
| Arterial hypertension | 16 (53.3) |
| Diabetes | 4 (13.3) |
| Hyperlipoproteinemia | 5 (16.6) |
| Tobacco smoking | 6 (20) |

**Table 3.** Correlation between patient characteristics and clinical data with symptoms' recurrence*

| Variable | B | Sig. |
|---|---|---|
| Sex | 1.135 | 0.443 |
| Type of VVS | 4.658 | 0.018 |
| Type of PM | -3.732 | 0.068 |
| Previously implanted ILR | -2.478 | 0.194 |
| PM with algorithm for treating VVS | 0.942 | 0.588 |

* Dependent variable: syncope
B – regression coefficient; Sig. – significance; VVS – vasovagal syncope; PM – pacemaker; ILR – implantable loop recorder

In our study, after pacemaker implantation, during the follow-up period, 36.6% of patients had syncope. Comparing to the results of the VPS II study where 31% of patients had syncope during follow-up, our results are in line for additional explanation. Pacemaker in VVI mode of stimulation was implanted in six (20%) patients and five of them had syncope during the follow-up. Although, in our study, mode of stimulation has not been identified as a risk factor associated with a statistically higher rate of symptoms after pacemaker implantation ($p = 0.068$), experience tells us that patients with pacemaker in VVI mode of stimulation have syncope after intervention significantly more often. We interpret our results as a consequence of the insufficient number of enrolled patients with this mode of stimulation to achieve statistical significance. In addition, eight (26.6%) of our patients received a device with a special algorithm for treating reflex syncope. This algorithm allows rapid increasing of heart frequency in case of significant drop in heart rate and thus prevents vasodilatation, a drop in blood pressure, and, finally, the occurrence of syncope [10, 11]. However, it is accepted that timely detection of paradoxical neural reflex, which is responsible for the occurrence of vasovagal syncope, at its afferent part, is most important for preventing syncope. Thus, the traditional function of pacemaker, preventing bradycardia development and acting at the efferent part of the neural reflex, is changed. Based on this idea, new pacemaker algorithms, which allow the pacemaker to react in accordance with cardiac contraction dynamics, measuring the change in intracardiac impedance, are developed. Increased myocardial contractility, that occurs in the initial pathophysiological segments of the development of vasovagal syncope, due to increased releasing of catecholamines and still insufficient venous return in the right ventricle, can be detected [12, 13, 14]. This allows us to stop the vicious circle that leads to the occurrence of vasovagal syncope with the pacing at this, afferent part of paradoxical reflex. In our study, only one patient with an implanted device with this special algorithm had syncope during the follow-up period. Unfortunately, in our center, we have not had the opportunity to implant more pacemakers with this algorithm, but we believe that their use in the future will improve the results of pacing therapy in this indication. Relatively high percentage of symptom recurrence in our study population must be considered from the viewpoint of the length of the patient's follow-up. Described studies had, in most cases, a twelve-month follow-up, and we had an average follow-up of 5.9 ± 3.0 years, which provides greater significance to our results. Additionally, mean age of our patients was under 49 years and was significantly lower than in other studies. Even before our study, many researchers questioned whether vasovagal syncope in the elderly had different pathophysiological mechanisms of development compared to younger people and whether that could provide greater efficacy of pacing therapy in this indication. Therefore, they noted that studies which promote the importance of pacemaker therapy in management of vasovagal syncope had enrolled patients with mean age significantly higher than that in studies whose results have challenged the effectiveness of pacing in this indication [15]. It should be noted that the nature of symptoms was different in patients who continued to have syncope after the pacemaker implantation. These patients stated that syncope after the pacemaker implantation compared to those before the intervention were less sudden, preceded by prolonged prodromal symptoms; also, none of these patients sustained any injures.

It is important to mention major conclusions of the meta-analysis, which included nine studies that assessed the role of pacemaker therapy in treatment of patients with vasovagal syncope, and which was published in 2007 [16]. In addition to the known fact that in the group of double-blind studies it is not possible to prove the efficacy of pacing therapy, it is also highlighted that results were not significantly changed when research was limited only to patients with cardioinhibitory type of vasovagal syncope confirmed during the HUTT [16]. In our study, however, three (15%) patients with cardioinhibitory type of disease had syncope and we showed that syncope after pacemaker implantation is statistically less likely to occur in patients with cardioinhibitory type of vasovagal syncope than in those with mixed type of the disease.

It is necessary to develop new ideas that will lead to better selection of patients with vasovagal syncope, who will gain from the pacemaker therapy. One such idea, used in our study, is related to the early implantation of ILR in patients with recurrent vasovagal syncope, in order to select patients with highly suspected cardioinhibitory type of disease, and then based on ILR records determine specific therapy [17]. Therefore, ILR is implanted in patients with recurrent vasovagal syncope and then the patients are observed for any development of significant bradycardia or significant asystolic pauses, which would be the indication for pacemaker implantation. In two large studies, ISSUE 2 and ISSUE 3, the rate of symptom persistence in a group of patients with an implanted pacemaker and in those without specific therapy, was compared [18, 19]. In both studies, with ISSUE 3 being a double-blind study with a placebo control group, statistically significant reduction of absolute and relative risk of symptom persistence in patients who were under specific therapy was demonstrated [18, 19, 20]. In our study, this new approach to patient selection, by early implantation of an ILR, was applied in 10 patients, and two (20%) of them had syncope during the follow-up period.

Our results indicate that pacemaker implantation is a safe procedure. There were two (6.7%) perioperative surgical complications, and a reintervention was required in one patient. In one perioperative surgical complication, atrial lead dislodgement occurred, which was resolved during the same hospitalization by implanting a new atrial lead. In the second case, iatrogenic apical pneumothorax was diagnosed; the patient was monitored by a thoracic surgeon, and after a somewhat prolonged hospitalization in our center, the patient was discharged in good general condition. In the patient who died during the follow-up period, noncardiovascular cause of death was found. Therefore, pacemaker implantation, like any other surgical procedure, has some risks, but it is important to emphasize that mentioned complications do not diverge in their type or in frequency from what is expected [21, 22].

## CONCLUSION

Our study has shown that pacemaker therapy is a safe treatment for patients with vasovagal syncope, whose efficacy can be improved by a strict selection of patients. We have shown that syncope is statistically more likely to occur after the pacemaker implantation in patients with mixed type of vasovagal syncope. Our results and permanent envelopment of new therapeutic models and new pacemaker algorithms assure us that efficacy of pacing therapy in this indication will be advanced in the near future.

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# Да ли је пејсмејкер терапија право решење за болеснике са вазовагалном синкопом?

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**КРАТАК САДРЖАЈ**

**Увод** Вазовагалне синкопе представљају најчешћи тип рефлексних синкопа. Постоје бројне дилеме везане за лечење болесника са вазовагалном синкопом, а пејсмејкер терапија у овој индикацији је још увек контроверзна.

**Циљ рада** Циљ рада је био да се испита ефикасност и безбедност пејсмејкер терапије у лечењу болесника са вазовагалном синкопом, да се процени допринос нових терапијских модела повећању њене успешности и да се одреде фактори ризика повезани са повећаном учесталошћу симптома након уградње пејсмејкера.

**Методе рада** Ретроспективном студијом обухваћено је 30 болесника са вазовагалном синкопом којима је у Пејсмејкер центру Клиничког центра Србије у Београду од новембра 2003. године до јуна 2014. године уграђен трајни антибрадикадни пејсмејкер. Дијагноза је постављена на основу резултата *head-up tilt* теста. Укључени су болесници са дијагнозом кардиоинхибиторног и комбинованог типа болести.

**Резултати** Просечна старост болесника била је 48,1 ± 11,1 година, а 18 (65,0%) болесника је било мушког пола. Просечан период праћења износио је 5,9 ± 3,0 година. Код 10 (33,3%) болесника најпре је уграђен имплантабилни *loop* рекордер. Код 20 (66,7%) болесника постављена је дијагноза кардиоинхибиторног, а код 10 (33,3%) комбинованог типа болести. У периоду праћења 11 (36,7%) болесника је имало синкопу. Користећи мултиплу логистичку регресиону анализу, показали смо да се синкопа након уградње пејсмејкера чешће јављала код болесника са комбинованим типом болести ($p = 0,018$). Регистроване су две (6,7%) перипроцедуралне хируршке компликације.

**Закључак** Пејсмејкер терапија је безбедна метода лечења болесника са вазовагалном синкопом, чија ефикасност може бити унапређена ригорозном селекцијом болесника. Показали смо да се након уградње пејсмејкера симптоми статистички чешће јављају код болесника са комбинованим типом вазовагалне синкопе.

**Кључне речи:** вазовагална синкопа; пејсмејкер терапија; *head-up tilt* тест

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# The role of the color Doppler ultrasonography and computed tomography in estimation of portal hypertension

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**SUMMARY**

**Introduction** Liver diseases with disturbances of hepatic and splanchnic circulation lead to the portal hypertension, with or without a portal vein thrombosis.

**Objective** This study was based on the testing of hypothesis that more data and more precise diagnosis in patients with disorders of portal circulation can be obtained by using color Doppler ultrasonography (CDU) and computed tomography (CT) with contrast.

**Methods** The study was conducted from February 2011 to May 2014 and it comprised 120 patients who were suspected to have portal hypertension or already had clinical confirmation of the portal hypertension, patients with hepatitis, and some patients with hematological diseases. The first group of 40 patients was examined by conventional ultrasonography and CDU, the second group by contrast CT, and the third group of patients was examined by both methods (CDU and contrast CT). After six months of adequate therapy, the patients had control examinations with the same diagnostic technique used during their first examination.

**Results** Retrospective analysis showed that CDU is more sensitive than CT in the assessment of presence and age of thrombi (CDU 93.9%; CT 86.1%). CT gives precise data in detection of portosystemic collaterals. Sensitivity of CT is 100% and its specificity is 67%. Cumulative sensitivity and specificity for most parameters were increased in patients with portal hypertension when both methods were applied.

**Conclusion** This study emphasizes the possibility of early and more accurate diagnosis achieved when combining two radiological techniques (CDU and contrast CT scan), which is not the case when these methods are used separately.

**Keywords:** color Doppler ultrasonography; portal hypertension; portal thrombosis; computed tomography

## INTRODUCTION

A hepatic circulation supplies blood to the liver parenchyma through the portal vein and the hepatic artery. The blood-supplying hepatic vessels are separated and independent. Only at the level of the liver acinus, as the functional unit, there is communication with hepatic veins and drain circulation system. In terms of understanding hemodynamic changes in portal–portal and portal–systemic circulation, it is very important to know this anatomical characteristic and its varieties. [1]. Portal hypertension occurs as a result of morphological changes at the level of parenchyma, which leads to increased resistance and pressure in the portal venous system. The most common causes of portal hypertension are diffused histopathological changes in liver parenchyma, vascular processes of the hepatic vein, decompensation of the right heart, etc. The specific etiological entities that cause portal hypertension include Budd–Chiari and Cruveilhier–Baumgarten syndrome. Increased vascular resistance in the portal circulation may occur at prehepatic, intrahepatic, and posthepatic levels [1–3]. Clinical diagnosis of portal hypertension is based on anamnesis, laboratory analysis, and endoscopy [4]. In daily practice it is necessary to introduce radiological non-invasive diagnostic techniques [ultrasonography (US), color Doppler ultrasonography (CDU), computed tomography (CT) with contrast administration, or magnetic resonance imaging (MRI)]. Invasive diagnostic techniques such as conventional or digital angiography with direct or indirect presentation of the portal vein are also used. Invasive diagnostic techniques have been suppressed by the development of non-invasive diagnostic techniques, i.e. CDU, CT, and MRI, which are now used for definitive determination of pathological changes of the liver and the portal circulation. The main advantage of CDU is its possibility to determine the morphologic and hemodynamic parameters which are classified as qualitative, quantitative, and semi quantitative [5]. These parameters allow us to determine the presence and direction of

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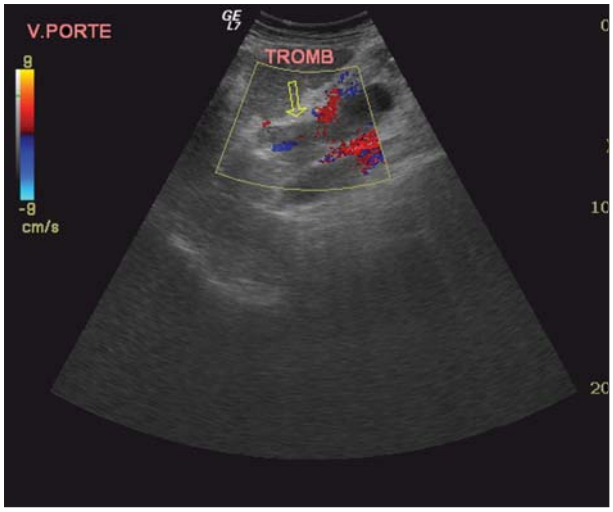


**Figure 1.** Color Doppler ultrasonography – obstructed lumen by thrombus of dilated portal vein

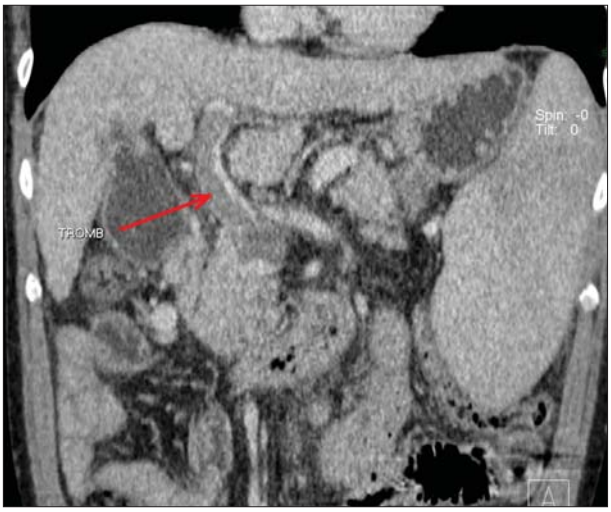


**Figure 2.** Contrast CT scan – portal thrombosis

flow in it and in its feeding branches, as well as in collaterals developed due to the portal hypertension.

Some of the signs of portal hypertension are the following: increased portal vein diameter (more than 13 mm), portal vein thrombosis (Figures 1 and 2), the presence and the detection of portosystemic collaterals. Increased diameter of the perigastric collaterals that exceeds 6–7 mm is a highly sensitive sign of portal hypertension; however, it is very rare and is seen in approximately 26% of patients with liver cirrhosis [6–10]. CT with contrast administration is a highly sophisticated diagnostic technique for displaying portal circulation. The advantage of CT scan compared with CDU is its high sensitivity and specificity in showing morphologic changes – both distal and proximal from location of the portal vein thrombosis, as well as existence of collaterals. X-ray radiation, iodine contrast agent and inability to determine the speed and direction of flow through blood vessels make CT inferior to CDU. MRI is used in situations when radiologist estimates that CDU and contrast CT scan data are insufficient for evaluation, or when there is a discrepancy in the findings of the previously mentioned techniques.

This study provides original contribution to the field of radiology in our region as it points to the possibility of early and accurate diagnosis when using combined radiological techniques (CDU and contrast CT scan), which is not the case when using these techniques separately.

## OBJECTIVE

This study was based on the testing of hypothesis that more data and more precise diagnosis in patients with disorders of portal circulation can be obtained by using CDU and CT with contrast. CDU was used as it shows hemodynamic parameters for early detection of portal hypertension, while CT with contrast was used as it is a technique that provides accurate data on morphological changes, especially in the detection of collateral network and any obstruction caused by thrombosis. With adequate selection of diagnostic and therapeutic algorithm, and with sophisticated choice of patients, what is achieved by combining CDU and contrast CT is that patients are not exposed to the unnecessary radiation. It has been found that the cumulative sensitivity and specificity of the tested parameters is greater, which justifies the combination of these diagnostic techniques. A relatively small number of articles have been published regarding this topic.

## METHODS

The study was designed as a prospective study that comprised 120 patients with liver disease and with high suspicion of portal hypertension. The study included patients who had a clinical diagnosis of liver cirrhosis, hepatitis, portal vein thrombosis, liver tumor, and/or splenomegaly caused by liver disease. The most frequent complications of portal hypertension were bleeding from gastroesophageal varices and ascites in patients with cirrhosis. Patients were divided into three equal groups comprising 40 patients each:

Group 1 – patients examined only by US and CDU;

Group 2 – patients examined only by contrast CT scan, and

Group 3 – patients examined using both CDU and CT.

All three groups had control examinations after six months of therapy and were examined by the same diagnostic technique used during their initial examination. The main inclusion criterion was clinical diagnosis of portal hypertension. The main exclusion criteria were low level of suspicion of portal hypertension, patients with renal insufficiency, and patients under the age of 30.

For statistical analysis we used Statistical Package for Social Sciences software – SPSS 21 for Windows (IBM Corp., Armonk, NY, USA). The analysis used standard methods of descriptive and differential statistics. Numerical characteristics are shown by mean values (arithmetic mean) and a measure of variability (range of values, standard deviation) and attributive characteristics by using frequencies and percentages.

Comparison of numerical values of characteristics between the groups was performed using Student's t-test; statistically significant values are considered to be at the level of $p < 0.05$. The results are presented in tables.

## RESULTS

At the Center of Radiology, Clinical Center of Vojvodina, Novi Sad, Serbia, during a period of three years patients with symptoms of gastrointestinal disease and/or biliary tract disease and patients with hematological symptoms were examined using conventional ultrasonography with CDU on Logiq 7 (GE Healthcare, Chicago, IL, USA) machine with a 3.5 MHz probe, and with multislice computed tomography using SOMATOM Sensation Cardiac 64 (Siemens Healthcare GmbH, Erlangen, Germany). A greater number of males were included in the study, i.e. there were 86 (71.7%) males and 34 (28.3%) females. The age of the patients is presented in Table 1. Percentage of patients with the diagnosis of liver cirrhosis amounted to 63.3%, of which 95% was of alcoholic etiology, while the remaining 5% of patients were with biliary, cryptogenic, and immunogenic cirrhosis (Table 2).

At the first examination, in the first group of patients diagnosed using CDU, the average value of the diameter of the portal vein was 12.73 mm, and at the control examination after six months the average lumen width was 12.05 mm. There was a statistically significant difference in the values of measurements ($t = 2.859$, $p < 0.01$). The results of CDU parameters are presented in Table 1. At the first examination, the average flow rate through the portal vein was 0.18 m/s, and at the control examination after six months it was 0.21 m/s. There was a statistically significant difference in the values of measurements ($t = -3.269$, $p < 0.01$). An important parameter in the assessment of portal hypertension is certainly a "congestion index" (CI) of the portal vein. The "congestion index" is used to mean the ratio between the cross-sectional area ($cm^2$) and the blood flow velocity (cm/s) of the portal vein, as determined by a CDU [11]. It is used for identification of the initial state of portal hypertension. CI values (cm × s) at the first examination and at the control examination after six months were displayed as increased CI (Table 3).

At the first examination, in the second group of patients examined only by contrast CT scan, the average value of the diameter of the portal vein was 14.21 mm, and at the control examination after six months the average lumen width was 12.75 mm. There was a statistically significant difference in the values of measurements ($t = 3.121$, $p < 0.01$). The parameters in surveying by contrast CT scan are presented in Table 3.

The third group of patients was examined by using both techniques, i.e. CDU and contrast CT, and the parameters are presented in Table 4. The average value of the diameter of the portal vein at the first examination was 12.65 mm when done by CDU and 13.42 mm when done by CT. At the control examination after six months the average lumen width was 12.57 mm when done by CDU and 13.17 mm when done by CT. There was a statistically significant difference in the measurement of the average diameter of the portal vein at the first examination between the values determined by the CDU and CT ($t = -2.215$, $p < 0.05$).

**Table 1.** Age distribution of patients

| Age groups (years) | Number of patients |
|---|---|
| 16–25 | 1 |
| 26–35 | 3 |
| 36–45 | 6 |
| 46–55 | 29 |
| 56–65 | 61 |
| 66–75 | 20 |

**Table 2.** Percentage of patients with referred diagnosis

| Referred diagnosis | % |
|---|---|
| Liver cirrhosis | 63.3 |
| Thrombosis of the portal vein | 10.0 |
| Hepatitis B and C | 9.2 |
| Portal hypertension | 7.5 |
| Thrombosis in the mesocaval shunt | 3.3 |
| Cavernous transformation | 3.3 |
| Preparation for transplantation | 3.3 |

**Table 3.** The results of values obtained by color Doppler ultrasonography (CDU) and by multi-slice computer tomography (MSCT) separately

| Parameter | Values obtained by CDU | | | | Values obtained by MSCT | | | |
|---|---|---|---|---|---|---|---|---|
| | First exam | | Follow-up at 6 months | | First exam | | Follow-up at 6 months | |
| | N | % | N | % | N | % | N | % |
| Increased diameter of the portal vein | 11 | 27.5 | 7 | 17.5 | 23 | 57.5 | 9 | 22.5 |
| Thrombosis of the portal vein | 1 | 2.5 | 2 | 5.0 | 12 | 30.0 | 5 | 12.5 |
| PV flow – reduced | 32 | 80.0 | 25 | 62.5 | - | - | - | - |
| Congestion index – increased | 12 | 30.0 | 4 | 10.0 | - | - | - | - |
| Hepatopetal flow | 37 | 92.5 | 37 | 92.5 | - | - | - | - |
| Hepatofugal flow | 2 | 5.0 | 2 | 5.0 | - | - | - | - |
| Flow not respiratory-dependent | 31 | 77.5 | 29 | 72.5 | - | - | - | - |
| Presence of portosystemic collaterals | 2 | 5.0 | 2 | 5.0 | 28 | 70.0 | 28 | 70.0 |
| Absence of portosystemic collaterals | 38 | 95.0 | 38 | 95.0 | 12 | 30.0 | 12 | 30.0 |
| Increased diameter of the splenic vein | 28 | 70.0 | 14 | 35.0 | 10 | 25.0 | 5 | 12.5 |
| Increased AP diameter of the liver | 18 | 45.0 | 13 | 32.5 | 30 | 75.0 | 24 | 60.0 |
| Increased AP diameter of the spleen | 25 | 62.5 | 21 | 52.5 | 25 | 62.5 | 15 | 37.5 |

N – number of patients; PV – portal vein; AP – anterior-posterior diameter

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**Table 4.** The results of values receive by using color Doppler ultrasonography (CDU) and contrast multi-slice computer tomography (MSCT) together

| Parameter | CDU + MSCT | | | |
|---|---|---|---|---|
| | First exam | | Follow-up at 6 months | |
| | N | % | N | % |
| Increased diameter of the portal vein | 13 | 32.5 | 9 | 22.5 |
| Thrombosis of the portal vein | 6 | 15.0 | 4 | 10.0 |
| Presence of portosystemic collaterals | 3 | 7.5 | 3 | 7.5 |
| Increased diameter of the splenic vein | 5 | 12.5 | 5 | 12.5 |
| Increased AP diameter of the spleen | 24 | 60.0 | 24 | 60.0 |
| Increased AP diameter of the liver | 28 | 70.0 | 25 | 62.5 |

N – number of patients; AP – anterior-posterior diameter

No significant difference in diameter measured by CDU and CT ($t = -1.220$, $p > 0.05$) was observed at the control examination (Table 4).

## DISCUSSION

Lumen width of the portal vein was checked in patients who underwent only the CDU examination. The results from this study are consistent with the results of Haag et al. [12]. In a group of 375 patients with liver cirrhosis, who were examined by CDU, Haag et al. [12] found that 112 (30%) patients had expanded lumen of the portal vein, as was found to be the case in our study as well. Our research showed that the sensitivity of CDU in the measurement of the width of the lumen was 85.7% and specificity 97.1%. Lim et al. [13] found that portal vein thrombosis is the most common cause of portal prehepatic hypertension. The authors state that idiopathic thrombosis is rare, but that it is always a complication of other diseases and syndromes. The three causes of thrombosis of the portal vein identified in our study, ordered in their frequency of occurrence, are as follows: liver cirrhosis, hepatocellular carcinoma, and Budd–Chiari syndrome. Since the mid-80s of the 20th century, several studies have been published regarding the functional measurement of portal flow velocity [14–16]. In our study, after the first CDU examination, 32 (80%) patients were found to have decreased flow due to portal hypertension determined by standardized clinical protocols according to Child–Pugh classification [17]. At the control examination after six months, a reduced flow rate was found in 25 (62.5%) patients. This improvement of the flow rate (80% vs. 62.5%) can be explained by a good therapeutic response of the patients to the treatment. Similar data in the literature have not been found. An inverse or hepatofugal flow is one of the indicators of the portal hypertension, which may be registered in some or all of the segmental veins. Our research showed that at the first and at the control CDU examination hepatofugal flow was diagnosed in 2 (5%) patients. Survey data are approximate to the results of the study conducted by Gaiani et al. [18], where out of 228 patients, 68 (3%) had hepatofugal flow. Portosystemic shunt can be seen in 80–90% of patients with portal hypertension [19–21]. Most often, the CDU could register flow in dilated umbilical vein, in gastroesophageal and splenorenal collaterals, as well as in mesenteric collaterals, which are very rare [22–26]. In our study, in both examinations, CDU showed low sensitivity of 6.7%, while specificity was 100%. The sensitivity of CDU was low, as most of the patients were inadequately prepared, i.e. they were meteoristic and/or had large ascites, which made visualization of organs difficult, particularly the presence of portosystemic collaterals. The duration of the hemodynamic disturbances affects the value of the CI as the pathological changes in vein progress, unless the adequate therapeutic treatment is applied [27]. In a study of 64 liver cirrhosis patients conducted by Moriyasu et al. [27] sensitivity was 55%. Sensitivity recorded in our study was 47.1%, which is slightly lower when compared to the results of Moriyasu et al. [11]. This difference is most likely due to the fact that the patients had varying degrees of damage of the liver parenchyma and variously developed portosystemic collaterals, which could affect differences in flow rate and thus the CI. In portal hypertension the flow is slow and complete loss of oscillation is observed, the curve is not respiratory dependent [28]. Results from our study correlate with the research of Safak et al. [29], which showed that 70% of patients did not have the respiratory-dependent flow. This stipulated fact indicates that ultrasound is a primary diagnostic technique in determination of the diameter of lienal vein in patients with portal hypertension. Sensitivity of this study was 80% and specificity was 93.3%. Splenomegaly is a common finding in patients with portal hypertension. Ultrasonography has high sensitivity (92%) and specificity (100%) in case of splenomegaly, as determined in our research, which correlates with the literature, which quotes similar results, i.e. sensitivity 95% and specificity 98% [30]. A combination of the two diagnostic techniques (CDU and contrast CT scan) gives more precise information on the morphology and hemodynamic changes in patients with portal hypertension, which is not the case when these techniques are used separately. In Group 3, in patients examined by CDU and CT, the diameter of the portal vein showed that the cumulative sensitivity was 93.3% and the specificity was 100%. Furthermore, sensitivity and specificity for the presence of thrombus in the portal vein and splenomegaly was 100%. In measuring the width of lienal vein lumen, the cumulative sensitivity was 71.5% and specificity was 100%. The study previously mentioned intra-and extrahepatic portosystemic collateral in patients with portal hypertension [31, 32]. In determination of portosystemic collaterals, we found that cumulative sensitivity of CDU and CT was 41.9% and specificity was 100%. Low cumulative sensitivity of both techniques in the diagnosis of portosystemic collaterals can be explained by low sensitivity of CDU, which directly affects cumulative value and is caused by the previously mentioned reasons regarding difficult visualization by CDU (due to presence of gas, ascites, etc.). Similar data was stated by Nelson et al. [33] in their study of 64 patients, where they had a good correlation in diagnosing esophageal varices found in 68% of the patients, while the percentage of patients with splenic and spleno-renal varices was significantly lower and was found in 22% of the patients.

## CONCLUSION

The advantage of CDU is possibility to determine the morphologic and hemodynamic parameters. These parameters allow us to determine the presence and direction of flow. CT with contrast administration is highly sophisticated diagnostic technique in the detection of morphologic changes like collateral network and any obstruction caused by thrombosis. X-ray radiation, iodine contrast agent, and inability to determine the speed and direction of flow through the blood vessels make CT inferior to CDU. A cumulative sensitivity and specificity in most parameters (diameter of the portal vein, the presence of thrombus in the portal vein, lienal vein diameter, anterior–posterior diameter of the liver and spleen) was higher than when these techniques are used separately, except for determination of portosystemic collaterals (sensitivity of CDU and contrast CT scan was 41.9%, and specificity of both techniques was 100%) in patients with portal hypertension.

This study emphasizes the possibility of early and accurate diagnosis when combining two radiological methods (CDU and contrast CT scan), which is not the case when these methods are used separately.

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# Улога колор доплер ултрасонографије и компјутеризоване томографије у процени портне хипертензије

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**КРАТАК САДРЖАЈ**

**Увод** Обољења јетре праћена поремећајем јетрене и спланхничне циркулације изазивају портну хипертензију, са тромбозом вене порте или без ње.

**Циљ рада** Ово испитивање је засновано на провери хипотезе о добијању већег броја података и прецизнијег постављања дијагнозе код болесника са поремећајем портне циркулације коришћењем колор допплер ултрасонографије (КДУ) и контрастне компјутеризоване томографије (КТ).

**Методе рада** Истраживање је обухватило 120 болесника, од фебруара 2011. до маја 2014. године, са сумњом или већ клинички потврђеном портном хипертензијом, болеснике са хепатитисом и неколико болесника са хематолошким обољењима. Прва група од 40 болесника је прегледана конвенционалном ултрасонографијом и КДУ, друга група контрастним КТ и трећа група болесника је прегледана уз помоћ обе методе (КДУ и контрастним КТ). Шест месеци након примењене адекватне терапије начињени су контролни прегледи.

**Резултати** Ретроспективна анализа је показала да је КДУ осетљивија од КТ у процени постојања и старости тромба (КДУ 93,9%, КТ 86,1%). КТ даје прецизне податке у откривању портосистемских колатерала. Осетљивост КТ је 100%, а специфичност 67%. Кумулативна осетљивост и специфичност у већини параметара су повећане у односу на методе понаособ.

**Закључак** Ова студија истиче могућност постављања ране и прецизније дијагнозе комбиновањем две дијагностичке методе (КДУ и КТ са интравенским контрастом), што није случај када се те методе примењују појединачно.

**Кључне речи:** колор доплер ултрасонографија; портна хипертензија; портна тромбоза; компјутеризована томографија

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# Prevalence and risk factors of vascular calcification in pre-dialysis patients with Balkan endemic nephropathy

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**SUMMARY**

**Introduction** Vascular calcifications (VC) are common in patients with chronic kidney disease and present one of manifestations of mineral and bone disorders in these patients.

**Objective** The aim of this pilot study was to examine the prevalence and risk factors of VC in pre-dialysis patients with Balkan endemic nephropathy (BEN) and other kidney diseases.

**Methods** The study involved 32 pre-dialysis patients, 15 with BEN and 17 with other kidney diseases. All the patients underwent an interview, objective examination, routine laboratory analyses and measurement of serum concentration of intact parathyroid hormone (iPTH), 25-hydroxyvitamin D3 [25(OH)D3] and osteopontin. VCs in iliac, femoral, radial, and digital arteries were evaluated and Adragao VC score was calculated. The samples of radial artery were collected during the first creation of an arteriovenous fistula, and expression of osteocalcin, bone morphogenic protein-2 osteopontin, and matrix Gla-protein in arterial wall were examined.

**Results** Patients with BEN were significantly older (71.1 ± 6.1 vs. 54.7 ± 11.1 years), but they had significantly lower systolic and mean blood pressure (95.7 ± 13.2 mmHg vs. 104.3 ± 7.4 mmHg) and lower serum concentration of phosphorus (1.32 ± 0.36 mmol/l vs. 1.65 ± 0.35 mmol/l) and cholesterol (4.3 ± 1.1 mmol/l vs. 5.2 ± 0.8 mmol/l) than patients with other kidney diseases. Mean VC score was significantly lower in patients with BEN than in those with other kidney diseases (2.8 ± 1.7 vs. 4.6 ± 1.8; p = 0.009), but expression of four examined proteins in arterial wall differed insignificantly between the two groups. VC score correlated significantly with serum concentrations of cholesterol, triglycerides (positively), and iPTH (negatively).

**Conclusion** Pre-dialysis BEN patients had a significantly lower mean score of VC than patients with other kidney diseases.

**Keywords:** vascular calcification; pre-dialysis patients; Balkan endemic nephropathy

## INTRODUCTION

Balkan endemic nephropathy (BEN) is a familial, chronic, tubule-interstitial disease that occurs in limited areas of the Balkan Peninsula. The disease is usually without symptoms, progresses slowly and today is mainly revealed in the sixth decade of life [1, 2]. Therefore, the diagnosis is usually reached in the advanced stage of the disease when tubular disorders, metabolic disorders characteristic for chronic renal failure including mineral and bone disorders as well as moderate hypertension and anemia are also present.

Vascular calcifications (VC) are common in patients with chronic kidney disease and represent a significant predictor of both general and cardiovascular mortality [3, 4, 5]. Pathogenesis of VC has been the subject of many studies that have found its association with mineral metabolism disorders and renal bone disease [5, 6]. Today, it is considered that VC is not the result of passive deposition of calcium phosphate, but the result of an active process similar to the process of bone calcification. This process presents a series of complex biochemical and cellular events in which a number of regulatory proteins that induce or inhibit the deposition of minerals in the blood vessels actively participate [4–7].

In BEN as chronic tubule-interstitial disease hyperphosphatemia, one of the main pathogenic factors of VC is uncommon even in patients on regular hemodialysis [8]. It could be proposed that the prevalence of VC in BEN is lower than in other diseases, although there is no published information on this topic.

## OBJECTIVE

The aim of this pilot study was to compare prevalence of VC and factors that may contribute to their development in pre-dialysis patients with BEN and other kidney disease.

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## METHODS

### Patients

The study involved 32 patients in the fifth stage of chronic kidney disease before starting dialysis treatment, examined at the moment of creation of arteriovenous fistula. The patients were divided into two groups; the first consisted of 15 patients with BEN as primary kidney disease, and the second of 17 patients with other kidney diseases (glomerulonephritis in six, diabetic nephropathy in four, pyelonephritis in four, and three patients with other kidney diseases). During the interview and objective examination, the following data of all the patients were registered: age, sex, duration of chronic kidney disease, and a risk factor of cardiovascular diseases (hypertension, diabetes mellitus, dyslipidemia, and smoking), body mass index, blood pressure.

### Laboratory analyses

Hemoglobin level as well as serum concentrations of calcium, phosphorus, alkaline phosphatase, iron, total cholesterol, LDL cholesterol, and triglycerides were measured by standard laboratory methods. Serum concentration of intact parathyroid hormone (iPTH) was measured by immunochemiluminescent method on Cobas 6000 modular (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) (normal value: 15–65 pg/ml), 25-hydroxyvitamin D3 [25(OH) D3] by electrochemiluminescence immunoassay – ECLIA on Cobas 6000 modular (normal value: 75–250 ng/ml) and osteopontin by ELISA technique using sandwich human osteopontin ELISA kit (Abcam plc., Cambridge, United Kingdom) and Microplate Reader RT-2100 C (Rayto Life and Analytical Sciences CO. Ltd., Shenzhen, China) (normal value ≥5 ng/ml). Glomerular filtration rate (eGFR) was estimated by the MDRD equation: eGFR = 175 × serum-creatinine$^{-1.154}$ × age$^{-0.203}$ × 0.742 (women) [9].

### Radiological examinations

VCs in the iliac, femoral, radial, and digital arteries were evaluated by one radiologist (S.R.) in plain radiographic films of pelvis and hands in all examined patients. A simple VC score was calculated as described by Adragao et al. [10]. In brief, radiographic films of the pelvis were divided into four sections by two lines – a horizontal line over the upper limit of both femoral heads, and a vertical line along the middle of the vertebral column. The films of each hand were divided by a horizontal line over the upper limit of the metacarpal bones. The presence of linear calcifications in each part of the film was counted as 1, and its absence as 0. The VC score was the sum of scores in all parts of films ranging from 0 to 8. VC were quantified as 0 = no calcification, 1–3 = mild calcification, and ≥4 severe calcification.

### Histopathological analysis

A sample of radial artery, 3–5 mm in length, was collected during the first creation of arteriovenous fistula for hemodialysis access. The sample was fixed in buffered formalin for 12–24 hours, then processed and embedded in paraffin. The sections of 5 μm were used for immunohistochemical analysis done by an experienced pathologist blinded to the clinical data. To demonstrate the expression of osteocalcin, bone morphogenic protein-2 (BMP-2), osteopontin and matrix Gla-protein (MGP) in the wall of the artery, immunohistochemical staining with the following human-specific antibodies was used: rabbit polyclonal antibodies to osteocalcin, BMP-2, and MGP, and rabbit monoclonal antibody to osteopontin, all produced by Abcam, Cambridge, United Kingdom. Protein expression was quantified using a semi-quantitative scoring system (0 = no expression; 1 = weak or moderate focal or diffuse weak expression; 2 = high focal or moderate diffuse expression; 3 = high diffuse expression) in high-power fields (×200 magnification). Scores of 0 and 1 were considered to be a negative finding, and scores 2 and 3 positive.

### Statistical analysis

Continuous variables are presented as mean and standard deviation, and categorical as frequencies. The Student's t-test was used to assess differences between continuous variables and the $\chi^2$ and Fisher's exact test between categorical variables, as appropriate. Correlation between continuous variables was calculated using Pearson's linear correlation coefficient, and correlation between categorical variables by Cramer's phi coefficient. All analyses were performed using the SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

Table 1 presents the main characteristics of the patients studied. Patients with BEN were significantly older than patients with other kidney diseases and had significantly lower systolic blood pressure as well as mean blood pressure (95.7 ± 13.2 mmHg vs. 104.3 ± 7.4 mmHg; p = 0.037). All but two patients in each group had hypertension and were treated with antihypertensive drugs, more frequently

**Table 1.** The basic characteristics of the studied patients

| Characteristics | Patients | | p |
|---|---|---|---|
| | With BEN | With other kidney diseases | |
| Number of patients | 15 | 17 | |
| Sex – male | 11 | 9 | 0.234 |
| Age (years) | 71.7 ± 6.1 | 54.7 ± 11.1 | <0.0001 |
| BMI (kg/m$^2$) | 25.0 ± 3.2 | 24.8 ± 5.1 | 0.903 |
| Systolic BP (mmHg) | 133.5 ± 22.3 | 151.5 ± 14.5 | 0.014 |
| Diastolic BP (mmHg) | 76.9 ± 8.9 | 79.2 ± 8.1 | 0.439 |
| Duration of CKD (years) | 3.5 ± 2.5 | 4.1 ± 2.8 | 0.543 |
| $CaCO_3$ (No. of patients treated) | 11 | 13 | 0.838 |
| Calcitriol (No. of patients treated) | 15 | 17 | 1.0 |

BEN – Balkan endemic nephropathy; BMI – body mass index; BP – blood pressure; CKD – chronic kidney disease

**Table 2.** Comparison of laboratory findings between patients with Balkan endemic nephropathy (BEN) and other kidney diseases

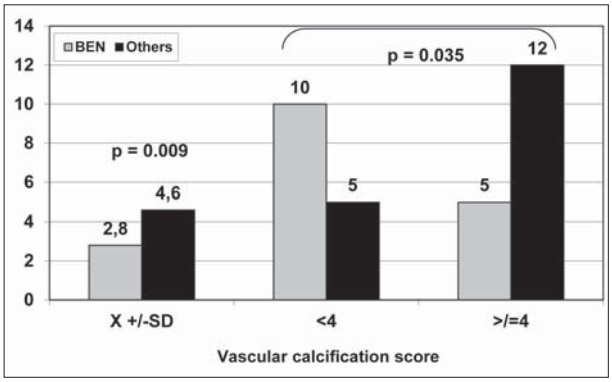
| Laboratory findings | Patients | | p |
|---|---|---|---|
| | with BEN | with other kidney diseases | |
| eGFR-MDRD (ml/min/1.73 m²) | 14.5 ± 2.8 | 7.5 ± 1.5 | 0.759 |
| Calcium (mmol/l) | 2.40 ± 0.24 | 2.33 ± 0.15 | 0.298 |
| Phosphorus (mmol/l) | 1.32 ± 0.36 | 1.65 ± 0.35 | 0.015 |
| Ca × P (mmol²/l²) | 3.19 ± 0.98 | 3.81 ± 0.78 | 0.059 |
| Alkaline phosphatase (U/l) | 74.5 ± 25.4 | 86.2 ± 22.7 | 0.179 |
| PTH (pg/ml) | 188.6 ± 105.4 | 150.0 ± 115.6 | 0.331 |
| Vitamin D (ng/ml) | 51.1 ± 22.1 | 53.0 ± 23.1 | 0.809 |
| Osteopontin (ng/ml) | 59.2 ± 32.5 | 87.3 ± 82.9 | 0.211 |
| Cholesterol (mmol/l) | 4.3 ± 1.1 | 5.2 ± 0.8 | 0.049 |
| LDL cholesterol (mmol/l) | 2.4 ± 0.8 | 2.6 ± 0.6 | 0.457 |
| Triglycerides (mmol/l) | 1.9 ± 0.9 | 2.3 ± 0.8 | 0.134 |
| Hemoglobin (g/l) | 114.3 ± 10.5 | 116.1 ± 8.1 | 0.575 |
| Iron (μmol/l) | 13.6 ± 5.3 | 15.3 ± 6.6 | 0.446 |

eGFR – estimated glomerular filtration rate; MDRD – Modification of Diet in Renal Disease; PTH – parathormone; LDL – low-density lipoprotein

with angiotensin-converting enzyme inhibitors. The differences between the two groups in body mass index, diastolic blood pressure, and duration of chronic kidney disease were not significant.

The results of laboratory analyses are presented in Table 2. There was no significant difference in eGFR between the two groups. Patients with BEN had a significantly lower serum concentration of phosphorus and cholesterol than patients with other kidney diseases, while the difference in the product of phosphorus and calcium was on the border of statistical significance. No statistical significance was found between the two groups in other laboratory findings. It should be noted that although mean serum calcium concentration was near the upper limit of target range proposed by The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines [11], analysis of individual values showed that about one third of patients had mean serum calcium concentration above the target range (5/15 of BEN patients, and 6/17 of others). Serum iPTH concentration was similar in both groups and about one half of patients of both groups had iPTH concentration below 150 pg/ml, the lower limit of KDOQI target range (seven BEN patients and nine others).

Graph 1 shows the score of VC estimated in X-ray films of hands and pelvis. When compared the number of patients with a score of VC less than or equal to 4 and greater than 4, it appeared that 10/15 of patients with BEN had score below 4, while this was the case with only 5/17 of patients with other kidney diseases. Pearson's $\chi^2$ test showed that this difference was statistically significant. In addition, patients with BEN had a significantly lower mean score of VC (2.8 ± 1.7) than patients with other kidney disease (4.6 ± 1–8; p = 0.009). Expression of four examined proteins in arterial wall differed insignificantly between the two groups of patients: 3/13 of BEN patients and 1/14 of patients with other kidney disease had an expression score equal to or greater than 2 for osteocalcin; 1/13 of BEN and 1/14 of others for BMP; 6/13 of BEN and 5/14 of others for osteopontin; and 3/13 of BEN and 5/14 of other patients for MGP.




**Graph 1.** Vascular calcification score in patients with Balkan endemic nephropathy (BEN) and other kidney diseases

Patients of both groups were divided into two subgroups – one with a VC score equal or less than 4, and the other with the score greater than 4. Characteristics of these subgroups were compared and are presented in Table 3. The number of BEN patients with VC score below 4 was significantly higher compared to patients with other kidney disease. Patients who had VC score below 4 had statistically significant lower concentrations of total cholesterol and triglyceride but higher concentration of iPTH, although the results bordered statistical significance. No significant difference was found between the patients with VC score less than 4 and equal to or greater than 4 in the expression of osteocalcin, BMP-2, osteopontin, and MGP in the wall of the radial artery.

Six patients from each group had a positive history of angina pectoris, signs of ischemia on ECG were recorded in seven patients with BEN and in nine patients with other kidney diseases, but only among patients with other kidney diseases three patients had a history of myocardial infarctions. Also, the number of smokers was higher in the group with other kidney diseases than in the BEN group, i.e. five vs. two patients. However, all these differences did not reach statistical significance.

Table 4 presents the results of correlation between VC score and all demographic, clinical, and laboratory variables determined in both groups. Only variables that correlated significantly with VC score are presented. Pearson's linear correlation coefficient showed a significant positive correlation between VC score and serum concentration of cholesterol and triglycerides and significant negative correlation with serum iPTH concentration. It is interesting that between body mass index and VC score a negative, but insignificant, correlation was found (r = -0.326; p = 0.069). Cramer's phi coefficient used to investigate the correlation between categorical variables showed significant correlation between VC score and presence of peripheral vascular disease.

## DISCUSSION

This study showed that patients with BEN had a significantly lower mean score of VC than patients with other kidney diseases. The number of patients with BEN who

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**Table 3.** Comparison of patients with vascular calcification score less than 4 and equal to or greater than 4

| Parameter | | Vascular calcification score | | p |
|---|---|---|---|---|
| | | <4 | ≥4 | |
| Sex | Male | 10 | 10 | 0.647 |
| | Female | 5 | 7 | |
| Age (years) | | 65.3 ± 9.0 | 60.4 ± 14.6 | 0.263 |
| Diagnosis | BEN | 10/15 | 5/15 | 0.035 |
| | Other | 5/17 | 12/17 | |
| eGFR (ml/min/1.73 m$^2$) | | 8.3 ± 2.3 | 6.9 ± 1.9 | 0.075 |
| Cholesterol (mmol/l) | | 4.3 ± 1.0 | 5.3 ± 1.0 | 0.01 |
| Triglycerides, mmol/l | | 1.6 ± 0.5 | 2.6 ± 0.9 | 0.001 |
| S-iron | | 12.6 ± 5.5 | 16.2 ± 6.1 | 0.093 |
| PTH (pg/ml) | | 208.0 ± 135.6 | 132.9 ± 70.2 | 0.051 |
| S-calcium >2.4 (mmol/l) | | 6/15 | 5/17 | 0.720 |
| S-phosphorus >1.8 (mmol/l) | | 2/15 | 3/15 | 0.714 |
| Expression score | Osteocalcin ≥2 | 1/12 | 3/15 | 0.762 |
| | BMP-2 ≥2 | 0/12 | 2/15 | 0.565 |
| | Osteopontin ≥2 | 3/12 | 8/15 | 0.274 |
| | MGP ≥2 | 3/12 | 5/15 | 0.962 |

Patients of both groups are included in this analysis.
eGFR – estimated glomerular filtration rate; BMP – bone morphogenic protein, MGP – matrix Gla-protein
Expression score – expression of listed proteins in the radial artery wall quantified semi-quantitatively as described in Methods.

**Table 4.** Variables that significantly correlate with vascular calcification score

| Parameter | Pearson's coefficient of linear correlation | |
|---|---|---|
| | r | p |
| Cholesterol (mmol/l) | 0.444 | 0.011 |
| Triglycerides (mmol/l) | 0.520 | 0.002 |
| PTH (pg/ml) | -0.394 | 0.026 |
| Parameter | Cramer's phi coefficient | |
| | Value | p |
| Peripheral VD | 0.373 | 0.038 |

had a VC score less than 4 was significantly higher than that in patients with other kidney diseases. Analysis of factors that could be associated with the development of VC showed that patients with BEN had a significantly lower systolic and mean blood pressure, as well as serum concentration of cholesterol and phosphorus, lower product of phosphorus and calcium (on the borderline of statistical significance), but were significantly older than patients with other diseases. Significant positive correlation was found between VC score and serum concentration of cholesterol and triglycerides, but negative with serum iPTH concentration.

Numerous risk factors are associated with VC in chronic kidney disease. In addition to "traditional" risk factors, such as age, male sex, hypertension, diabetes, and dyslipidemia, "non-traditional" factors, such as uremic toxins, disorders of mineral metabolism and their regulatory hormones (PTH, vitamin D), excessive use of calcium salts as phosphate binders, inflammation, malnutrition, and oxidative stress substantively participate in the VC development [12]. BEN patients included in the present study were significantly older than patients with other kidney diseases and it was the only risk factor for VC that was more pronounced in patients with BEN than in others. Many authors reported that the age of patients with manifested BEN has shifted to the older ages [13, 14]. While most of the patients in the first descriptions of the disease were in the fourth decade of life [13, 15], in 1980s, most patients with manifested disease were in their sixth decade [1, 2]. Although age is a well known risk factor for VC, our BEN patients, in spite of older age than those with other kidney diseases, had less VC. However, several other risk factors for VC were less pronounced in patients with BEN than in those with other kidney diseases: BEN patients had significantly lower systolic and mean blood pressure, lower serum concentration of cholesterol and phosphorus than patients with other kidney diseases, and the difference in product of phosphorus and calcium between the groups was on the borderline of statistical significance. Some of these characteristics of BEN have already been described and our results confirmed these findings. Thus, in contrast to the earlier studies that reported usually normal blood pressure in patients with BEN [15], recent studies have shown that the prevalence of hypertension in BEN patients is similar to those with other kidney diseases but it is easier to regulate [16, 17, 18]. In our studies, all but two patients in each group had hypertension, which was, however, better regulated by antihypertensive drugs in BEN patients.

The present study also showed significantly lower serum concentration of cholesterol in BEN patients compared to patients with other kidney diseases. Pavlović et al. [19] found significantly lower total cholesterol and free cholesterol serum concentration in BEN family members than in members of non-BEN families living in the same location and healthy controls living outside the BEN region. The authors explained this lower concentration of cholesterol by lecithin-cholesterol acyltransferase deficiency found in BEN family members.

In addition to lower values of the two abovementioned risk factors for VC (blood pressure and serum cholesterol concentration), BEN patients also had a significantly lower serum phosphorus concentration in comparison to patients with other kidney diseases. The significantly lower serum phosphorus concentration in BEN patients led to lower production of calcium and phosphorus in BEN patients. Disorders of mineral metabolism present the most important pathogenic factors for VC in chronic kidney disease [20]. Experimental studies have shown that increasing phosphorus concentrations can induce human arterial vascular smooth muscle cells to transdifferentiate towards an osteoblastic phenotype [5, 21]. There is little data on mineral disorders in BEN. Bukvić et al. [8] were the only ones who described that hyperphosphatemia appeared very rarely in BEN patients on hemodialysis and that normal serum phosphorus concentrations in BEN patients were maintained even without the use of phosphate binders. Normal and low phosphorus level is uncommon in patients in advanced stages of chronic kidney disease. It can be caused by chronic tubular disorders and increased phosphate excretion, but also by malabsorption of phosphorus or phosphate binders overdosage [22, 23, 24]. Although we have no evidence, we can assume the increased phosphate excretion in our BEN patients as the cause of

lower phosphorus concentration. The majority of patients of both groups used calcium carbonate as phosphate binders but in similar moderate doses (BEN: 2.8 ± 0.51 g/day; others: 3.1 ± 0.81 g/day; p = 0.227). The use of calcium carbonate, the only available phosphate binder in our country, was one of the factors that caused serum calcium concentration above target range proposed by KDOQI guidelines in one third of the examined patients. This was undoubtedly one of the factors that contributed to the fact that the mean iPTH level in both groups was near the lower limit of KDOQI target guideline range for iPTH, and that about one half of patients of both groups had iPTH below the target range. Proportion of patients with iPTH below the target range is higher in our patients than in patients involved in several European studies, but significantly lower percentage of their patients used calcium-based phosphate binders [25, 26]. Comparative studies showed that the use of calcium-based phosphate binders is more frequently associated with episodes of hypercalcemia and low iPTH level than the use of calcium-free phosphate binders [27]. Low iPTH is one of risk factors for the development of VC and low bone turnover [28, 29, 30]. KDOQI guidelines suggest that, if the intact PTH levels fall below the lower target limit, calcitriol, vitamin D analogs, and/or calcimimetics should be reduced or stopped. All this suggests that in our limited conditions a careful follow-up of serum calcium and phosphorus concentration is required in order to maintain iPTH in the target range.

Our investigations of risk factors for VC were complemented with measuring serum osteopontin concentration, one of the inhibitors of extraskeletal calcification whose effect is independent of serum phosphorus and alkaline phosphatase concentration [30]. Also, the expression of osteocalcin, BMP-2, osteopontin, and MGP in the wall of the radial artery was examined. The results presented showed that there was no statistically significant difference in the serum concentration of osteopontin in patients with BEN and those with other kidney diseases. However, patients with BEN had a lower serum concentration of osteopontin than others, and in the radial artery of BEN patients osteopontin was expressed in six samples, while the expression of other examined proteins was found in less than three samples (data not presented). Nevertheless, no significant difference was found between the patients with VC score <4 and ≥4 in the expression of all four proteins in the wall of the radial artery. The recent analysis performed on the Amiens CKD database on VC biomarkers found traditional cardiovascular risk factors as a reliable predictor of VC but not new stimulators and inhibitors of VC previously suggested as major participants in VC in experimental studies [31]. In addition, several studies found traditional risk factors and serum concentration of calcium and phosphorus as the main risk factors for VC in patients with diabetes [32, 33]. Our results are in accordance with these data showing significant positive correlation between VC score and serum concentration of cholesterol and triglycerides and significant negative correlation with serum iPTH, regulatory hormone of mineral metabolism.

## CONCLUSION

Patients with BEN had a significantly lower mean score of VC than patients with other kidney diseases. Traditional risk factors and mineral disorders were found as the main risk factors for VC and patients with BEN had significantly less pronounced three of these factors – lower systolic blood pressure, lower serum cholesterol, and phosphorus concentration, but insignificantly lower product of calcium and phosphorus than patients with other kidney diseases. A significant difference in VC score between BEN patients and those with other kidney diseases obtained in this pilot study requires to be confirmed in a larger, multicenter study.

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# Преваленција и фактори ризика васкуларних калцификација код болесника са балканском ендемском нефропатијом у одмаклој хроничној инсуфицијенцији бубрега

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**КРАТАК САДРЖАЈ**

**Увод** Васкуларне калцификације (ВК) честе су код болесника са хроничним болестима бубрега и представљају једну од манифестација поремећаја метаболизма минерала ових болесника.

**Циљ рада** Циљ овог рада је био испитивање преваленције и фактора ризика ВК код болесника са балканском ендемском нефропатијом (БЕН) и другим болестима бубрега у терминалној бубрежној инсуфицијенцији пре започињања лечења дијализама.

**Методе рада** Испитивање је обухватило 32 болесника, 15 са БЕН и 17 са другим болестима бубрега. Поред анамнезе и објективног прегледа свим болесницима су урађене рутинске лабораторијске анализе, мерење концентрације интактног паратхормона (*iPTH*), 25-хидроксивитамина Д3 [*25(OH)D3*] и остеопонтина. ВК у илијачним, феморалним и дигиталним артеријама су процењене по методи *Adragao*. Узорак радијалне артерије узет је током операције прве артериовенске фистуле и у њему је методама имунохистологије испитана експресија остеокалцина, морфогеног протеина кости-2, остеопонтина и Гла-протеина матрикса.

**Резултати** Болесници са БЕН су били значајно старији (71,1 ± 6,1 вс. 54,7 ± 11,1 година), имали су значајно нижи систолни и средњи артеријски притисак (95,7 ± 13,2 *mm Hg* вс. 104,3 ± 7,4 *mm Hg*), нижу концентрацију фосфора (1,32 ± 0,36 *mmol/l* вс. 1,65 ± 0,35 *mmol/l*) и холестерола у серуму (4,3 ± 1,1 *mmol/l* вс. 5,2 ± 0,8 *mmol/l*) него болесници са другим болестима бубрега. Просечан скор ВК био је значајно мањи код болесника са БЕН у односу на болеснике са другим болестима бубрега (2,8 ± 1,7 вс. 4,6 ± 1,8; $p = 0,009$), али није било значајне разлике у експресији четири испитана протеина и зиду артерије. Утврђена је значајна корелација између скора ВК и концентрације холестерола, триглицерида (позитивна) и *iPTH* (негативна).

**Закључак** Болесници са БЕН у одмаклој хроничној инсуфицијенцији бубрега имали су значајно нижи скор ВК од болесника са другим болестима бубрега.

**Кључне речи:** васкуларне калцификације; хронична инсуфицијенција бубрега; балканска ендемска нефропатија

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# Biochemical liver function test parameter levels in relation to treatment response in liver metastatic colorectal patients treated with FOLFOX4 with or without bevacizumab

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**SUMMARY**

**Introduction** Combined use of bevacizumab and conventional anticancer drugs leads to a significant improvement of treatment response in patients with metastatic colorectal carcinoma (CRC). Conventional treatment protocols exert undesired effects on the liver tissue. Hepatotoxic effects are manifested as a disturbance of liver function test parameters. The relation between clinical outcome and disorder of biochemical parameters has not been completely evaluated.

**Objective** The objective of our study was to examine whether clinical outcome in patients with liver metastatic CRC correlates with the level of liver function test parameters.

**Methods** The study included 96 patients with untreated liver metastatic CRC who received FOLFOX4 protocol with or without bevacizumab. Biochemical liver parameters were performed before and after the treatment completion. Treatment response was evaluated as disease regression, stable disease, and disease progression. The patients were divided into three groups according to the accomplished treatment response.

**Results** In the group of patients with disease regression the post-treatment levels of aspartate aminotransferase, alanine aminotransferase, and bilirubin were statistically significantly increased. In contrast to this, gamma-glutamyltransferase and protein post-treatment values were significantly lower in relation to initial values. In patients with stable disease, difference was found only in the level of proteins being lower after the treatment. In patients with disease progression, values of aspartate aminotransferase and bilirubin were significantly increased after completed treatment.

**Conclusion** Treatment responses are not completely associated with the level of liver function test parameters. The only parameter which correlated with treatment response is gamma-glutamyltransferase. Its decrease is accompanied with disease regression.

**Keywords:** bevacizumab; colorectal liver metastases; hepatotoxicity; liver function test parameters; treatment response

## INTRODUCTION

Colorectal carcinoma is the second leading cause of cancer death among all malignant diseases [1, 2, 3]. Irinotecan- or oxaliplatin-based regimens combined with fluorouracil and leucovorin (FOLFOX4) are established as first-line conventional chemotherapy protocols for metastatic colorectal carcinoma (mCRC) [4–7]. The development and addition of novel biological therapy to standard anticancer agents have significantly expanded treatment options in these patients. Results of the performed studies have shown that the addition of bevacizumab, a monoclonal antibody against vascular endothelial growth factor (VEGF), to first-line standard chemotherapy treatment protocols in patients with liver mCRC remarkably improved their therapeutic effect. This is reflected in clinically significant improvement of treatment response rate as well as in overall and progression-free survival [8, 9, 10].

Conventional chemotherapies exert direct hepatotoxic effect. The production of free oxygen radicals is considered to be the key event in chemotherapy-induced hepatic injury, which is manifested as a disturbance of liver biochemistry parameter values, or it can be histologically confirmed [11–18]. However, except for studies' data which emphasize the clinical benefit of combined use of bevacizumab and conventional anticancer drugs, knowledge about their influence on liver function status is limited. So far there are no data about the association between treatment response and biochemical liver function status in patients treated with conventional anticancer agents with or without bevacizumab.

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## OBJECTIVE

The objective of this study was to answer whether treatment responses correlate with the level of tested biochemical liver function tests parameters and how the addition of bevacizumab influences it. It has been found that in spite of the favorable therapeutic effect of the treatment, except for gamma-glutamyltransferase (GGT) level decrease, the other six liver function test parameters were unchanged or even aggravated.

## METHODS

The study group consisted of 96 patients with histologically confirmed liver mCRC, one or more unidimensionally measurable lesions (>1 cm according to the RECIST 1.1 criteria) [19], without the possibility for curative liver resection. The diagnosis of potentially resectable liver metastatic disease was based on computed tomography (CT) scan evaluation. The patients were treated with FOLFOX4/FOLFOX4 + bevacizumab as a first line chemotherapy protocol. The treatment was conducted at the Institute for Radiology and Oncology in Belgrade, Serbia. Demographic and clinical data were obtained by reviewing medical records for period from January 2009 to December 2014.

The study included only patients with previously untreated liver metastatic disease. Prior chemotherapy and radiotherapy for CRC treatment was allowed if they were completed at least one month before the patient inclusion in the study. Other inclusion criteria were as follows: the Eastern Cooperative Oncology Group (ECOG) (Eastern Cooperative Oncology Group)performance status score of 0–2, >18 years of age, normal hematologic, liver, and kidney function, and no contraindications for the drugs administration. Exclusion criteria were the following: previous other malignant disease except cervical carcinoma *in-situ* and basal cell skin cancer, known brain metastases, and clinically significant cardiovascular disease. The study was carried out in accordance with the Declaration of Helsinki, Good Clinical Practice Guidelines and institutional ethics committee requirements. All the patients gave their written informed consent before their participation in the study.

The patients were assigned to FOLFOX4 or FOLFOX4 + bevacizumab treatment protocol according to physician decision. FOLFOX4 chemotherapy protocol consisted of a two-hour infusion of leucovorin (20 mg/m$^2$) followed by a 5-FU iv bolus (400 mg/m$^2$) and a 22-hour infusion (600 mg/m$^2$) during the first two days, with oxaliplatin (135 mg/m$^2$) as a two-hour infusion on day 1 of a two-week cycle. Patients additionally treated with bevacizumab received it on the first day of therapy in a dose of 5 mg/kg.

The duration of bevacizumab administration was determined by a physician decision. In a case of grade 2/3 of nonhematologic toxicity (mucositis, diarrhea, and proteinuria), the chemotherapy was delayed for one week or until the patient's full recovery. In patients with grade 4 of mucositis, diarrhea, proteinuria (nephrotic syndrome), hypertension, thromboembolic events, and grade 3/4 of hemorrhagic events, as well as in those with gastrointestinal perforation, the treatment protocol was stopped and such patients were excluded from the study. In case of hematologic toxic events grade ¾, the hematology parameters were determined daily and the treatment was postponed until the patient's complete recovery. Assessment of adverse events during the treatment was performed using National Cancer Institute Common Toxicity Criteria (version 2.0) [20]. Each patient received at least four and at most twelve cycles of certain chemotherapy protocol. Patients were followed up until the end of the treatment or until the disease progression and switch to a second-line treatment protocol.

Before their enrolment into the study, assessments of vital signs, ECOG performance status, height, weight, endoscopic and radiologic examinations (abdominal ultrasound, chest X-ray, and multislice computerized tomography were performed for all the patients. Routine liver function test parameters [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, GGT, lactate dehydrogenase] relevant for monitoring chemotherapy hepatotoxic effects, were performed before and after the completion of the treatment. Their determination was performed using commercial biochemical tests on the Advia 1800 (Siemens Healthcare GmbH, Erlangen, Germany) biochemical analyzer.

Treatment response was evaluated after every fourth cycle until the completion of the study treatment. Response Evaluation Criteria in Solid Tumors guidelines version 1.1 were used to define all the responses. They were determined as disease regression (complete or partial regression), stabilization, and progression of the disease. Tumor responses were assessed by members (surgeon, medical oncologist, radiologist, and pathologist) of the joint interdisciplinary committee for gastro-intestinal tumors of the host institutions, who were not involved in the study.

Assuming that the addition of bevacizumab to standard FOLFOX4 protocol would lead to moderate difference of values of some biochemical liver parameters between the two groups of patients (effect size 0.30), a minimum of 82 evaluable patients was required. Statistical analyses were performed by using commercially available SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA) software package. The intent-to-treat patient population included all the patients who participated in the study. The usual descriptive statistic parameters were used in statistical analysis of the obtained results (median with interquartile range 25–75 percentiles). Depending on the normality of distribution of the observed parameters, Student's t-test for dependent or independent parametric characteristics and Wilcoxon signed-rank test and Mann–Whitney U-test for non-parametric characteristics were performed.

## RESULTS

All the included patients were treated and followed up in the study. There were 41 female (42.7%) and 55 (57.3%) male patients with median age of 60 years (range: 35–79

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years). Out of 96 enrolled patients, 52 (54.2%) were treated with combined use of FOLFOX4 and bevacizumab, while 44 patients (45.8%) received the FOLFOX4 protocol.

According to treatment response, the patients were divided into three groups: (1) patients with disease regression, (2) patients with stable disease, and (3) patients with disease progression.

Results of the study are summarized in Table 1 and Graphs 1, 2, and 3. The obtained results are given before and after the treatment.

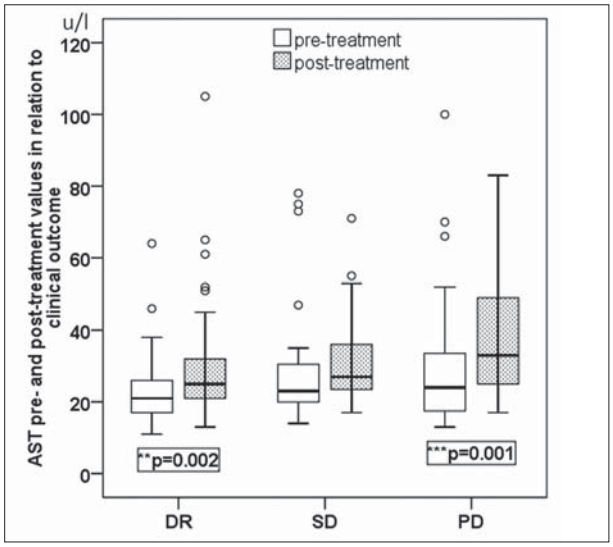
In the group of patients with disease regression as a post-treatment response, 7.3% achieved complete (CR) and 93.7% partial regression (PR) of the disease (not shown). Complete response was accomplished only in patients treated with combined use of FOLFOX4 and bevacizumab. At the same time partial regression was observed in patients on both treatment protocols, with the largest number of patients (68.2%) treated with FOLFOX4 + bevacizumab (not shown). Comparison of pre- and post-treatment values of tested biochemical parameters in these group of patients has shown that used anticancer agents led to the statistically significant increase in AST ($p = 0.002$), ALT ($p = 0.002$) and bilirubin ($p = 0.001$). In contrast to this, the level of GGT after the treatment was statistically significantly lower ($p = 0.035$) in relation to corresponding pre-treatment values (Table 1).

**Table 1.** Pre- and post-treatment values of biochemical liver test parameters in relation to clinical outcome in patients treated with conventional anticancer drugs (Group 1) and with bevacizumab added to conventional anticancer agents (Group 2)

| Biochemical parameters | Value | Disease regression (n = 41) | | Stable disease (n = 23) | | Progressive disease (n = 32) | |
|---|---|---|---|---|---|---|---|
| | | Pre | Post | Pre | Post | Pre | Post |
| AST (U/L) | Median | 21 | 25** | 23 | 27 | 24 | 33*** |
| | IQR | 17–26.5 | 21–33.5 | 20–32 | 23–36 | 17.2–33.7 | 25–50 |
| ALT (U/L) | Median | 21 | 26** | 20 | 25 | 23 | 26.5 |
| | IQR | 14–30 | 18–38.5 | 17–31 | 19–29 | 18–34.7 | 20.2–40.2 |
| ALP (U/L) | Median | 86 | 95 | 122 | 105 | 134 | 142.5 |
| | IQR | 69–116 | 73.5–127.5 | 91–222 | 89–143 | 104–206 | 111–184.5 |
| GGT (U/L) | Median | 42 | 36* | 77 | 61 | 87.5 | 77 |
| | IQR | 27–88.5 | 25–61.5 | 35–185 | 28–127 | 44.2–129.5 | 49–146.2 |
| LDH (U/L) | Median | 347 | 372 | 402 | 431 | 492 | 486 |
| | IQR | 288.5–387.5 | 325–419 | 347–926 | 345–624 | 320.2–944 | 432.5–732.7 |
| Bilirubin (μmol/L) | Median | 8.2 | 10.1** | 8.6 | 10.1 | 8.4 | 11.8*** |
| | IQR | 6.9–11.1 | 7.2–13.7 | 6.9–14 | 7.5–13.3 | 6.3–11.2 | 8.9–15.6 |
| Proteins (U/L) | Median | 73 | 72* | 74 | 71** | 73 | 72 |
| | IQR | 71–76 | 69–73.5 | 71–78 | 70–74 | 70–76.7 | 68–75.5 |

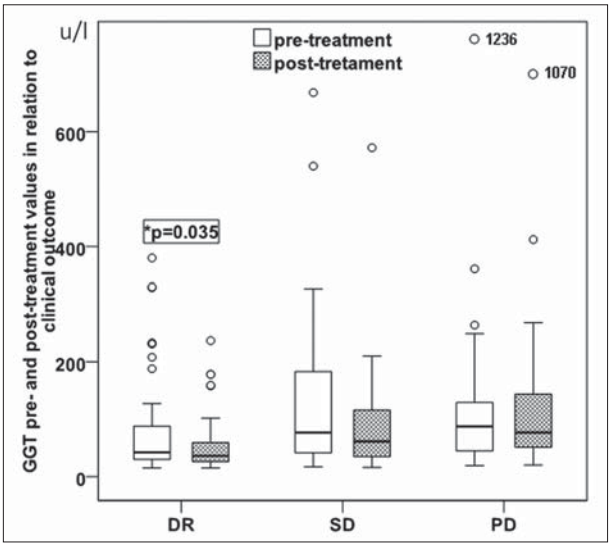
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$ intragroup pre- and post-treatment comparison

n – number of patients; AST – aspartate aminotransferase; U/L – units per liter; ALT – alanine aminotransferase; ALP – alkaline phosphatase; GGT – gamma-glutamyltransferase; LDH – lactate dehydrogenase; IQR – interquartile range



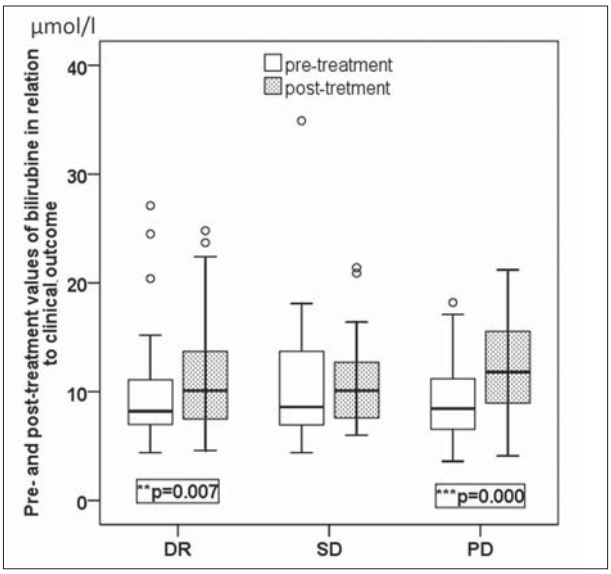

**Graph 1.** Pre- and post-treatment values of aspartate aminotransferase (AST) in relation to clinical outcome

** $p < 0.01$
*** $p < 0.001$ intragroup pre- and post-treatment comparison
DR – disease regression; SD – stable disease; PD – disease progression




**Graph 2.** Pre- and post-treatment values of gamma-glutamyltransferase (GGT) in relation to clinical outcome

* $p < 0.05$ intragroup pre- and post-treatment comparison;
** Extreme values of GGT in gruop of patients with PD are presented in the upper right corner
DR – disease regression; SD – stable disease; PD – disease progression




**Graph 3.** Pre- and post-treatment values of bilirubin in relation to clinical outcome

** p < 0.01
*** p < 0.001 intragroup pre- and post-treatment comparison
DR – disease regression; SD – stable disease; PD – disease progression

Of the 23 patients with stable disease, 56.5% (13 patients) received FOLFOX4 and the rest received FOLFOX4 + bevacizumab. The analysis of pre- and post-treatment results of tested liver function test parameters in these group of patients has shown that out of seven tested parameters, statistically significant difference was found only in the amount of protein. In this group of patients, as well as in those with disease regression, the level of protein after the treatment was statistically significantly decreased ($p = 0.012$ and $p = 0.010$, respectively).

Progression of the disease was much more pronounced in patients treated with FOLFOX4 chemotherapy protocol (71.9%). In patients with disease progression, an increase after the treatment was found in only two parameters – AST ($p = 0.001$) and bilirubin ($p = 0.000$) (Table 1).

Results of the intergroup analysis of difference of post and pre-treatment values have shown that statistically significant difference was found only in bilirubin values between groups with stable and progressive disease ($p = 0.017$) (not shown).

Pre- and post-treatment results of biochemical liver function test parameters were also compared between patients with the same treatment response, but on different treatment protocol. In this manner, groups comprising small number of patients were formed, which presents a limitation in presenting the obtained results. The results of the analysis have shown that there was no statistically significant difference between the tested parameters.

The absolute values of statistically significant results obtained after intragroup comparison of pre- and post-treatment values are graphically plotted (Graphs 1–3). Graph 1 shows that statistically significant difference between pre- and post-treatment results of ALT was found in groups of patients with disease regression and disease progression. Opposite to this, ALT pre- and post-treatment results in the group of patients with stable disease did not differ significantly. Graph 2 shows that statistically significant decrease of GGT post-treatment values was found only in the group of patients with disease regression. In two other groups of patients, no difference between GGT values before and after the treatment was found. Graph 3 shows that level of bilirubin after the treatment was significantly increased in patients with disease regression and in those with disease progression.

## DISCUSSION

The results of the study have shown that combined use of bevacizumab and FOLFOX4 was oncologically more effective than FOLFOX4 alone, demonstrated by better treatment response. Namely, stabilization of the disease was prominent in FOLFOX4 chemotherapy protocol, while complete or partial regressions were inherent in FOLFOX4 + bevacizumab. These findings are consistent with the results of other studies which have examined the efficacy of bevacizumab added to conventional cytotoxic therapy.

Also, it was observed that both treatment protocols led to an increase of some of the tested liver function parameters (AST, ALT, and bilirubin). These findings might indicate that according to biochemical liver status there was no significant difference in chemotherapy-induced liver injury between these two treatment protocols.

On the other side, when levels of tested biochemical parameters were correlated with the treatment response, significant disparity was noted. Favorable clinical outcome did not always respond with the improvement of the tested liver function parameters. On the contrary, it was found that the most pronounced increase of liver biochemical parameters (AST, ALT, and bilirubin) was observed in patients with disease regression in comparison to those with stable or disease progression (AST and bilirubin). These findings demonstrate that both liver metastases as the basic disease and conventional anticancer agents used for their treatment have significantly impaired liver tissue as the result and lead to the disturbance of some of biochemical liver parameters.

Presented results of the study indicate that GGT is the only parameter which correlates with treatment response. Level of this enzyme is in relation with patients' clinical improvement being decreased after the treatment.

Several studies have shown a relation between FOLFOX4 chemotherapy protocol and severe hepatic injury manifested as hepatic sinusoidal obstruction syndrome or steatosis [21–28]. In these diseases it is not uncommon that biochemical parameters remain normal despite the underlying histopathological liver damage. Unique liver potential to regenerate and its capacity to compensate disruption of biochemical parameters could be a possible explanation for these findings [11–14]. In accordance with this are GGT results obtained in the study. Alongside clinical improvement, reduction of post-treatment GGT values may be a result of a liver compensatory mechanism. Nevertheless, several liver parameters were increased after the

treatment. The biggest concern that needs to be clarified is what causes such discrepancy between the levels of biochemical parameters. In other words, there is a dilemma as to why the value of one of the most specific liver parameter (GGT) is decreased in FOLFOX4 treated mCRC patients, while other parameters remain increased or unchanged.

## CONCLUSION

To our knowledge, this is the first study to analyze the association between treatment response in patients with mCRC and biochemical liver function test parameters relevant for chemotherapy hepatotoxicity assessment. Recognizing GGT as a possible prognostic parameter for therapy effectiveness makes the study findings more important. However, it is necessary to conduct further investigations to confirm this claim.

## NOTE

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# Ниво биохемијских параметара функционалног статуса јетре у односу на клинички одговор код болесника са метастатским колоректалним карциномом који су лечени *FOLFOX4* протоколом са бевацизумабом или без њега

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**КРАТАК САДРЖАЈ**

**Увод** Удружена примена бевацизумаба и конвенционалних антиканцерских лекова доводи до значајног побољшања клиничког одговора код пацијената са метастатским колоректалним карциномом (*CRC*). Конвенционални протоколи лечења испољавају нежељене ефекте на ткиво јетре. Хепатотоксични ефекти хемиотерапије се манифестују у виду поремећаја вредности биохемијских параметара функционалног статуса јетре. Корелација клиничког исхода и поремећаја вредности биохемијских параметара још увек није у потпуности позната.

**Циљ рада** Сходно наведеном, циљ нашег рада је да провери да ли клинички исход код *CRC* болесника са метастазама на јетри корелира са вредностима биохемијских параметара јетре или не.

**Методе рада** У студију је укључено 96 болесника оболелих од *CRC* са метастатским променама на јетри који су третирани *FOLFOX4* протоколом са бевацизумабом или без њега. Биохемијски параметри јетре су анализирани пре почетка и на крају спровођења терапијског протокола. Клинички одговор болесника је процењен као регресија, стабилизација или прогресија болести. Болесници су према постигнутом клиничком одговору подељени у три групе.

**Резултати** У групи болесника са регресијом болести ниво *AST*, *ALT* и билирубина на крају лечења је статистички значајно повишен. Супротно томе, вредности гама-глутамил трансферазе (ГГТ) и протеина након спроведеног лечења су статистички значајно ниже у односу на иницијалне вредности. Код болесника са стабилизацијом болести разликује се само ниво протеина, који је значајно нижи на крају лечења у односу на иницијалне вредности. Код болесника са прогресијом болести вредности *AST* и билирубина су биле значајно повишене након спроведеног лечења.

**Закључак** Клинички одговор код болесника са метастатским *CRC* није у потпуности у корелацији са вредностима биохемијских параметара јетре. Једини параметер који корелира са клиничким налазом је ГГТ. Смањење његове вредности праћено је регресијом болести.

**Кључне речи:** бевацизумаб; колоректалне метастазе на јетри; хепатотоксичност; параметри функционалног статуса јетре; клинички одговор

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# Negative-pressure wound therapy for deep groin vascular infections

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**SUMMARY**

**Introduction** Infection of synthetic graft in the groin is a rare but devastating complication. When it occurs, several possibilities of treatment are available. Extra-anatomic reconstruction and *in-situ* implantation of new, infection resistant grafts are associated with high mortality and morbidity. Therefore, more conservative approach is needed in some cases. Negative-pressure wound therapy is one of the options in treating such patients.

**Objective** The aim of this study was to assess the outcome for deep groin vascular graft infection treated with negative-pressure wound therapy.

**Methods** Seventeen patients (19 wounds), treated for Szilagyi grade III groin infections between October 2011 and June 2014, were enrolled into this observational study.

**Results** Majority of the wounds (11/19) were healed by secondary intention, and the rest of the wounds (8/19) were healed by primary intention after initial negative-pressure wound therapy and graft substitution with silver-coated prostheses or autologous artery/vein implantation. No early mortality was observed. Minor bleeding was observed in one patient. Reinfection was noted in three wounds. Only one graft occlusion was noted. Late mortality was observed in three patients.

**Conclusion** Negative-pressure wound therapy seems to be safe for groin vascular graft infections and comfortable for both patient and surgeon. However, the rate of persistent infection is high. This technique, in our opinion, can be used as a "bridge" from initial wound debridement to definitive wound management, when good local conditions are achieved for graft substitution, either with new synthetic graft with antimicrobial properties or autologous artery/vein. In selected cases of deep groin infections it can be used as the only therapeutic approach in wound treatment.

**Keywords**: groin infection; synthetic graft infection; negative-pressure wound therapy

## INTRODUCTION

Majority of reconstructions in the field of arterial vascular surgery are associated with synthetic graft implantation. Among different complications that accompany their use, one of the most devastating is graft infection. It can occur in up to 5% of patients [1].

When diagnosed, several possibilities for treatment are available. The traditional treatment consists of the graft excision and extra-anatomic reconstruction, which is a definitive solution, although there are some circumstances when these reconstructions are not feasible, such as the occlusion of the popliteal artery, which does not allow transobturator bypass for groin infection. Also, more infection-resistant conduits can be utilized for *in-situ* reconstruction, such as autologous veins or arteries. Although not intended for use in an infected area, synthetic grafts with antimicrobial properties such as silver-coated and rifampicin-soaked prostheses could be an option when dealing with this devastating complication. All these procedures are associated with high morbidity and mortality [2]. In these settings patients are exposed to stress from surgery and other possible complications associated with it. Furthermore, reinfection rate is significant, and amounts to 18.5% for *in-situ* silver-coated grafts in infrainguinal localization [3]. Because of this, various graft preservation techniques have been increasingly utilized in an attempt to improve outcome [4, 5].

Argenta and Morykwas [6] as well as Morykwas et al. [7] studied negative-pressure wound therapy in plastic and reconstructive surgery, as well as in the treatment of mediastinitis after sternotomy for heart surgery. Now, it has emerged as a new therapeutic approach in dealing with infected wounds, including perivascular and arterial graft infections. Today, negative-pressure wound therapy has become routine in many hospitals worldwide. However, there are a limited number of papers dealing with Szilagyi grade III infections treated in this manner.

Negative-pressure wound therapy creates a moist wound-healing environment; it drains excessive fluid from the wound, reduces tissue edema, cleans deep wounds from bacteria, accelerates the formation of granulation tissue and induces faster approximation of wound edges [8, 9].

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Graft preservation may be an option when the anastomosis is intact – only a small part of the graft is exposed, the patient has no systemic signs of sepsis, the graft is patent, and the causative microorganism is not *Pseudomonas aeruginosa* [5]. It is reasonable to assume that in these circumstances even a clinically healed infection is transformed into a low-grade state, which can be reactivated during the course of time.

## OBJECTIVE

The aim of the present study was to assess early and short-term results obtained for deep groin vascular graft infection treated with negative-pressure therapy, as well as to compare treatment outcome between patients with early and late graft infections.

## METHODS

All patients with Szilagyi grade III infections in the groin who were treated at our institution from October 2011 to June 2014 were included in this observational prospective study with a retrospective analysis of the primary operation. Patients treated with extra-anatomic bypass or who underwent major amputation for vital indications were excluded.

Patient demographics, type of previous intervention, type of graft materials used in the second procedures performed, duration of negative-pressure wound therapy, early and short-term morbidity and mortality, complications of negative-pressure therapy, reinfection, type of wound healing, causative microorganisms, graft patency, and follow-up information (the condition of the groin, graft, ipsilateral limb, and overall health) were recorded.

The diagnosis of graft infection was established by interview, clinical examination, wound cultures, elevated white blood cell count ($>10 \times 10^9$/l), and multi-slice computed tomography to determine the extent of infection.

Broad-spectrum antibiotics were given initially and changed, accordingly, when appropriate wound cultures were obtained. Debridement of all wounds was performed prior to the start of negative-pressure wound therapy. In some patients, due to graft occlusion, new silver-coated grafts were inserted in the groin, as well as in patients who underwent lymphostasis. However, due to persistent infection, few patients underwent autologous vein/arterial graft insertion in order to achieve wound healing. Usually, two days after surgery negative-pressure wound therapy was started, when hemostasis was achieved. Vacuum therapy and wound dressing was performed in the manner suggested by the manufacturer (KCI Medical, San Antonio, TX, USA) with continuous negative pressure of 125 mmHg. Vaseline gauze was used to cover grafts and native arteries to avoid direct contact with the polyurethane sponge. The sponges were changed three times a week. All the patients were treated in hospital, and during the course they received adequate antibiotic treatment, which continued after their discharge with oral therapy for one month at the most.

Follow-up examination was performed after one, three, and six months thereafter. Clinical examination and ankle-brachial index determination were performed.

### Statistical analysis

Results are presented as number (%) for categorical data. Student's t-test and Fisher's exact test were used for analysis. For the difference between age, a bootstrapping method was used, and for the duration and costs, exact tests were performed. A two-sided $p < 0.05$ was considered significant. All the data were analyzed using SPSS 20.0 (IBM Corp, Armonk, NY, USA).

## RESULTS

Mean age of patients was 63.1 ± 10.7 years (range 42–85 years). Thirteen patients were male and four were female. Four patients were classified according to American Society of Anesthesiology (ASA) as IV, and 13 of them as ASA III stage. In all 17 patients, infection involved previously implanted synthetic grafts (and all of them were Dacron grafts), and was confined to the groin. Part of the anastomosis was exposed in 5/19 wounds after initial debridement. Diabetes mellitus was present in 70.6% of patients, and five (29.4%) patients had fever without signs of sepsis. In total, 19 wounds were treated with negative-pressure wound therapy. Type of previous surgery is shown in Table 1.

In 12 patients infection was early (up to one month after the initial surgery, 15.58 ± 7.07 days), and in five patients it was late (after one month from the initial surgery performed, 8.40 ± 3.91 months). In all the patients multislice computed tomography was performed, and in all of them examination showed perigraft fluid, with collection confined only to the groin. Causative microorganisms are shown in Table 2.

Five patients had polymicrobial infection, and in one patient the wound swab was sterile.

Mean duration of negative-pressure wound therapy was 45 days (5–100 days), and costs ranged from €150 to €4,000, with average value of €1,100.

Majority of wounds (11/19) healed by secondary intention with granulation tissue covering exposed graft over time. Rest of wounds (8/19) healed by primary intention after initial negative-pressure wound therapy. Table 3 shows type of surgery performed in infected groin wounds.

No early mortality, up to 30 days, was observed. Complications during their hospital stay were recorded in six patients. Three of them had lymphatic cyst, one patient developed acute coronary syndrome and percutaneous coronary intervention was performed. One patient suffered ischemic stroke, and one had epistaxis and an epileptic seizure. Complications of negative-pressure wound therapy were noted in only one wound (1/19) and consisted of minor bleeding from wound edge. No other complications were observed.

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**Table 1.** Type of surgery previously performed in the study patients

| Type of surgery | Number of patients |
|---|---|
| Aortobifemoral bypass | 11 |
| Iliacofemoral bypass | 3 |
| Femoropopliteal bypass | 2 |
| Femorofemoral cross-over bypass | 1 |

**Table 2.** Microorganisms cultured from infected wounds

| Microorganism | Number of patients |
|---|---|
| Vancomycin resistant enterococcus | 2 |
| Klebsiella enterobacter | 3 |
| Staphylococcus epidermidis | 1 |
| MSSA | 2 |
| Proteus vulgaris | 2 |
| Enterococcus | 2 |
| Staphylococcus species | 2 |
| Escherichia coli | 1 |
| Enterobacter | 1 |
| Proteus mirabilis | 1 |
| Acinetobacter | 1 |

MSSA – methicillin-sensitive *Staphylococcus aureus*

**Table 3.** Grafts used for the reconstruction of groin blood vessels

| Type of surgery performed | Number of wounds |
|---|---|
| Graft preservation | 7 |
| Silver graft implantation | 6 |
| Autologous vein/artery implantation | 6 |

Persistent infection was observed in six patients (six wounds) and all of them had virulent microorganisms isolated from the wounds (vancomycin-resistant enterococcus, *Klebsiella*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter*, *Proteus mirabilis* and *vulgaris*).

Reinfection occurred in three groin wounds (three patients) in total. These patients had *Enterococcus* and *Staphylococcus epidermidis* isolated from their wounds. The same pathogens were isolated when primary graft infection was treated in those patients. Only one graft occlusion was noted during the follow-up period. Table 4 summarizes abovementioned data and shows that no statistically significant difference was observed when comparison was performed between outcome of early and late graft infections.

Late mortality was observed in 3/17 patients who were admitted to hospital several months after negative-pressure wound therapy due to reinfection and sepsis. Cause of death was multiple organ failure in all three cases.

Also, no statistically significant difference was observed when comparison between early and late infection outcome was performed regarding age, duration of negative-pressure therapy, and costs. Table 5 summarizes these data.

Eventually, all wounds healed (100%). Seven grafts were preserved (36.8%), and in 12 (63.2%) cases we had to substitute infected grafts with silver coated grafts (six cases) and autologous artery/vein (also six cases). In four

**Table 4.** Outcome of negative-pressure therapy of early and late synthetic graft infection ($p < 0.05$ is considered significant)

| Parameter | | Infection | | | | p value |
|---|---|---|---|---|---|---|
| | | Early | | Late | | |
| | | N | % | N | % | |
| Gender | Male | 9 | 75 | 4 | 80 | 1.000 |
| | Female | 3 | 25 | 1 | 20 | |
| Early mortality (up to 30 days) | No | 12 | 100 | 5 | 100 | - |
| | Yes | 0 | 0 | 0 | 0 | |
| Early morbidity (up to 30 days) | No | 7 | 58.3 | 4 | 80 | 0.600 |
| | Yes | 5 | 41.7 | 1 | 20 | |
| Complications of negative-pressure therapy | No | 12 | 100 | 4 | 80 | 0.294 |
| | Yes | 0 | 0 | 1 | 20 | |
| Persistent infection (up to 30 days) | No | 8 | 66.7 | 3 | 60 | 1.000 |
| | Yes | 4 | 33.3 | 2 | 40 | |
| Reinfection | No | 10 | 90.9 | 2 | 50 | 0.154 |
| | Yes | 1 | 9.1 | 2 | 50 | |
| Graft patency | No | 1 | 8.3 | 0 | 0 | 1.000 |
| | Yes | 11 | 91.7 | 5 | 100 | |

**Table 5.** Analysis of age, duration, and costs of negative-pressure therapy ($p < 0.05$ is considered significant)

| Parameter | Infection | N | Mean | SD | Med. | Min. | Max. | p-value |
|---|---|---|---|---|---|---|---|---|
| Age (years) | Early | 12 | 64.17 | 11.496 | 61.50 | 42 | 85 | 0.549 |
| | Late | 5 | 60.60 | 9.236 | 63.00 | 50 | 71 | |
| | Total | 17 | 63.12 | 10.723 | 62.00 | 42 | 85 | |
| Duration of negative-pressure therapy (days) | Early | 12 | 47.42 | 29.623 | 45.00 | 9 | 100 | 0.574 |
| | Late | 5 | 38.00 | 30.943 | 45.00 | 5 | 80 | |
| | Total | 17 | 44.65 | 29.364 | 45.00 | 5 | 100 | |
| Cost of negative-pressure therapy (euros) | Early | 11 | 1,000 | 1,100 | 450 | 150 | 3,700 | 0.583 |
| | Late | 5 | 1,300 | 1,400 | 1,000 | 200 | 3,800 | |
| | Total | 16 | 1,100 | 1,200 | 650 | 150 | 3,800 | |

N – number of patients; Mean – mean value; SD – standard deviation; Med. – median value; Min. – minimum value; Max. – maximum value

cases when silver-coated graft was implanted, wound dehiscence (probably caused by inadequate debridement) occurred and negative-pressure therapy continued with graft preservation. In other two cases of silver graft implantation, the wound was closed after graft substitution in the usual manner and eventually healed *per primam,* in six cases due to persistent infection (the probable cause of such high rate of persistent infection is more virulent pathogens and overall poor health condition of these patients). In these six cases, all wounds healed *per primam intentionem* after infected grafts were substituted with autologous artery/vein.

## DISCUSSION

The use of negative-pressure wound therapy in vascular surgery started with pioneer works of Demaria et al. [10] and Giovannini et al. [11], who were the first authors that applied this technique on exposed blood vessels. They successfully treated two patients with groin infections. After that, several publications have appeared with good early and midterm results regarding this technique [5, 8,12]. Overall mortality in these series was reported to be 0% up to 18 months of follow-up. However, Dosluoglu et al. [5] reported 27% of mortality during follow-up of 33 months. Mayer et al. [13] also found a high rate of long-term mortality (41%). This could be explained by a longer follow-up period in his study.

Overall mortality in our series was 18%. No early death was noted, and all of them were observed in the follow-up period and were related to reinfection. Similar results were obtained by Mayer et al. [13], who reported 30-day and one-year mortality to be 0% and 16%, respectively.

Overall amputation and reinfection rates were 0–5% reported in previously mentioned publications. Although we found no amputations in our study, the rate of reinfection was much higher, but acceptable in our opinion, and during the follow-up we found 16% (3/19 wounds) of reinfection in previously healed wounds. Persistent infection was observed in 31% (6/19 wounds), and those patients were treated with autologous artery/vein *in-situ* insertion, after the removal of the infected synthetic graft.

Regarding causative pathogens, Calligaro et al. [14] reported 40% of limb salvage when involved microorganism was *Pseudomonas,* which is reported to be very aggressive. In our series we had no *Pseudomonas* infection. However, Mayer et al. [13] found no association between *Pseudomonas* infection and the need for graft excision. In their opinion, patients presenting with *Pseudomonas* infection are not poorer candidates for any type of graft preservation technique.

In our study, only one (1/19 wounds, 5%) complication of negative-pressure wound therapy occurred, and it presented as minor bleeding from wound edge. Mayer et al. [13] reported also one (2%), but serious bleeding due to inadequate previous resection of infected artery. Svensson et al. [15] reported bleeding complications in two patients (2/33 wounds, 6%). Authors of that study also reported the median total cost per patient for the negative-pressure therapy to be about €1,300 [15]. In our study, the average cost of such therapy was €1,100 per patient.

All the patients in our study experienced complete wound healing. Majority of wounds (11/19, 58%) healed by secondary intention with granulation tissue covering exposed graft over time. The rest of the wounds (8/19, 42%) healed by primary intention after the initial wound debridement and negative-pressure therapy. After that, graft substitution with autologous artery/vein or silver-coated grafts was performed and the wounds were closed in the usual manner.

## CONCLUSION

Negative-pressure wound therapy seems to be safe for groin vascular graft infections with no early mortality, virtually no complications of its use, and excellent patency rate of 95%.

Comfortable for both patient and surgeon, this approach emerges as a new and promising therapeutic option in these difficult patients. However, high rate of persistent infection which we found mandates careful selection of patients for this approach. This technique, in our opinion, can be used as a "bridge" from initial wound debridement to definitive wound management, when good local conditions are achieved for graft substitution, either with a new synthetic graft with antimicrobial properties or autologous artery/vein. In selected cases of deep groin infections, this technique can be used as the only therapeutic approach in wound treatment.

The limitation of this study is the small number of patients and short follow-up period of average 10.0 ± 5.8 months (range of 1–24 months). Also, there was no comparative group in the study. Therefore, no statistically significant differences were observed when comparing outcome of early and late synthetic graft infections. Despite this fact, we observed better results in patients with shorter duration of infection caused by less virulent microorganisms, in whom negative-pressure wound therapy was commenced early. New studies with larger number of subjects are needed to clarify the role and establish the algorithm for negative-pressure wound therapy in the field of vascular surgery.

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# Терапија негативним притиском у лечењу инфекције графта у препони


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**КРАТАК САДРЖАЈ**

**Увод** Инфекција синтетског графта у препони је ретка али озбиљна компликација. Може се лечити на више начина. Екстраанатомски бајпас и *in situ* имплантација новог, на инфекцију отпорног графта су повезани са значајним морбидитетом и морталитетом. Због тога је у неким случајевима потребан конзервативнији приступ у лечењу. Терапија негативним притиском је једна од могућности у лечењу тих болесника.

**Циљ рада** Циљ рада је био да се прикажу резултати лечења инфекције синтетског графта у препони употребом негативног притиска.

**Методе рада** У ову опсервациону студију је укључено 17 болесника (19 рана) који су лечени због инфекције графта у препони (*Szilagyi* III) између октобра 2011. и јуна 2014. године.

**Резултати** Већина рана (11/19) зарастала је *per secundam*, док су остале ране зарасле *per primam* после иницијалне примене терапије негативним притиском, а онда је следила замена инфициране протезе силвер графтом или аутологном артеријом/веном. Није забележен ниједан рани смртни случај. Мање крварење је забележено код једног болесника. Реинфекција је настала код три болесника. Забележена је једна оклузија графта. Касни морталитет је регистрован код три болесника.

**Закључак** Примена терапије негативним притиском је, чини се, безбедна код инфекције графта у препони и подобан метод и за болесника и за хирурга. Ипак, учесталост перзистентне инфекције је висока. По нашем мишљењу, ова техника може бити употребљена као „премошћење“ од иницијалног дебридмана до дефинитивног збрињавања ране, када постоје добри локални услови за супституцију графта било новом протезом са антимикробним својствима или аутологном артеријом/веном. Код селектованих случајева инфекције графта у препони може бити коришћена као једини начин лечења.



**Кључне речи**: препонска инфекција; инфекција синтетског графта; терапија негативним притиском

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# Risk factors for severe influenza A virus infections in post-2009 pandemic period

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**SUMMARY**

**Introduction** Literature data concerning risk factors for severe influenza in post-2009 pandemic period, from low- and middle-income Central and Eastern European countries are very limited.

**Objective** The aim of this study was to investigate the risk factors for severe A(H1N1)pdm09 and A(H3N2) influenza during the post-2009 pandemic period.

**Methods** During four consecutive seasons of 2010/2011–2013/2014, nasopharyngeal or nasal and pharyngeal swab samples from 153 patients with mild and 147 patients with severe influenza were tested using real-time reverse transcription polymerase chain reaction (real-time RT PCR) assays.

**Results** The study indicated three statistically significant risk factors of influenza severity, including presence of chronic underlying illness/condition [odds ratio (OR) of 15.2, 95% confidence interval (CI) of 1.8–125.4, p = 0.001), age ≥15 years (OR 9.2, 95% CI 3.5–24.1, p < 0.001), and delay in medical care of more than two days after the symptoms onset (OR 3.2, 95% CI 1.6–6.4, p = 0.001).

**Conclusion** Obtained results confirmed that patients with chronic underlying illness/condition and older than 15 years had the highest risk for serious complications from influenza and highlighted the importance of start of antiviral therapy within the first two days of illness in order to reduce the risk for the most severe outcomes of influenza, such as acute respiratory distress syndrome and lethal outcome.

**Keywords:** influenza A; acute respiratory infections; real-time RT PCR

## INTRODUCTION

Influenza is usually mild-to-moderate, self-limited, acute upper respiratory tract disease, but each year severe complications develop in about three to five million people worldwide [1]. During the pandemic of 2009, it became clear that the existing systems of influenza surveillance provide very limited data on severity of an influenza season. To overcome this problem, WHO recommended strengthening the monitoring of influenza viruses and underlying risk conditions that are specifically associated with severe clinical presentations of influenza [2]. According to the WHO case definition, influenza-like illness (ILI) is an acute respiratory illness with the onset within the previous 10 days, body temperature of ≥38°C and cough, while severe acute respiratory illness (SARI) is a type of ILI with pronounced difficulties in breathing, demanding hospitalization [3]. SARI may be presented as primary viral or secondary bacterial pneumonia and exacerbation of chronic diseases. In some cases SARI may progresses to the most severe form of acute lung injury named acute respiratory distress syndrome (ARDS).

Manifestations and clinical outcome of influenza virus infection is a result of complex interplay between viral and host factors. Development of severe influenza virus infections depends on viral type- and strain-specific virulence determinants, immunological and physiological characteristics of host, and may also be influenced by health-related behavior and medical treatment of patients [4, 5, 6]. Advanced age, chronic medical conditions including chronic respiratory and cardiovascular diseases, diabetes mellitus, chronic liver or renal diseases and immunodeficiencies, as well as pregnancy, are well known factors for complications of influenza [1]. During the pandemic influenza outbreak in 2009, obesity was also associated with severe A(H1N1)pdm09 infections [7]. Most of the literature data concerning risk factors for severe outcome of influenza are focused on the influenza A(H1N1)pdm09 infections during the 2009 pandemic outbreak in developed, high-income countries, while data for other influenza subtypes and from low- and middle-income Central and Eastern European countries are scarce [8, 9].

## OBJECTIVE

The aim of this study was to investigate the risk factors for severe A(H1N1)pdm09 and A(H3N2) influenza during the post-2009 pandemic period in Vojvodina province, Serbia.

## METHODS

### Patients and real-time RT PCR testing

Centre for Virology at the Institute of Public Health of Vojvodina conducts continuous viro-

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logical surveillance of influenza in the Vojvodina region in collaboration with primary healthcare institutions, hospitals and institutes of public health in the Vojvodina region, Serbia. During four consecutive seasons, from 2010/2011 to 2013/2014, nasal and throat or nasopharyngeal swab samples were taken from 887 patients when the WHO definitions for ILI or SARI [3] were met. Patients were asked a set of questions about their age, height, weight, vaccine status, and pre-existing health-conditions including malignancies, diabetes, chronic cardiovascular, respiratory, liver, and kidney diseases, immunosuppressive conditions, and pregnancy. The time from symptom onset to the first medical contact and antiviral treatment was also recorded. The body mass index (BMI) was calculated for each patient, except for pregnant women, using self-reported weight and height and the following formula: weight in kilograms divided by height in meters squared. Among adults aged >19 years obesity was defined as BMI ≥30. For patients aged up to 19 years, obesity was determined using appropriate WHO BMI-for-age charts (http://www.who.int/dietphysicalactivity/childhood/en/) and criteria that BMI value of more than 2 SD above the mean is equivalent to BMI >30 at 19 years.

Samples were transported to the Centre for Virology and tested by real-time reverse transcription polymerase chain reaction (real-time RT PCR) assays. Extraction of viral RNA was done by QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Real-time RT PCR testing was performed using influenza A type-specific and A(H1N1)pdm09 and A(H3N2) subtype-specific primer and probe sets provided by Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) and AgPath-ID™ One-Step RT-PCR Reagents (Applied Biosystems, Foster City, CA, USA). Real-time RT PCR assays were performed on the Applied Biosystems 7500 thermocycler according to the protocol developed by the CDC (enclosed with the reagents). A total of 300 patients with real-time RT PCR-confirmed influenza A infections were included in this study.

### Statistical analyses

Descriptive statistics was performed for all study variables. For continuous variables, the data were reported as mean [with 95% confidence interval (CI)] and median (with range), and for categorical variables as percentage. For comparison of different variables between patients with ILI and patients with SARI/ARDS, Student's t-test and Fisher's exact test were used where appropriate. Variables with p-values <0.05 were entered into logistic regression models to identify factors associated with influenza severity. Severe influenza was assigned as a dependent variable in logistic regression analysis, while independent variables were the following: age group, influenza A subtype, the average time from symptoms onset to the first medical contact, the presence of any chronic underlying illness/condition (CUI/C), the presence of more than 1 CUI/C, and the presence of individual CUI/C. Age of the patient and time from symptoms onset to hospitalization were classified as two categories (<15 or ≥15 years, and ≤2 or >2 days, respectively). Odds ratios (OR) and 95% CI were calculated for each variable. Identified risk factors with a p-value <0.05 in the univariate analysis were included in a multivariate logistic regression model to assess independent association with severity. A factor was defined significant for $p < 0.05$ in the multivariate analysis. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

The age distribution, distribution of influenza A virus subtypes and underlying illnesses/conditions by clinical manifestations of influenza are summarized in Table 1. In the group of 300 influenza A positive patients, there was approximately equal number of those who experienced severe disease (49%, 153/300), and those with mild forms of illness (51%, 147/300). A highly significant difference ($p < 0.001$) between the mean and median age of those who had uncomplicated influenza (mean age of 20.7 years, 95% CI 17.6–23.8, median age of 13 years, range of 1–81 years) and of those with severe illness (mean age of 47.8 years, 95% CI 44.7–50.9, median age of 51 years, range of 2–81) was found. Children aged 5–14 years dominated among ILI cases (47.1%, 72/153), while most of the SARI/ARDS cases were 30–64 (58.5%, 86/147) years of age.

Among 300 positive cases, A(H1N1)pdm09 and A(H3N2) subtypes were almost equally represented (48%, 144/300 and 52%, 156/300, respectively). Significantly more SARI/ARDS cases were caused by influenza A(H1N1)pdm09 (66%, 97/147, $p < 0.001$), while significantly more ILI cases were associated with A(H3N2) (69.3%, 106/153, $p < 0.001$) subtype.

The presence of one or more CUI/C was recorded in 40.7% (122/300) of patients positive for influenza A. Chronic cardiovascular disease was the most common underlying medical condition in positive patients (14.7%, 44/300). CUI/C were significantly more present in patients with complications (73.5%, 108/147) compared to those with mild form of disease (9.1%, 14/153, $p < 0.001$). All detected comorbidities were statistically more common in patients with SARI/ARDS than in patients with ILI.

Differences in age distribution, and mean and median age, between patients with severe A(H1N1)pdm09 and A(H3N2) influenza, were not significant, with the exception of children aged 5–14 years, which were recorded only among A(H3N2) SARI/ARDS patients (10%, 5/50, $p = 0.004$) (Table 2). Persons 30–64 years old predominated in the group of influenza A(H1N1)pdm09 with SARI/ARDS (57.7%, 56/97), as well as in the A(H3N2) SARI/ARDS group of patients (60%, 30/50). CUI/C were more prevalent in the group of influenza A(H1N1)pdm09 SARI/ARDS patients (80.4%, 78/97) then in A(H3N2) SARI/ARDS patients (60%, 30/50, $p = 0.0105$). In regard to the individual CUI/C, the only significant difference was observed in the case of malignant diseases which were detected

**Table 1.** Age distribution, distribution of influenza A virus subtypes, and underlying illnesses/conditions among patients with mild and severe influenza

| Characteristic | | Positive n = 300 | ILI (%) n = 153 (51) | SARI/ARDS (%) n = 147 (49) | p |
|---|---|---|---|---|---|
| Age (years) | 0–4 | 18 (6) | 16 (10.5) | 2 (1.4) | 0.001 |
| | 5–14 | 77 (25.7) | 72 (47.1) | 5 (3.4) | <0.001 |
| | 15–29 | 47 (15.7) | 20 (13.1) | 27 (18.4) | >0.05 |
| | 30–64 | 127 (42.3) | 41 (26.8) | 86 (58.5) | <0.001 |
| | ≥65 | 31 (10.3) | 4 (2.6) | 27 (18.4) | <0.001 |
| | Mean (95% CI) | 34 (31.3–36.7) | 20.7 (17.6–23.8) | 47.8 (44.7–50.9) | <0.001 |
| | Median (range) | 33 (1–84) | 13 (1–81) | 51 (2–84) | <0.001 |
| Influenza A virus subtypes | H1N1pdm09 | 144 (48) | 47 (30.7) | 97 (66) | <0.001 |
| | H3N2 | 156 (52) | 106 (69.3) | 50 (34) | <0.001 |
| CUI/C* | With CUI/C | 122 (40.7) | 14 (9.1) | 108 (73.5) | <0.001 |
| | One CUI/C | 87 (29) | 13 (8.5) | 74 (50.3) | <0.001 |
| | More than one CUI/C | 35 (11.7) | 1 (0.6) | 34 (23.2) | <0.001 |
| | Cardiovascular disease | 44 (14.7) | 11 (7.2) | 33 (22.4) | 0.0003 |
| | Respiratory disease | 20 (6.7) | 2 (1.3) | 18 (12.2) | 0.001 |
| | Diabetes mellitus | 13 (4.3) | - | 13 (8.8) | <0.001 |
| | Malignancy | 22 (7.3) | - | 22 (14.9) | <0.001 |
| | Immunodeficiency | 35 (11.7) | 1 (0.6) | 34 (23.1) | <0.001 |
| | Liver/renal disease | 23 (7.7) | - | 23 (15.6) | <0.001 |
| | Obesity** | 8 (2.7) | - | 8 (5.4) | 0.003 |
| | Pregnancy | 9 (3) | 1 (0.6) | 8 (5.4) | 0.0177 |

ILI – influenza-like illness; SARI – severe acute respiratory illness; ARDS – acute respiratory distress syndrome; CI – confidence interval; CUI/C – chronic underlying illness/condition

* Patients with multiple chronic underlying illnesses or conditions were counted for each
** Body mass index equal to or greater than 30

**Table 2.** Age distribution and distribution of underlying illnesses/conditions among patients with severe A(H1N1)pdm09 and A(H3N2) influenza

| Characteristic | | Severe A(H1N1) pdm09 influenza n = 97 | Severe A( H3N2) influenza n = 50 | p |
|---|---|---|---|---|
| Age (years) | 0–4 | 2 (2.1) | - | >0.05 |
| | 5–14 | - | 5 (10) | 0.004 |
| | 15–29 | 22 (22.7) | 5 (10) | >0.05 |
| | 30–64 | 56 (57.7) | 30 (60) | >0.05 |
| | ≥65 | 17 (17.5) | 10 (20) | >0.05 |
| | Mean (95% CI) | 47.06 (43.28–50.85) | 49.14 (43.44–54.84) | >0.05 |
| | Median (range) | 51 (2–83) | 51.5 (6–84) | >0.05 |
| CUI/C* | With CUI/C | 78 (80.4) | 30 (60) | 0.0105 |
| | One CUI/C | 52 (53.6) | 22 (44) | >0.05 |
| | More than one CUI/C | 26 (26.8) | 8 (16) | >0.05 |
| | Cardiovascular disease | 23 (23.7) | 10 (20) | >0.05 |
| | Respiratory disease | 11 (11.3) | 7 (14) | >0.05 |
| | Diabetes mellitus | 10 (10.3) | 3 (6) | >0.05 |
| | Malignancy | 19 (19.6) | 3 (6) | 0.0298 |
| | Immunodeficiency | 23 (23.7) | 11 (22) | >0.05 |
| | Liver/renal disease | 17 (17.5) | 6 (12) | >0.05 |
| | Obesity** | 5 (5.1) | 3 (6) | >0.05 |
| | Pregnancy | 4 (4.1) | 4 (8) | >0.05 |

CI – confidence interval; CUI/C – chronic underlying illness/condition

* Patients with multiple risk factors were counted for each
** Body mass index equal to or greater than 30

in 19.6% (19/97) of severely ill patients with A(H1N1) pdm09 infections, but in only 6% (3/50, p = 0.0298) of patients with severe A(H3N2) influenza.

Differences in health seeking behavior and antiviral treatment of patients with different clinical manifestation of influenza are presented in Table 3. The mean time from the onset of influenza symptoms to the first medical contact was 2.3 days (95% CI 2.1–2.5 days) with a median of two days (range 1–6) for ILI patients, and 3.3 days (95% CI 2.9–3.6) with a median of three days (range 1–10 days) for SARI/ARDS patients, which was significantly different (p < 0.001). Patients with ARDS had the longest mean delay before consultation (3.5, 95% CI 2.8–4.2). Data on antiviral treatment were available for 91 patients with ILI and 113 patients with SARI/ARDS. Antivirals were administrated to 41.6% (47/113) of SARI/ARDS patients, but

**Table 3.** Health care behavior and antiviral treatment of patients with different clinical manifestation of influenza

| Characteristic | | ILI | SARI/ ARDS | SARI** | ARDS | Fatal cases*** | p* |
|---|---|---|---|---|---|---|---|
| Health care behavior | Total number of patients | 153 | 147 | 126 | 21 | 9 | |
| | Mean time from the symptoms onset to the first medical contact in days (95% CI) | 2.27 (2.1–2.5) | 3.31 (2.9–3.6) | 3.27 (2.9–3.6) | 3.52 (2.8–4.2) | 2.89 (1.8–3.9) | <0.001 |
| | Median time from the symptoms onset to the first medical contact in days (range) | 2 (1–6) | 3 (1–10) | 2.5 (1–10) | 3 (1–6) | 3 (1–5) | <0.001 |
| Antiviral therapy | Total number of patients | 91 | 113 | 99 | 14 | 7 | |
| | Number of patients who received the antiviral treatment (%) | 6 (6.6) | 47 (41.6) | 40 (40.4) | 7 (50) | 5 (71.4) | <0.001 |
| | Number of patients who received the antiviral treatment within 48h from symptoms onset (%) | 4 (66.7) | 31 (65.9) | 25 (62.5) | 1 (14.3) | 1 (20) | >0.5 |
| | Number of patients who received the antiviral treatment >48h from symptoms onset (%) | 2 (33.3) | 16 (34.1) | 15 (37.5) | 6 (85.7) | 4 (71.4) | |

ILI – influenza-like illness; SARI – severe acute respiratory illness; ARDS – acute respiratory distress syndrome; CI – confidence interval

* Significance of difference between ILI and SARI/ARDS cases

** SARI cases without ARDS

*** SARI and ARDS cases with fatal outcome

**Table 4.** Results of the logistic regression analysis with severe influenza as depended variable

| Risk factor | | Univariate regression analysis | | | Multivariate regression analysis | | |
|---|---|---|---|---|---|---|---|
| | | OR | 95% CI | p | OR | 95% CI | p |
| Age | ≥15 | 27.1 | 11.8–61.7 | <0.001 | 9.2 | 3.5–24.1 | <0.001 |
| Influenza A subtype | A(H1N1)pdm09 | 4.4 | 2.7–7.1 | <0.001 | 1.7 | 0.8–3.3 | 0.146 |
| Health care behavior | More than two days from the symptom onset to the first medical contact | 2.6 | 1.6–4.2 | <0.001 | 3.2 | 1.6–6.4 | 0.001 |
| CUI/C | With CUI/C | 27.5 | 14.2–53.2 | <0.001 | 15.2 | 1.8–125.4 | 0.011 |
| | More than 1 CUI/C | 5.9 | 0.7–47.5 | 0.091 | - | - | - |
| | Cardiovascular disease | 3.9 | 1.9–8.1 | <0.001 | 0.7 | 0.3–2.8 | 0.107 |
| | Respiratory disease | 10.5 | 2.4–46.3 | 0.002 | 0.9 | 0.4–3.6 | 0.382 |
| | Diabetes mellitus | 6.5 | 1.4–29.7 | 0.016 | 0.5 | 0.2–4.5 | 0.575 |
| | Malignancy | 28.4 | 3.8–113.4 | 0.001 | 1.6 | 0.1–24.3 | 0.741 |
| | Immunodeficiency | 45.7 | 6.2–339.1 | <0.001 | 4.7 | 0.3–70.4 | 0.262 |
| | Liver/renal disease | 31.5 | 4.2–135.9 | 0.001 | 2.0 | 0.2–24.0 | 0.583 |
| | Obesity* | 3.4 | 0.7–17.1 | 0.138 | - | - | - |
| | Pregnancy | 8.7 | 1.1–70.8 | 0.042 | 0,7 | 0.1–13.5 | 0.815 |

OR – odds ratio; CI – confidence interval; CUI/C – chronic underlying illness/condition

* Body mass index equal to or greater than 30

only 6.6% (6/91, p>0.001) of ILI patients received antiviral therapy. Among cases with serious complications of influenza, more patients with SARI received antivirals within two days from the symptoms onset (62.5%, 25/40), comparing to the patients with ARDS (14.3%, 1/7, p = 0.0347), and the patients who died (20%, 1/5). Out of 300 patients, only five patients (1.7%) – four with ILI and one with SARI – had a history of vaccination against influenza.

Three percent (9/300) of all positive cases and 6.1% (9/147) of all severe cases had fatal outcome. All patients who died had A(H1N1)pdm09 influenza, and most of them were 30–64 years old (66.7%, 6/9) and had the CUI/C (88.9%, 8/9 (data not shown in the table). The mean time from the onset of symptoms to hospital admission was 2.9 days (95% CI 1.8–3.9) with a median of three days (range of 1–5 days). The mean time from symptoms onset to death was 8.9 days (5.5–12.3) with a median of 9 days (range of 1–15 days) (data not shown in the table).

The univariate logistic regression analysis revealed that age ≥15 years (OR 27.1, 95% CI 11.8–61.7), being infected with influenza A(H1N1)pdm09 (OR 4.4, 95% CI 2.7–7.1), having CUI/C (OR 27.5, 95% CI 14.2–53.2), and more than two days from the symptoms onset to the first medical contact (OR 2.6, 95% CI 1.6–4.2) were significantly (p < 0.001) associated with severe diseases (Table 4). All individual CUI/C, except obesity, were associated with complicated forms of influenza. Among them, immunocompromised patients had the highest risk of having SARI/ARDS (OR 45.7, 95% CI 6.2–339.1, p < 0.001). In the multivariate analysis, three variables remained statistically significant prediction factors of influenza severity, including CUI/C (OR 15.2, 95% CI 1.8–125.4, p = 0.001), age of ≥15 years (OR 9.2, 95% CI 3.5–24.1, p < 0.001), and delay in medical care more than two days after the symptoms onset (OR 3.2, 95% CI 1.6–6.4, p = 0.001).

## DISCUSSION

The results of our study demonstrated that almost 60% of ILI cases were children aged <15 years, and more than 90% of these children had an uncomplicated form of influenza. The lack of preexisting immunity together with factors facilitating the virus transmission (shedding the greater

quantities of virus for longer periods of time than adults; prolonged stay in crowded indoor environments such are schools and daycare facilities), make children more susceptible to influenza virus infections. However, their efficient innate immune mechanisms and adaptive T cell immune response, most likely, protect them from developing severe disease [10]. On the other hand, children younger than five years of age are at higher risk from influenza complications due to the immature immune system [8]. Still, in the present study, the children aged <5 years were significantly more present among ILI (10.5%) than among SARI/ARDS (1.4%) cases, and almost 90% of the children of that age had a mild form of illness.

It is well known that despite having no greater risk of infection comparing to younger adults, older adults suffer the highest rates of hospitalization and mortality due to influenza [11]. In this study, almost 60% of SARI/ARDS and almost 70% of fatal cases were patients 30–64 years old. About two thirds of patients from that age group had SARI/ARDS. Moreover, patients ≥65 years old accounted for only about 10% of the whole sample, but almost 90% of them had complicated forms of illness. Similarly to our findings, Zhang et al. [12] discovered that severe influenza virus infections occurred most frequently in the oldest population (>60 years old), but the absolute number of adults aged 20–60 with severe illness was higher than in those >60 years old. It can be assumed that adults are partially protected against influenza due to cross-reactive immunity generated during previous exposures to influenza A viruses. However, such relative protection is probably impaired by underlying diseases which are more common in adults than in children. Chronic diseases themselves and low immunity caused by them can induce progression of influenza to more serious forms, and influenza might in turn aggravate patients' chronic illness. In this investigation, CUI/C were present in almost 75% of severely ill patients and in less than 10% of patients with mild form of the disease. Moreover, eight out of nine fatal cases had one or more CUI/C, which was in accordance with the McCullers's and Hyden's [13] suggestion that seasonal influenza rarely kills without a secondary cause.

Of all patients with severe influenza enrolled into our study, 5.4% were pregnant, 5.4% were obese, and obesity was recorded in 25% of patients who died. The specific changes in physiology (increased heart rate, stroke volume, oxygen consumption, and decreased lung capacity) and alterations in cell-mediated immunity in pregnancy increase the risk for severe influenza [7]. Obesity is associated with various comorbidities that are risk factors for influenza complications, such as diabetes, chronic cardiovascular or pulmonary diseases [14], and it is also an independent risk factor for severe influenza probably due the decreased pulmonary function and immunodysregulation involving adipokines [15]. Van Kerkhove et al. [11] reported that, according to pooled data from 19 countries and regions, during the pandemic of 2009, pregnant women were about seven times more likely to be hospitalized comparing to non-pregnant women, and that the proportion of obese patients increased with disease severity and represented a median of 6%, 11.3%, and 12% of all hospitalized, ICU-admitted, and fatal cases, respectively.

This investigation revealed that serious complications developed in 26.5% of previously healthy individuals without CUI/C. That finding may be explained by the immunosenescence in the elderly (a decline in functionality of the innate and adaptive immune system) [16], or by heritable predisposition to severe outcome of influenza independent of viral strain, preexisting immunity and the age, leading to pathological immune response (the so-called cytokine storm) [17].

The present study demonstrated that patients with ILI had significantly shorter delay before medical consultation than patients with SARI/ARDS (2.3 vs. 3.3 days). In relation to our results, Van Cauteren et al. [5] reported slightly shorter (1.9 days) mean time from the symptoms onset to the first medical consultation for ILI patients, while Kusznierz et al. [14] recorded 4.3 days for non-fatal hospitalized patients. Among patients who died, the disease progression was rapid, with a median time of three days from the symptoms onset to hospital admission, and a median time of nine days to death. During the pandemic of 2009, Santa-Olalla Peralta et al. [18] recorded that fatal cases had the median time of three days from the onset of the illness to the hospitalization, and a median time of 13 days to death.

The time from the onset of symptoms to initiation of antiviral treatment is the key factor in reducing the severity of influenza infections [6, 7]. In this study, among severely ill cases, antiviral therapy was administrated within two days from the onset of symptoms significantly more often to patients with SARI (62.5%) than to patients with ARDS (14.3%) and, although nonsignificantly, to patients who died (20%). These results suggest that prompt administration of antivirals could reduce the risk for most severe complications of influenza, ARDS and the fatal outcome. Although flu vaccine effectiveness can vary depending on characteristics of the person being vaccinated (such as their age and health) and antigenic similarity between the vaccine and circulating influenza viruses, the vaccination can significantly reduce the risk of getting ill from flu and the risk of more serious outcomes [19]. The proportion of vaccinated persons in this study was only 1.7%, which is consistent with overall low flu vaccine coverage in our country and in some other Eastern European countries [9].

Both influenza subtypes were approximately equally represented among positive patients, but their distribution in groups with different clinical manifestations of influenza varied significantly. Two thirds of SARI/ARDS cases were caused by influenza A(H1N1)pdm09 subtype, while almost 70% of ILI cases were associated with A(H3N2). There are few published comparisons of manifestation and outcomes of A(H1N1)pdm09 and A(H3N2) influenza. During the pandemic of 2009, it was recorded that infections caused by the new A(H1N1)pdm09 virus appear to be indistinguishable in severity and symptoms from seasonal A(H1N1) or A(H3N2) influenza [4]. Most patients experienced uncomplicated illness, while severe disease developed in only a small subset of patients. The majority of the A(H1N1)pdm09 viruses contain none of the known

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genetic hallmarks of highly pathogenic influenza viruses and there is no evidence for the significant antigenic changes [4, 15]. Only minor proportion of currently circulating A(H1N1)pdm09 strains (<1.8%) contain D222G mutation in hemagglutinin gene, which allows deep access to the lungs [20]. However, recent studies have reported changes in the epidemiology of the A(H1N1)pdm09 infection which are reminiscent of outbreaks in historical post-pandemic periods. Namely, the age-specific shift of A(H1N1)pdm09 infections towards adults, consistent with a development of A(H1N1)pdm09-specific immunity in younger populations that had the highest infection rates during the pandemic of 2009, has been observed [21]. The synergism between A(H1N1)pdm09 viruses and chronic underlying conditions mostly prevalent in older adults resulted in increased severity of influenza A(H1N1) pdm09 infections, which has been detected in our study and which is in line with the results of other researchers [21]. In contrast, high levels of immunity against A(H1N1) pdm09 viruses naturally acquired during the pandemic of 2009 made children more susceptible to infections with A(H3N2) viruses in subsequent years.

Our conclusions were also confirmed by the results of logistic regression analysis. Three independent risk factors for severe clinical disease were identified. Patients with CUI/C had about 15 times, and patients aged ≥15 years had about nine times higher the risk from severe influenza. The odds of severe disease in patients who asked for medical help more than two days after the symptoms onset (OR 3.2) was lower, but still significant.

This study had a number of limitations. The patients enrolled into this study represented only a small fraction of the total number of influenza cases in the population of Vojvodina, due to limitations of the passive surveillance system. Passive sentinel influenza surveillance system is simple and cost-effective, but it detects only symptomatic individuals who seek medical care and obtain laboratory tests. Moreover, selection of patients, data collection, and submission of samples to virological testing is performed voluntarily by physicians and might be influenced by their willingness and capacity to perform them. It should also be noted that BMI was calculated using self-reported height and weight, which are subject to substantial measurement error. Self-reported BMI tend to have lower values than true BMI based on physical measurement of height and weight, resulting in underestimates of the prevalence of obesity in study population [22]. The relatively small number of children younger than five years and patients with certain CUI/C identified through surveillance, reduced the statistical power of our results and probably affected their precision. Thus, our findings need to be interpreted with some caution because of the selection bias and small number of cases in certain groups.

## CONCLUSION

In conclusion, our study confirms that during the first four consecutive seasons after the 2009 pandemic, individuals with CUI/C, older than 15 years, and who delayed medical consultation for more than two days after the illness onset, had significantly higher risk for serious complications from influenza. Furthermore, they highlight the importance of an early start of antiviral therapy, in order to reduce the risk for the most severe outcomes of influenza such as ARDS and the fatal outcome. Results from this study may help in targeting vulnerable populations and improvement of patient management. This is especially relevant in the light of an overall low vaccination coverage and very limited use of antivirals in our country.

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## Фактори ризика за настанак тешких инфлуенца А вирусних инфекција после пандемије 2009. године

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**КРАТАК САДРЖАЈ**

**Увод** У литератури постоје веома оскудни подаци о факторима ризика за настанак тешких облика инфлуенце после пандемије 2009. године у земљама Централне и Источне Европе.

**Циљ рада** Циљ овог рада био је да се испитају фактори ризика за настанак тешких A(*H*1*N*1)*pdm*09 и A(*H*3*N*2) инфлуенца вирусних инфекција после пандемије 2009. године.

**Методе рада** Узорци назофарингеалних брисева или брисева носа и грла 153 пацијента са благим и 147 пацијената са тешким обликом инфлуенце тестирани су *real-time RT PCR* тестовима на присуство инфлуенца A(*H*1*N*1)*pdm09* и A(*H*3*N*2) вируса.

**Резултати** Истраживање је указало на четири статистички значајна фактора ризика за настанак тешких случајева инфлуенце, укључујући присуство хроничне болести/стања (*OR* 15,2; 95% *CI* 1.8–125,4; $p = 0,001$), узраст ≥15 година (*OR* 9,2; 95% *CI* 3,5–24,1; $p < 0,0001$) и одлагање медицинске консултације више од два дана након почетка болести (*OR* 3,2; 95% *CI* 1,6–6,4; $p = 0,001$).

**Закључак** На основу резултата овог истраживања може се закључити да су пацијенти са хроничним обољењима/стањима и старији од 15 година били под највећим ризиком од озбиљних компликација инфлуенце, као и да је примена антивирусне терапије у прва два дана болести значајна за смањење ризика од најтежих компликација.

**Кључне речи:** инфлуенца А; акутна респираторна инфекција; *real-time PCR*

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# Quality of osteoarthritis care in family medicine – A cross-sectional study

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**SUMMARY**

**Introduction** Effective treatments for osteoarthritis are available, yet little is known about the quality of primary care in the Republic of Srpska for this disabling condition.

**Objective** The main objective of this study was to analyze the overall quality of osteoarthritis treatment in a family medicine setting, as well as to explore whether the achievement of quality indicators was associated with particular patient characteristics and severity of osteoarthritis.

**Methods** The cross-sectional study included 120 patients with confirmed hand, knee, and hip osteoarthritis, recruited at seven family practices in the town of Ugljevik, Republic of Srpska, Bosnia and Herzegovina. Data were extracted from a patient questionnaire on quality indicators, as well as from their electronic and paper records, to assess care against 14 indicators. The included quality indicators were based on the Arthritis Foundation's Quality Indicator set for Osteoarthritis. Summary achievement rates for hip, knee, or hand osteoarthritis, as well as for the total sample, were calculated.

**Results** The mean achievement rate for all 14 quality indicators obtained from medical records was 74%, and 77% obtained from patient interview. The quality indicators concerning referral for weight reduction (23%) and pharmacological treatment (24%) had the lowest achievement rates, whereas the highest achievement rates were related to physical examination (100%), pain and functional assessment (100%), and education (90.8%). Patients physical functioning was significantly associated with the quality indicator achievement rate ($p = 0.001$).

**Conclusion** Pharmacological therapy and the referral of osteoarthritis patients in need of weight reduction seem to have the greatest potential for improvement in primary health care.

**Keywords:** osteoarthritis; quality of care; quality indicator; family medicine

## INTRODUCTION

Osteoarthritis (OA) is the most common chronic disorder, which usually results in joint pain and deformity, ultimately leading to chronic disability. Hence, it becomes a significant medical and financial burden in a world with ageing population [1]. Symptoms of OA are often insidious and can be highly variable, depending on the affected joint and the severity and the number of joints affected, with the joint pain as the first and predominant symptom. Other manifestations are self-limited morning stiffness, the crepitus on palpation, tenderness over the affected joint on palpation and frequently reduction in joint range of motion. As the disease progresses, patient gradually experiences progressively severe joint discomfort and increasing difficulty with activities of daily living [1, 2, 3].

OA is a highly prevalent disease, but little attention has previously been paid to the quality of health care delivered for this disease, and to performance in the processes of care. Routine audit and feedback on provided care is needed to improve the quality of that care. Several studies have demonstrated regional and subspecialty variations in the use of pharmacological, non-pharmacological, and surgical treatment modalities in patients with OA [4, 5]. Although systematic measurement of health care quality can lead to improvements in terms of care delivered to patients with OA [6, 7, 8], it is difficult to define what constitutes quality because standard sets of measures to assess quality vary considerably. A systematic review by Edwards et al. [9] identified a range of indicators for OA which have a good evidence base, are consistent with international guidance, and many of which have been implemented previously.

The quality of care is also influenced by patients' declared and real expectations. Patient often express expectations about information and treatment, for instance. Nevertheless, this expectation is variable, not uniformly shared between patients and physicians or during the therapeutic course. This expectation can be less a need for more information than a need for re-assurance and pain control [10].

However, very little is known about the quality of care family physicians provide for this disabling condition.

## OBJECTIVE

The main objective of this study was to analyze the overall quality of OA treatment in a family medicine setting in one town in the Republic

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of Srpska as well as to explore whether the achievement of quality indicators (QIs) was associated with particular patient characteristics and severity of OA.

## METHODS

### Patients

A cross-sectional study included patients with confirmed hand, knee, and hip OA, recruited at seven family practices in the Primary Health Care in Ugljevik, the Republic of Srpska, Bosnia and Herzegovina, with the inclusion period from January 1, 2014 to May 31, 2014.

The sample size for the population of 398 patients with OA included in regional Osteoarthritis Registry, with the confidence interval of 7.67% and confidence level of 95% was calculated to be 116.

Inclusion criteria were age of ≥55 years, clinical diagnosis of primary hand, hip, and knee OA based on joint pain on most days for at least one month in the previous year (with at least two of the following symptoms: stiffness, crepitus, bony tenderness, and bony enlargement) and radiological diagnosis of OA (joint space narrowing, osteophytes, subchondral cysts, and bony sclerosis). Individuals with any evidence of secondary OA, inflammatory arthritis, and those with neurologic diseases were excluded. The patients were contacted for permission to be included in the study and were asked to give their written informed consent.

The study was conducted in accordance with the World Medical Association Declaration of Helsinki of 1964, with the approval of the Ethical Committee of the Medical Faculty of Foča, University of East Sarajevo.

### Instruments

A standardized questionnaire was used to collect data regarding the patients' characteristics such as gender, age, occupational status, smoking habits, physical activity, body mass index, duration of the disease, and self-reported comorbidities.

Depending on the type of the osteoarthritic joint most affected, questionnaires on physical functioning were chosen as follows: the Knee Injury and Osteoarthritis Outcomes Score (KOOS-PS), the Hip Disability and Osteoarthritis Outcomes Score (HOOS-PS) and the Michigan Hand Outcomes Questionnaire (MHQ). The KOOS-PS is a shortened version of KOOS, developed by Perruccio et al. [11] in 2008, consisting of seven questions about the knee physical functions, scored by summing the responses to the seven items of the KOOS-PS score, with the results interval scored from 0 to 100. The HOOS-PS is the shortened version of HOOS, developed by Davis et al. [12] in 2008 and Bond et al. [13]. It comprises five questions including climbing down the stairs, getting in or out of bath and sitting, running and twisting on loaded leg. Responses in KOOS-PS and HOOS-PS were graded on a five-point Likert scale. A score from 0 to 100 was determined by crosswalk table of raw scores for each subscale, with 0 representing the best results. The MHQ covers the following six domains: (1) overall hand function, (2) activities of daily living, (3) pain, (4) work performance, (5) aesthetics, and (6) patients' satisfaction with hand function. The last four of these domains are scored for the right and left hand separately. Each item is scored on a 1 to 5 scale, with the domain scores ranging from 0 to 100. If both hands are affected, the left and right hand scale scores are averaged to obtain the score. For every domain, a higher score indicates better hand function, except for the pain domain, where a higher score means more pain. The total score (the average of all domains) ranges from 0 to 100, with a higher score indicating a better hand function [14].

According to the level of the scores, physical function of the joints was classified as normal or with mild, moderate, severe, or very severe impairment.

The patients completed the questionnaire after consultation with a family physician. The questionnaires were administered and handled by the researchers.

The MHQ, KOOS-PS, and HOOS-PS were translated into Serbian and linguistically validated [15]. The internal consistency reliability of the Serbian version of the questionnaires was assessed by Cronbach's alpha coefficient, while their convergent validity was assessed by Spearman's correlation coefficient.

In testing for internal consistency, Cronbach's alphas ranged from 0.78 for the HOOS-PS, 0.82 for the KOOS-PS, to 0.91 for the MHQ (values >0.7 for Cronbach's alpha are considered a good internal consistency). The reliability using Spearman's correlation demonstrated substantial agreement, ranging from 0.86 for the HOOS-PS to 0.94 for the MHQ.

The Serbian versions of the MHQ, KOOS-PS, and HOOS-PS were shown to be reliable and valid tools for assessing of the hand, knee, and hip osteoarthritic joint physical functioning in Serbian-speaking patients.

### Derivation of QIs

In order to assess the quality of care of patients with OA, questionnaires based on the Arthritis Foundation's Quality Indicator set were used [16]. This indicator set covers regular physical examination and assessment of pain and functioning (QI 1–3), the provision of education (QI 4), instructions on exercise and weight management (QI 5–8), assessment of need for assistive devices (QI 9–10), the provision of pharmacological and surgical treatment (QI 11–13), and the provision of radiographs (QI 14). The data were extracted from the patient questionnaire on QI as well as from their electronic and paper records, to assess care against the 14 indicators. All aspects of the electronic medical records were included in the search for evidence of QIs, such as free text. The entire paper record from the date of diagnosis was also included.

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### Statistical analysis

Statistical analyses were carried out using SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA). Means and standard deviations (SD) for continuous variables and frequency and percentage for categorical variables were used to describe the data.

The mean scores of KOOS-PS, HOOS-PS, and MHQ were calculated for the different studied subgroups, and the normality of their distributions was tested by the Kolmogorov–Smirnov test. Differences between patients with hip, knee, and hand OA were analyzed by one-way analysis of variance (ANOVA) and between medians by the Kruskal–Wallis test. P-values less than 0.05 were considered significant.

QI achievement rates were calculated for each QI separately and for the study sample as a whole, in which the numerator represents the number of patients achieving the indicators and the denominator represents the number of eligible persons. Correspondingly, summary achievement rates for each person were calculated as the total number of QIs they passed, divided by the total number of QIs for which they were eligible. Summary achievement rates were calculated using the data obtained from medical records as well as from patients' interviews. The achievement rates are presented as percentages.

We also analyzed factors for variation in QI achievement rates in bivariate regression analyses, employing the following independent variables: age, gender, occupation, place of living, duration of disease and health-related quality of life measured by the HOOS-PS/HOOS-PS/MHQ scores.

**Table 1.** Patients' characteristics (n = 120)

| Characteristic | | n | % |
|---|---|---|---|
| Gender | Female | 83 | 69.0 |
| | Male | 37 | 31.0 |
| Occupation | Farmer | 24 | 20.0 |
| | Housewife | 47 | 39.0 |
| | Age retiree | 10 | 8.0 |
| | Blue collar jobs | 22 | 18.0 |
| | White collar jobs | 17 | 15.0 |
| Place of living | Town | 45 | 37.5 |
| | Rural region | 75 | 62.5 |
| Smoking | Yes | 21 | 17.5 |
| | No | 99 | 82.5 |
| Physical activity | Yes | 16 | 13.0 |
| | No | 104 | 87.0 |
| Localization | Hip | 29 | 24.0 |
| | Knee | 56 | 47.0 |
| | Hands | 35 | 29.0 |
| Comorbidity* | Other chronic disease | 97 | 81.0 |
| | None | 23 | 19.0 |
| Time since diagnosis (year) | <1 | 8 | 6.7 |
| | 1–3 | 38 | 32.0 |
| | 4–6 | 36 | 30.0 |
| | 7–10 | 22 | 18.0 |
| | >10 | 16 | 13.3 |

*Hypertension, diabetes mellitus, asthma, chronic obstructive pulmonary disease

**Table 2.** Distribution of joints affected according to intensity of impairment in physical functioning (KOOS-PS/HOOS-PS/MQH score)

| Impairment in physical function | Joint affected | | | | | |
|---|---|---|---|---|---|---|
| | Hands | | Knee | | Hips | |
| | n | % | n | % | n | % |
| Mild | 5 | 14.70 | 7 | 12.50 | 4 | 13.16 |
| Moderate | 10 | 29.40 | 14 | 25.00 | 7 | 23.68 |
| Severe | 12 | 32.40 | 20 | 35,42 | 11 | 36.84 |
| Very severe | 8 | 23.50 | 15 | 27.08 | 7 | 25.32 |
| Total | 35 | 100 | 56 | 100 | 29 | 100 |

## RESULTS

The total number of registered patients with hip, knee, and hand OA as recruited from family medicine practices was 127. Five patients were excluded from the study due to the co-presence of rheumatoid arthritis, and two due to secondary OA. For the patients who fulfilled criteria to be included in the study, the response rate was 100%. Demographic characteristics of the patients are presented in Table 1.

All three, knee, hip, and hand OA groups, reported high scores on the KOOS-PS/HOOS-PS/MHQ scales. More than one third (35%) of patients had severely impaired physical functioning. Differences between means of the three groups were not found (F = 1.992; p = 0.920) (Table 2).

The Kruskal–Wallis one-way analysis of variance showed that housewives and farmers had more severe functional impairments compared to the respondents with other occupations (H = 20.868; p < 0.001). The respondents with longer duration of the disease had more problems with physical functioning (H = 14.847; p = 0.011). A statistically significant difference in terms of severity of functional impairments was found in relation to the value of body mass index (H = 4.005, p = 0.009). Obese patients had more severe OA, not only in the hips and the knees, but also in non-weight bearing joints, hands (Table 3).

There were large variations in terms of achievement rates for different QI items (Table 4). The mean QI achievement rate for all 14 QIs obtained from medical records was 74%, and 77% obtained from patient interview. Quality measures using patient interview almost mirrored medical record findings. The QI concerning referral for weight reduction (QI 8) had the lowest level achievement rate, with 23% of the self-reported overweighed persons being referred to weight loss program. The achievement rates for pharmacological treatment (QIs 11 and 12) were 40.4% and 24%, respectively. Although only 13% of the respondents reported being physically active, the achievement rate for receiving information about the importance of physical activity and exercise was 78%.

The results of bivariate regression analysis show that patient physical functioning is significantly associated with QI summary achievement rate (p = 0.001), with non- standardized B = 5.9 (95% CI 2.3–8.7). Patients with higher scores on HOOS-PS/KOOS-PS and MHQ questionnaires had higher achievement rates. Age, gender, occupation, place of residence, and duration of the disease were not associated with the QI achievement rate.

**Table 3.** Distribution of severity of functional impairments according to body mass index (BMI) value, smoking status, physical activity, duration of the disease, and occupation

| Characteristic | | Mild | Moderate | Severe | Very severe | H* | p |
|---|---|---|---|---|---|---|---|
| BMI | 19–24.9 | 5 | 6 | 4 | 0 | 4.005 | 0.009 |
| | 25–29.9 | 8 | 21 | 10 | 8 | | |
| | >30 | 3 | 4 | 28 | 23 | | |
| Smoking | Yes | 3 | 10 | 12 | 13 | 2.935 | 0.402 |
| | No | 13 | 21 | 30 | 18 | | |
| Physical activity | Yes | 2 | 6 | 4 | 4 | 1.458 | 0.482 |
| | No | 14 | 25 | 38 | 27 | | |
| Duration of the disease (years) | <1 | 4 | 1 | 2 | 1 | 20.868 | <0.001 |
| | 1–3 | 4 | 15 | 17 | 2 | | |
| | 4–6 | 4 | 9 | 14 | 9 | | |
| | 7–10 | 4 | 3 | 4 | 11 | | |
| | >10 | 0 | 2 | 6 | 8 | | |
| Occupation | Farmer | 2 | 6 | 7 | 9 | 14.847 | 0.011 |
| | Housewife | 4 | 9 | 15 | 19 | | |
| | Age retiree | 4 | 1 | 4 | 1 | | |
| | Blue collar jobs | 5 | 8 | 9 | 0 | | |
| | White collar jobs | 3 | 7 | 6 | 1 | | |

*Kruskal–Wallis test

**Table 4.** Achievement rate of quality indicators (QI)

| QI [16] | | Number of patients eligible | Achievement rate in medical records (%) | Achievement rate in patient interview (%) |
|---|---|---|---|---|
| Physical examination | 1 | 120 | 100.0 | 100.0 |
| Pain and functional assessment | 2 | 120 | 100.0 | 100.0 |
| | 3 | 120 | 100.0 | 100.0 |
| Education | 4 | 120 | 90.8 | 100.0 |
| Exercise | 5 | 120 | 78.0 | 78.0 |
| Weight loss | 6 | 85 | 84.7 | 86.0 |
| | 7 | 85 | 84.7 | 86.0 |
| | 8 | 71 | 23.0 | 23.0 |
| Assistive devices | 9 | 97 | 83.0 | 83.0 |
| | 10 | 104 | 90.4 | 92.0 |
| Pharmacologic therapy | 11 | 47 | 40.4 | 42.0 |
| | 12 | 30 | 24.0 | 26.0 |
| Surgery | 13 | 54 | 75.9 | 78.0 |
| Radiographs | 14 | 73 | 79.0 | 79.0 |
| Mean QI achievement rate | | | 74.0 | 77.0 |

## DISCUSSION

This study used 14 indicators to measure the quality of primary care for OA, and the data on quality were obtained using patient interview and medical records. We found that the mean QI achievement rate was 74%, which is higher than the findings in other studies [17, 18, 19]. The achievement rates or pharmacological treatment was lower than in the other studies [19]. Around 40% of eligible patients used acetaminophen as the first drug choice to treat the OA pain. Data available from both medical records and patient interview showed that only 24% of patients had a trial of maximum acetaminophen dosage before switching to a different oral analgesic. Since the treatment of OA pain is such a common clinical problem, it seems an obvious area in which evidence-based treatment decisions should be directed towards the implementation of the existing guidelines or used to build stronger clinical guidelines. On the other hand, patients have to play central roles in determining their own care and have different preferences when choosing treatment. This may vary across cultural and ethnic backgrounds, relating to beliefs about healthcare in general and treatment in particular. Frequently, OA patients have preferences for complementary therapy, alternative medicine or invasive treatments such as injections [20]. Adherence is another barrier to treatment success. It is suggested that adherence to any intervention in OA is between 50% and 95%, but as these estimates are mainly derived from clinical trials, the real levels in clinical practice are likely to be much lower.

The finding that the referral for weight reduction had the lowest pass rate is in accordance with other studies [18]. The fact that only 23% of those who were overweight had been referred to weight reduction counseling might simply reflect that the majority of the respondents came from rural settings where community-based service for overweight people is not available. On the other hand, the high achievement rate for QI on exercise might reflect that

81% of the respondents had at least one chronic disease other than OA, such as hypertension or diabetes, and that there are many similarities between recommendation for non-pharmacological treatment of comorbidities and OA.

Bivariate regression analysis showed that the patient's physical functioning was significantly associated with the QI summary achievement rate. The effects of OA severity or the severity of functional impairment on the achievement rate might be explained by aspects of the condition and of the service, such as the age-related nature of OA and the likelihood of patients with a more severe condition consulting more often.

Although bivariate regression analysis did not show any significant association between occupation, body mass index, or duration of the disease and severity of functional impairments, the Kruskal–Wallis test showed that obese patients, farmers, and housewives, as well as patients with longer duration of the disease, had more severe impairments or worse score on the HOOS-PS/KOOS-PS/MQH questionnaires compared to the other respondents, indicating that these groups should be given special attention [18, 19].

Other authors have also assessed the quality of the OA treatment using different QI sets than what we used in the present study. For example, Askari et al. [21] reviewed the use of the ACOVE QIs in 17 studies and found that the interquartile range score of 29–41% for OA was the lowest score among the diseases reviewed [22], while Li et al. [23] reported the achievement rate on four QIs to be 22%. Although the QI achievement rate in the present study was higher, it is difficult to make a comparison because the study samples, settings and methods differ. However, large variations in achievement rates for different QI items point out that there is substantial room for improvement of OA care in the town of Ugljevik and that more attention should be paid to the education of family physicians in the field of rheumatology, such as OA management, as well as to the improvement of collaboration with rheumatologists. Future research and efforts for improving OA care should be directed towards the development and implementation of clinical guidelines for OA care.

The strength of this study is that the data on QI achievement rate were obtained from patients and had been validated against medical records, making it possible to assess the care received and perceived. The study sample was not subjected to selection bias related to the family physicians recruiting participants. The indicators refer to health care processes rather than outcomes, and as such may be more sensitive measures of quality, and are more clearly linked to further quality improvement actions. As the burden of OA is high, much of it is presented clinically to family physicians, the incorporation of the set of QIs at national and international level in the realm of routine primary care practice is therefore recommended. Interventions are to be designed to improve achievement of these indicators. Furthermore, it is necessary to define and analyze the boundaries of responsibility for care in the context of the physician–patient relationship and identify specific elements, such as providing adapted and formalized information to patients, adopting more comprehensive assessment and therapeutic approaches, dealing more with patients' views, ideas, and expectations, that are to be preserved in order to maximize patient outcomes without compromising the quality of care for patients with OA.

The present study has several limitations. The QI achievement rate may be overestimating the quality of OA care due to the characteristics of the participants, such as poor socioeconomic backgrounds, as well as the intensity of OA. The included indicators encompass only a small proportion of care, and it is important to note that QIs cannot represent the full spectrum of patient-centered care. Also, the study did not analyze physicians' views on the quality of care and implementation of guidelines.

## CONCLUSION

The quality of care for patients with OA in our study was suboptimal. The summary achievement of QIs was significantly associated with patient physical functioning. Pharmacological therapy and the referral of patients with OA in need of weight reduction seem to have the greatest potential for improvement. Continuous evaluation and implementation of improvement strategies are required. More attention should be paid to patients' views and expectations to increase quality of care and treatment adherence. The future studies need to determine the quality of care for patients in the whole region of the Republic of Srpska.

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## Квалитет лечења остеоартритиса у породичној медицини – студија пресека


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**КРАТАК САДРЖАЈ**

**Увод** Ефективни третмани за остеоартритис су на располагању, али мало се зна о квалитету бриге за ово стање у примарној здравственој заштити Републике Српске.

**Циљ рада** Главни циљ истраживања је анализирати квалитет третмана остеоартритиса у породичној медицини, као и испитати да ли је постизање индикатора квалитета повезано са одређеним карактеристикама болесника и тежином остеоартритиса.

**Методе рада** Студија пресека је обухватила 120 болесника са дијагностификованим остеоартритисом шака, колена и кукова, регистрованим у седам тимова породичне медицине у Угљевику. Подаци за процену неге кроз 14 индикатора квалитета су прикупљени анкетирањем пацијената и из њихових здравствених картона. Избор индикатора је заснован на сету индикатора квалитета Фондације за артритис (*The Arthritis Foundation's Quality Indicator set for Osteoarthritis*). Стопе остварења су израчунате за остеоартритис кука, колена или шаке, као и за укупни узорак.

**Резултати** Средња стопа остварења за свих 14 индикатора квалитета добијених из медицинске документације износила је 74% и 77% добијених анкетирањем пацијента. Најниже стопе су постигнуте у погледу упућивања на саветовање ради смањења тежине (23%) и фармаколошког третмана (24%), док су се највише стопе односиле на физикални преглед (100%), бол и функционалну процену (100%), те образовање (90,8%). Физичко функционисање пацијента је било значајно повезано са стопом остварења индикатора ($p = 0,001$).

**Закључак** У примарној здравственој заштитити треба побољшати фармаколошки третман остеоартритиса, као и повећати усмеравање пацијената са повећаном телесном тежином на њену редукцију.



**Кључне речи:** остеоартритис; квалитет неге; индикатор квалитета; породична медицина

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# The onset of systemic lupus erythematosus and thyroid dysfunction following Graves' disease – A case report and literature review

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**SUMMARY**

**Introduction** Graves' disease is a multifactorial autoimmune thyroid disease, with the presence of typical circulating autoantibodies that can activate the thyroid hormone receptors, resulting in hyperthyroidism, goiter, and ophthalmopathy. Systemic lupus erythematosus is a multi-systemic autoimmune disease that involves almost all the organs of the human body and is characterized by autoantibodies formation. Several studies have reported that autoimmune thyroid and rheumatic disorders can present an unusual relationship.

**Case Outline** We report a case of a middle-aged woman who presented with systemic lupus erythematosus one year after being diagnosed with Graves' disease. Prednisone and cyclophosphamide were administered to control the development of systemic lupus erythematosus. Furthermore, a percutaneous thyroid biopsy was performed for further confirmation of Graves' disease. Methimazole instead of propylthiouracil was added into the therapeutic scheme. A month later, the patient's clinical manifestation and laboratory tests got significant improvement, except that new thyroid dysfunction appeared opposite to the original one. The administration of anti-thyroid drug was discontinued. With a period of decreased administration of prednisone, the patient's thyroid function gradually got back to normal levels without any levothyroxine replacement.

**Conclusion** In conclusion, the clinical use of prednisone and antithyroid drugs may result in instability of the hypothalamus–pituitary–thyroid axis, and thyroid function should be carefully monitored in such patients.

**Keywords:** Graves' disease; systemic lupus erythematosus; thyroid dysfunction

## INTRODUCTION

Graves' disease (GD) is a multifactorial autoimmune thyroid disease (AITD), with the presence of typical circulating autoantibodies which could activate the thyroid hormone receptor, resulting in hyperthyroidism, goiter, and ophthalmopathy [1]. Systemic lupus erythematosus (SLE), as a multi-organ autoimmune disorder, is characterized by a loss of self-tolerance and organ dysfunction. The autoantibodies are mistakenly directed to attack healthy tissue [2, 3]. The association between thyroid disease and SLE was first mentioned in 1961 by White et al. [4] and Hijmans et al. [5]. Moreover, thyroid disorders appear to be more frequent in SLE patients [6]. Several studies have reported that a pathogenic association of AITD with SLE may exist in a wide range of variability [7, 8, 9]. The mechanisms by which AITD may be linked to systemic autoimmune diseases have not been fully clarified yet; however, alterations of common pathways are suggested by shared genetic variants affecting autoantigen presentation and regulation of the immune response [8].

Herein, we report a case of a diagnosis of GD and SLE, followed by the development of a new thyroid dysfunction after prednisone and cyclophosphamide (CTX) treatment. We also reviewed the medical literature on thyroid problems induced by the glucocorticoids usage.

## CASE REPORT

A 48-year-old woman who complained of palpitation, hyperidrosis, for one year and edema of lower extremities for two months was admitted to Qilu Hospital of Shandong University in March 2015. The woman complained of weakness, dysphoria, chest tightness and wheezes after exercise, irregular menstruation for one year, and weight loss of 5 kg in two months. She was diagnosed with GD, hypertension, and coronary heart disease in the local hospital. She received propylthiouracil (PTU), metoprolol, valsartan, and indapamide for treatment and the symptoms were soon relieved. However, two months later, edema of lower extremities, alopecia, skin erythema, irregular fever, and foam urine started presenting themselves and the patient was referred to Qilu Hospital of Shandong University for further treatment.

On physical examination, her temperature was 36.2°, pulse was 101 beats/min. and regular, respiratory rate was 23 breaths/min., and blood pressure was 161/89 mmHg. The physi-

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cal examination revealed poor spirit, angular face, and erythematous patches on her neck. Diffuse goiter and axillary lymph nodes were palpated. No tremor or vascular murmur could be found in the thyroid. Increased breath sounds were listened to on both sides of the chest. The abdomen was remarkable for splenomegaly. There was marked edema of lower extremities.

After the admission on March 25, 2015, the patient underwent a series of laboratory tests and imaging examinations. The laboratory data are listed in Table 1. Chest and abdomen computed tomography revealed focal fibrosis and hydrothorax of double lung, hydropericardium, cholecystitis, hydrocholecystis, pancreatitis, splenomegaly, and the mediastinal lymph node enlargement. Doppler sonography of the thyroid showed multiple thyroid nodules (goiter with adenoma).

The patient was diagnosed as GD; SLE, lupus nephritis; hypertension (Grade 2; extremely high risk group); and coronary heart disease. The disease activity index of SLE (SLEDAI) was 14. Percutaneous renal biopsy was performed. The histological findings were membranoproliferative glomerulonephritis along with "full-house" deposits by immunofluorescence staining, which was considered lupus nephritis (WHO IV-Ga, Figure 1). The electron microscope further showed the deposits were electron-dense and diffusely located in the subendothelial, subcutaneous, and mesangial area consistent with lupus nephritis (WHO IV-Ga, Figure 2). The patient was treated with prednisone 1 mg/kg/day along with CTX 0.6 g/m$^2$ per month. Fine-needle aspiration biopsy (FNAB) of the thyroid was performed. FNAB showed cellular smear with similar features to hyperplastic nodule (Figure 3). PTU was discontinued and methimazole (10 mg bid) was added in case of PTU-induced lupus.

After two weeks, the patient was alleviated with disappeared erythematous patches, fever and relieved edema. Urinalyses revealed white blood cell count (WBC) 30 p/ul, red blood cell count (RBC) 444.3 p/μL, proteinuria/24h 11.29 g. Blood routine showed hemoglobin (HGB) 128 g/L (115–150). Hepatic and renal function demonstrated serum creatinine 75 μmol/L (53–97), albumin 22.1 g/L (40–55). Her treatment was continued according to the original plan. A month later, the laboratory analysis revealed urine WBC 10.56 p/μL, RBC 24.42 p/μL, proteinuria/24h 5.43 g; HGB 112 g/L (115–150); serum albumin 18.3 g/L (40–55). Thyrotropin (TSH) 0.01 μIU/ml (0.35–4.94), free triiodothyronine (FT3) 1.54 pmol/L (2.63–5.70), and free thyroxine (FT4) 5.15 pmol/L (9.01–19.05) were all below the normal level. Methimazole was discontinued. She continued to receive prednisone 1 mg/kg/day plus CTX 0.6 g/m$^2$ again. Two months later, her clinical manifestation and laboratory index improved significantly. Laboratory test results included proteinuria/24h 3.35g; serum albumin 24.6 g/L (40–55) and antinuclear antibodies (ANAs) positive for (1:160). TSH 0.211 μIU/ml (0.35–4.94), FT3 1.54 pmol/L (2.63–5.70) and FT4 5.15 pmol/L (9.01–19.05), were still below the normal level. Amounts of prednisone were reduced by 10% of the original amount every two weeks and she continued to receive CTX 0.6 g/m$^2$. After three months, laboratory data showed proteinuria/24h 0.95 g, HGB 112

**Table 1.** Laboratory data on the patient's first admission

| Parameter | | Value |
|---|---|---|
| Blood routine | WBC (×109/L) | 5.13 |
| | RBC (×1012/L) | 3.24 |
| | Hb (g/L) | 90 |
| | HCT (%) | 25.1 |
| | Platelet (×109/L) | 146 |
| Urine | pH | 5.0 |
| | Glucose | - |
| | Occult blood | 3+ |
| | RBC (p/μl) | 46.2 |
| | WBC (p/μl) | 58.2 |
| | Ketone body | - |
| | ACR (g/g) | 8.01 |
| | κ/λ (0.75–4) | 1.56 |
| Urine renal tubular function | Albumin (mg/L) | 1960 |
| | β2-MG (mg/L) | 0.22 |
| | α1-MG (mg/L) | 166 |
| | IgG (mg/L) (0–14) | 159 |
| Hepatitis and tumor marker | Hepatitis B surface antigen | - |
| | Hepatitis C antibody | - |
| | Tumor marker | - |
| Biochemistry | Total protein (g/L) | 35.5 |
| | Albumin (g/L) | 15.6 |
| | ALT (IU/L) | 8.0 |
| | AST (IU/L) | 17.0 |
| | γ-GTP (IU/L) | 19.0 |
| | LDL-C (mmol/L) | 2.95 |
| | TG (mmol/L) | 1.41 |
| | HDL-C (mmol/L) | 1.21 |
| | BUN (mmol/L) | 11.72 |
| | Cre (umol/L) | 70 |
| | eGFR (mg/min/1.73m2) | 88.2 |
| | UA (μmol/L) | 590 |
| | K (mmol/L) | 4.29 |
| | Cl (mmol/L) | 108 |
| | Na (mmol/L) | 136 |
| | Ca (mmol/L) | 1.68 |
| Thyroid-related | TSH (μIU/ml) | <0.003 |
| | FT3 (pmol/L) | 8.39 |
| | FT4 (pmol/L) | 15.12 |
| | TPO-Ab (IU/ml) | 650.81 |
| | TG-Ab (IU/ml) | 245.95 |
| | TR-Ab (μIU/ml) (0–1.22) | 5.66 |
| Rheumatic-related | ANAs | 1:320 |
| | dsDNA(IU/ml)(<100) | 82.97 |
| | c-ANCA (U/ml) (<5) | 4.0 |
| | p-ANCA (U/ml) (<5) | 0.53 |
| | GBM-Ab | - |
| | ACL-IgG (U/ml)(<10) | 2.49 |
| | ACL-IgM (U/ml)(<10) | 2.84 |
| | SSA-Ab | `- |
| | SSB-Ab | - |
| | Sm-Ab | - |
| | Jo-1-Ab | - |
| | Scl-70-Ab | - |
| | nRNP-/Sm-Ab | - |
| | AMA (<1:20) | - |
| | GPI (mg/L) (<0.2) | 0.12 |
| | ACPA (Ru/ml) (<25) | 4.15 |
| | Serum C3(g/L) | 0.42 |
| | Serum C4(g/L) | 0.094 |
| | IgG (g/L) | 7.12 |
| | IgA (g/L) | 3.25 |
| | IgM (g/L) | 0.80 |
| Type of T cells | CD3+ cells (%) | 73.4 |
| | CD3+CD4+/CD3+CD8+ | 0.84 |
| | CD3+CD4+ cells (%) | 32.28 |
| | CD3+CD8+ cells (%) | 38.54 |

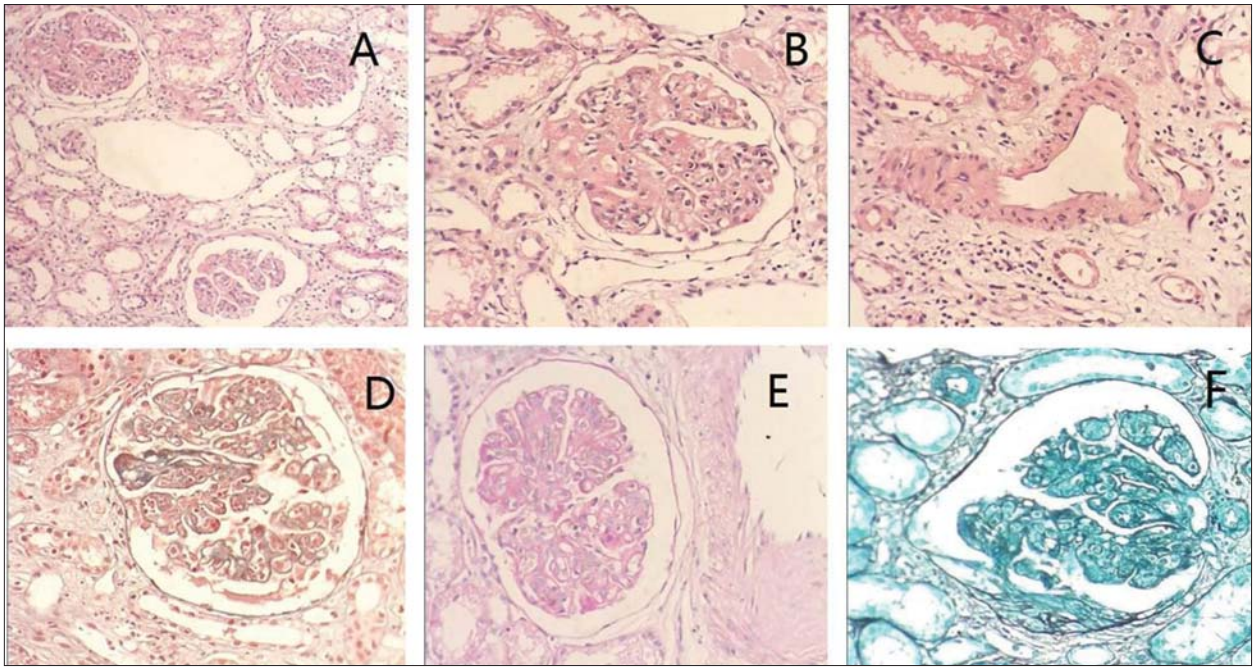



**Figure 1.** Histopathology of renal sections with HE (hematoxylin-eosin), Masson, PAS (periodic acid-Schiff staining), and PASM (periodic acid silver methenamine) staining; A: HE staining (×100); B: HE staining (×400); C: HE staining of tubulointerstitial (×400); D: Masson staining (×400); E: PAS staining (×400); F: PASM staining (×400)

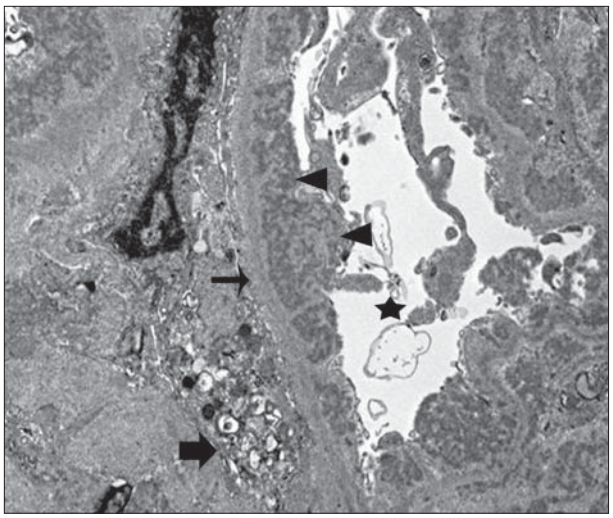


**Figure 2.** Renal electron microscopy (magnification ×6,000): glomerular basement membrane (thin arrow), podocyte (thick arrow), electron deposits (arrowheads), vessel lumen (star)

g/L (115–150), serum albumin 30.8 g/L (40–55), ANAs <:80 (<1:80), anti-double stranded DNA antibody (anti-dsDNA) 3.97 U/ml (0–100), TSH 0.684 μIU/ml (0.35–4.94), FT3 1.56 pmol/L (2.63–5.70), FT4 6.38 pmol/L (9.01–19.05), which all showed significant improvement. On the follow-up four months later, kidney and thyroid function were essentially back to normal range, and proteinuria was approximately 0.18 g / 24 h. The patient's thyroid function from the hospitalization to the last follow-up is shown in Graph 1. Changes of serum albumin and urine protein of the previous seven months are shown in Graph 2.

## DISCUSSION

Several studies have revealed a conceivable relationship between thyroid disease and SLE [7, 8, 9]. Both GD and SLE are multi-systemic autoimmune disorders sharing common genetic basis. The strongest association for both

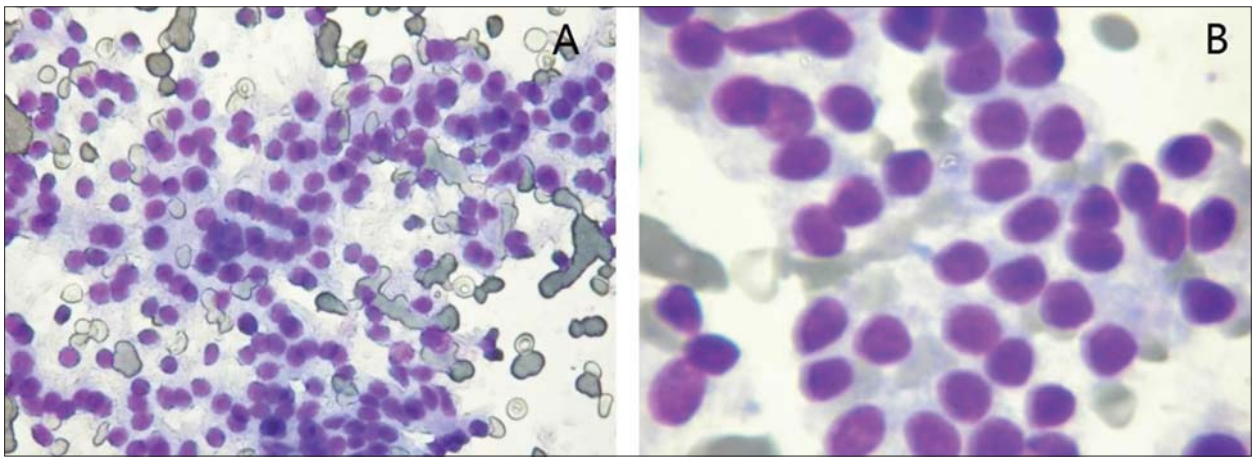
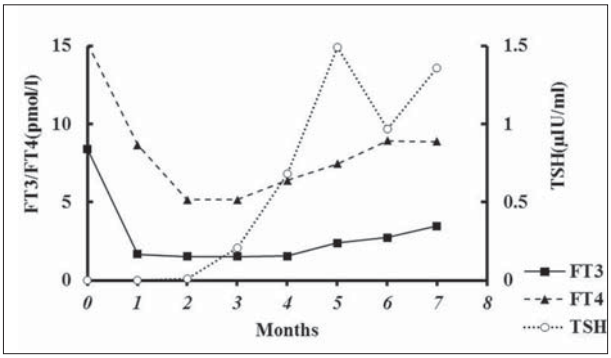
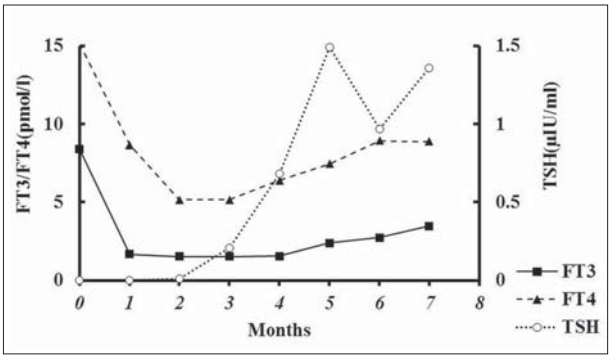



**Figure 3.** Thyroid tissue fine-needle aspiration biopsy with HE staining; A: magnification ×40; B: magnification ×100




**Graph 1.** Patient's thyroid function change from hospitalization in March 2015 to the last follow-up

FT3 – free triiodothyronine; FT4 – free thyroxine; TSH – thyrotropin




**Graph 2.** Corresponding change in serum albumin and urine protein from March 2015 to the last follow-up

GD and SLE are HLA haplotypes including HLA-B8, HLA-DR3 and inositol 1,4,5-triphosphate receptor type 3 (ITPR3) [8, 10]. Sex hormones have been considered to be responsible for susceptibility to autoimmune disease through modulation of Th1/Th2 response. Estrogens appear to promote autoimmune disease with a type 2 cytokine profile, such as in GD and SLE [11]. Therefore, our case with both GD and SLE is not accidental.

Drug-induced lupus (DIL) related to PTU is not rare, therefore correct diagnosis of idiopathic or drug induced lupus is very important for patients treated with PTU. DIL is characterized as musculoskeletal (joint and muscle) pain, serositis and constitutional manifestations such as fever, fatigue, and loss of appetite [12]. Laboratory findings of DIL, specifically serum positivity for ANAs and antihistone antibodies, along with negative anti-Smith antibodies, anti-dsDNA and normal complement profile are common. Classic mucocutaneous signs including malar erythema, discoid lesions, hair loss, and oral ulcers are also common in DIL. However, renal or neurologic manifestations are not usually involved in it. The course of DIL is usually benign and remission over several weeks after discontinuation of the inducing drug is usually seen. There is less chance of DIL in this middle-aged woman with PTU for one year, considering unmatched clinical and serologic manifestations.

In addition, GD can present with similar manifestations of SLE due to overlapping clinical and laboratory criteria. The differentiation between these two diseases requires careful laboratory evaluation. Diagnosis of SLE should be suspected if patients are diagnosed with GD or have received PTU with characteristic symptoms of SLE and positive ANA. Patients with GD who display SLE but not enough for a diagnosis of SLE should be closely followed up for avoiding misdiagnoses and mistreatments.

Glucocorticoids commonly used for the treatment of SLE can inhibit the secretion of serum TSH, and reduce the thyroid hormone by several mechanisms. There are numerous targets of drug interaction on the pathways of thyroid hormone at different phases of synthesis, secretion, and transport in the circulation and metabolism [13]. Being the single best marker of thyroid function, serum TSH values are usually low or normal in patients with hyperthyroidism, but they are usually high due to the biologically inactive TSH secretion [13, 14]. Some experts have reported that high-dose glucocorticoids suppress the secretion of TSH in hypothyroid patients and normal subjects [15]. Glucocorticoids may suppress the secretion of TSH depending on protein kinase C [16]. Recently, high doses of glucocorticoids have been found to play a role in decreasing the level of TRH mRNA in human hypothalamus, which may illustrate the mechanism by which lower TSH levels are secreted from the pituitary gland [17]. In addition, glucocorticoids can impair peripheral 5'-deiodination of T4 and lower serum thyroid binding globulin (TBG) level [18]; therefore, serum concentrations of TT4, FT4, and TT3 might decrease.

Glucocorticoids are commonly used for treatment of SLE due to its immunosuppressive effects, but their use can lead to thyroid dysfunction, especially in patients receiving antithyroid agents. We reviewed the literature of patients with thyroid dysfunction induced by use of various glucocorticoids and related data is shown in Table 2. Most of the patients were female with mean age of 43 years. It is believed that thyroid dysfunction induced by glucocorticoid cessation may be the rebound of immune activity [19, 20, 21]. Bartalena et al. [22] reported that Graves' hyperthyroidism and ophthalmopathy occur during chronic low-dose glucocorticoid therapy. They suggested that a high dose of glucocorticoids for the treatment of severe Graves' ophthalmopathy might indeed suppress the disease as well. However, the low dose use might aggravate the disease [22]. Glucocorticoids can lower serum potassium and induce thyroid periodic paralysis by several mechanisms such as an increased Na+/K+-ATPase pool in skeletal muscles, steroid-induced hyperinsulinemia, and hyperglycemia [23]. Interestingly, we observed the patient developed a new onset of thyroid dysfunction in which FT3, FT4, and TSH are all below the normal level and may be called "central hypothyroidism" after receiving glucocorticoid and CTX in our case. To our knowledge, there are no reported cases of developing "central hypothyroidism" with simultaneous treatment with glucocorticoids and antithyroid agents. The possibility of "central hypothyroidism" in our case could be speculated upon as follows: glucocorticoid can decrease secretion of TSH and lower serum TSH level through direct effects on TRH in the hypothalamus [18]. Glucocorticoid and methimazole

**Table 2.** Thyroid dysfunction induced by the use of glucocorticoids

| Reference | Maruyama et al. [19] | Nagai et al. [20] | Morita et al. [21] | Bartalena et al. [22] | Wongraoprasert et al. [23] |
|---|---|---|---|---|---|
| Age (years) | 51 | 42 | 53 | 38 | 23 |
| Sex | F | F | F | F | M |
| Primary disease | RA, CLT | Uveitis | AR | PV | CIDP, GD |
| Inducing factors | Dexamethasone cessation | Prednisone cessation | Celestamine cessation | Oral prednisone | Pulse methylprednisolone |
| Manifestations | Thyrotoxicosis | Goiter | Hand tremor | Thyrotoxicosis | Paralysis |
| Pathology | CLT | HT | None | None | None |
| Diagnosis | CLT | HT | PT | GD | TPP |
| Treatment | None | None | Thiamazole | Radioiodine | Potassium |
| Recovery time (months) | 2 | 2 | 14 | 4 | Several hours |
| Follow-up time (months) | 9 | 3 | 18 | Not given | Not given |
| Outcome | Hypothyroidism | Euthyroid | Euthyroid | Euthyroid | Euthyroid |

F – female; M – male; RA – rheumatic arthritis; CLT – chronic lymphocytic thyroiditis; AR – allergic rhinitis; PV – pemphigus vulgaris; CIDP – chronic inflammatory demyelinating polyneuropathy; GD – Graves' disease; HT – Hashimoto's thyroiditis; PT – painless thyroiditis; TPP – thyrotoxic periodic paralysis

decreased the level of thyroid hormone at the beginning of treatment; however, thyroid-stimulating hormone level does not immediately increase because of glucocorticoid inhibitory effect on hypothalamus. Therefore, reduced use of anti-thyroid drugs should be considered in patients who developed GD with other diseases received both glucocorticoid and anti-thyroid drugs to avoid instability of the hypothalamus–pituitary–thyroid axis. We didn't administer levothyroxine replacement since the patient had suffered from GD before. Her thyroid function improved markedly along with prednisone reduction as expected. As the pattern of thyroid dysfunction may actually reflect a sick euthyroid state that may normalize after hypercortisolism is resolved, we recommend withholding thyroid hormone replacement under this condition.

In summary, SLE and Graves' disease are systemic autoimmune diseases sharing common genetic basis and similar clinical manifestations. The clinical use of prednisone and antithyroid drugs may result in instability of the hypothalamus-pituitary-thyroid axis and the thyroid function should be carefully monitored in such patients

## ACKNOWLEDGEMENTS

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## NOTE

The patient's written consent was obtained according to the Declaration of Helsinki.

Yuanyuan Zhang and Xiaoyan Xiao contributed equally to this work.

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## Јављање системског еритемског лупуса и дисфункције штитасте жлезде током Грејвс–Базедовљеве болести – приказ болесника и преглед литературе

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**КРАТАК САДРЖАЈ**

**Увод** Грејвс–Базедовљева болест је мултифакторна аутоимуна болест штитасте жлезде, уз присуство типичних циркулишућих аутоантитела која могу активирати рецептор хормона штитасте жлезде, што резултира хипертироидизмом, гушавошћу и офталмопатијом. Системски еритемски лупус је мултисистемска аутоимуна болест која утиче на скоро све органе људског тела, а коју карактерише формирање антитела. У неколико студија је наведено да аутоимуни тироидни и реуматски поремећаји могу успоставити необичан однос.

**Приказ болесника** Приказујемо случај средовечне жене којој се јавио системски еритемски лупус годину дана пошто јој је успостављена дијагноза Грејвс–Базедовљеве болести. Преписани су јој преднисон и циклофосфамид како би се ограничио развој системског еритемског лупуса. Уз то је извршена перкутана биопсија штитасте жлезде за потврду дијагнозе Грејвс–Базедовљеве болести. У терапију је уведен метимазол уместо пропилтиоурацила. Месец дана касније клиничка слика и лабораторијски налази значајно су се побољшали, с тим што се нова дисфункција штитасте жлезде јавила као супротност првобитној дисфункцији. Прекинута је примена антитироидних лекова. Уз смањену примену преднисона функција штитасте жлезде болеснице постепено се вратила на нормални ниво без увођења левотироксина.

**Закључак** Клиничка примена преднисона и антитироидних лекова може резултирати нестабилношћу осе хипоталамус – хипофиза – штитаста жлезда, те би код таквих болесника требало пажљиво пратити функцију штитасте жлезде.

**Кључне речи:** Грејвс–Базедовљева болест; системски еритемски лупус; дисфункција штитасте жлезде

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# Synchronous advanced pulmonary tuberculosis and acute virus myocarditis mimicked Wegener granulomatosis in a 26-year-old man – A case report

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**SUMMARY**

**Introduction** Tuberculosis patients are rarely asymptomatic. Acute virus myocarditis presents with a wide range of symptoms, from mild dyspnea or chest pain to cardiogenic shock and death.

**Case Outline** A 26-year-old Caucasian man non-smoker presented with one-week history of lower extremities' swelling. The patient's medical history also revealed a two-day episode of subfebrile temperature with scanty hemoptysis three weeks prior to admission. The episode had not provoked him to seek medical care. Physical examination revealed generalized oedema, and laboratory analysis showed signs of acute renal insufficiency. Enlarged heart and hilar shadows, bilateral massive cavitary pulmonary opacities and pleural effusion were found at chest radiography. Sputum smears were *Mycobacteria* negative on direct microscopy. Electrocardiogram changes and echocardiography were suggestive of acute myocarditis with dilated cardiomyopathy. IgM titer to adenovirus was positive. Under diuretics, angiotensin-converting-enzyme inhibitor, beta-blocker, antibiotics and bed rest, fast heart compensation and renal function repair were achieved. Radiographic pulmonary changes promptly regressed except for a cavity in the right upper lobe. Bronchial aspirate from the affected lobe was *Mycobacteria* positive on direct microscopy and culture positive for *Mycobacterium tuberculosis*. Standard anti-tuberculosis drug regimen led to recovery.

**Conclusion** In the unusual common existence of two diseases whose presentation initially mimicked Wegener's granulomatosis, acute dilated cardiomyopathy contributed to pulmonary tuberculosis detection. To prevent diagnostic delay in tuberculosis, further efforts in population education are necessary together with continual medical education.

**Keywords:** tuberculosis, pulmonary; myocarditis, viral; cardiomyopathy; diagnostics; oedema; renal insufficiency

## INTRODUCTION

Tuberculosis (TB), declared global emergency by the World Health Organization, is an infectious disease caused by *Mycobacterium tuberculosis complex*. A total of 9.6 million people worldwide developed active TB and 1.5 million people died from the curable disease in 2014 [1]. Although TB may affect any organ, in 96% of the cases it starts in the lungs [2]. TB prevalence in a community influences the risk of being infected and the risk of developing TB in infected patients depends on many factors that determine human immunity [3]. Bacteriologic confirmation is the gold diagnostic standard.

Myocarditis is inflammation of the myocardium accompanied by myocellular necrosis, usually caused by infectious agents [4]. It represents a significant cause of death especially in young patients [5]. Viral myocarditis is most frequently caused by parvovirus B19, human herpes virus 6, coxsackievirus, and adenovirus [6]. Acute myocarditis should be considered in patients who present with recent onset of cardiac failure and/or arrhythmia [6]. Although endomyocardial biopsy with viral genome detection is considered the gold standard in the diagnostics of viral myocarditis, it is not routinely used in all suspected cases [7, 8]. Presence of viral genome in the myocardium of patients with acute dilated cardiomyopathy has no functional and prognostic relevance [9].

We aimed to describe a rare condition: synchronous existence of two atypically presented diseases that initially mimicked Wegener's granulomatosis. The Ethics Board of the Clinical Centre of Serbia in Belgrade has approved the case report to be published in a medical journal.

## CASE REPORT

A previously healthy 26-year-old non-smoker Caucasian man presented to emergency room with one-week history of rapidly progressive low extremities' swelling without other complaints. The patient's medical history revealed a two-day episode of subfebrile temperature and scanty hemoptysis three weeks prior to admission. He never went to see a doctor for that and denied history of alcohol abuse, toxic exposure or sick contact. He was a guest to his relatives, coming from a high TB prevalence and a post-war country.

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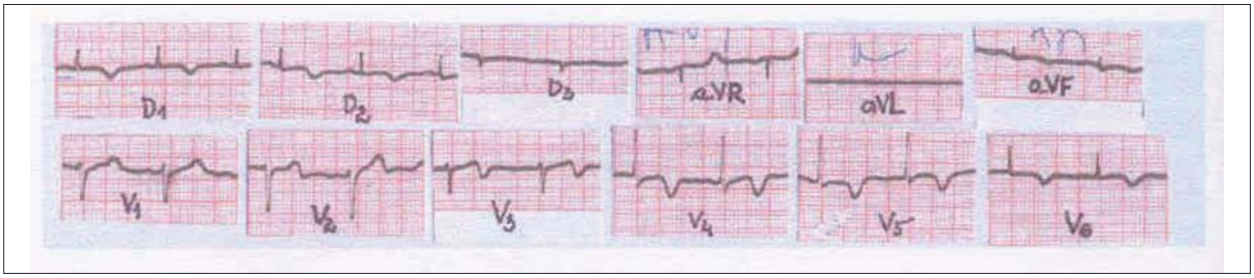


**Figure 1.** Electrocardiography on admission: low QRS complex voltage, depressed and inverted T wave in the standard and left precordial leads

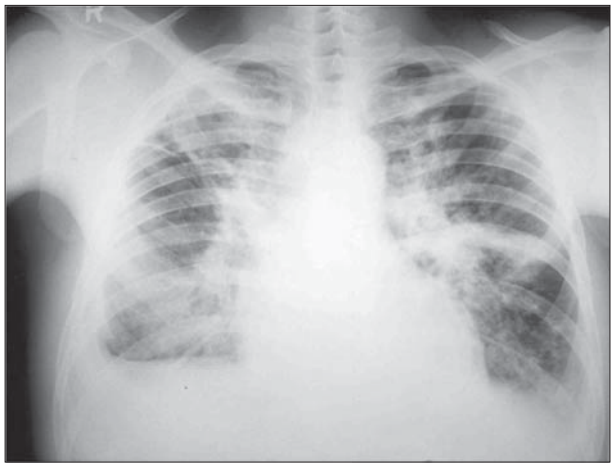

**Figure 2.** Posteroanterior chest radiography on admission: enlarged heart and hilar shadows, diffuse pulmonary parenchymal opacities, partially excavated, especially expressed in the right infraclavicular and left perihilar fields with bilateral obliteration of costophrenic sinus

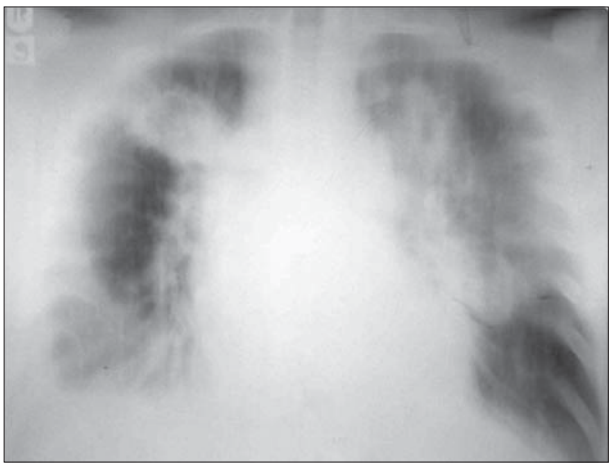

**Figure 3.** Frontal chest radio tomography on admission (9 cm): bilateral massive lung patchy infiltrates, partly excavated, together with renal insufficiency, might suggest Wegener's granulomatosis

Inspection showed heavy generalized edema especially expressed in lower extremities. The patient's vital signs were as follows: 1) blood pressure: 105/70 mmHg; 2) body temperature: 36.4°C; 3) respiratory rate: 16 breaths/min.; 4) heart and pulse rate: 88 beats/min.; and 5) oxygen saturation 95% while breathing room air. Auscultation revealed decreased pulmonary sound and crackles over pulmonary bases. Results of peripheral blood laboratory tests were within normal limits, including complete blood count, except for signs of renal insufficiency [urea: 12.2 mmol/L (range: 2.5–7.5), creatinine: 193 μmol/L (range: 53–124), $sK^+$: 5.6 mmol/L (range: 3.5–5.1)]. Electrocardiogram (ECG) changes are shown in Figure 1 and standard posteroanterior chest radiography in Figure 2. Apart from pulmonary and pleural changes, radiographic findings suggested that cardiomegaly was possible by the ratio of the heart diameter to that of the chest. The findings were completed with chest tomography (Figure 3), which was suggestive of granulomatosis Wegener. Negative vasculitis serology made the diagnosis of vasculitis less likely.

Echocardiography revealed signs of acute myocarditis: significant enlargement of the left ventricle following dilatative cardiomyopathy pattern with significantly reduced its systolic function (ejection fraction: 37%); changed hypoechogenic myocardial structure with thicker wall and septum that correlated with myocardial oedema during acute myocarditis; low degree mitral regurgitation due to dilatation of the left ventricle and pericardial dissociation with minimal pericardial effusion as signs of cardiac failure. Immuno-serologic examination was negative for systemic disease and IgM titer to adenovirus was positive.

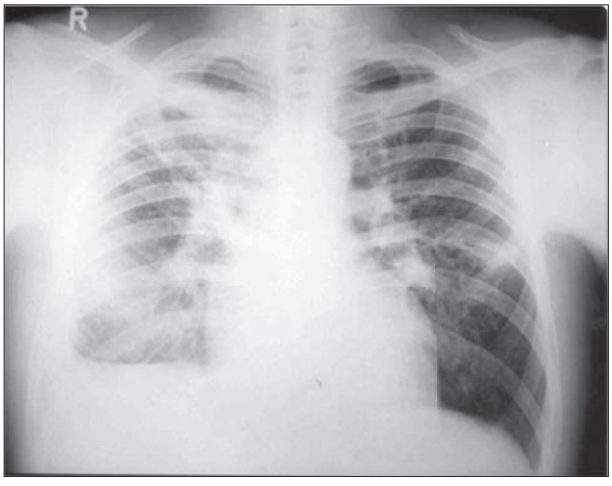

**Figure 4.** Chest radiography seven days after admission; under diuretic (and antibiotic) treatment, left-sided pleural effusion disappeared (clearly shaped left diaphragm and costophrenic sinus) together with majority of pulmonary shadows that belonged to hydrostatic pulmonary oedema

The patient responded well to furosemide, angiotensin-converting-enzyme inhibitor, calcium channel blocker, bed rest, and antibiotic. Generalized oedema promptly diminished and disappeared in several days and radiographic changes regressed significantly (Figure 4). Cardiac compensation was achieved and renal function normalized. ECG and echocardiography findings suggested re-

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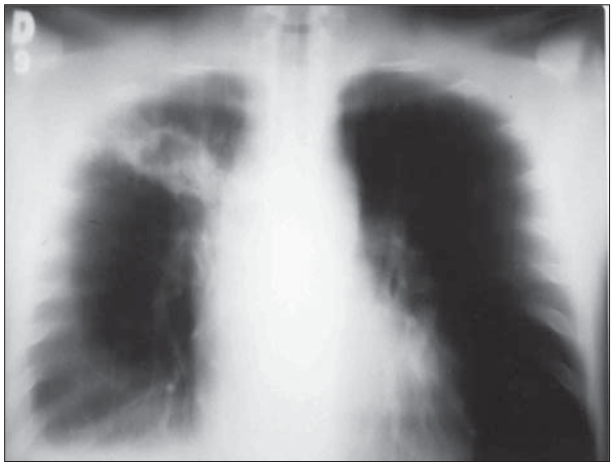

**Figure 5.** Chest tomography six weeks later showed irregularly shaped excavated thickened wall shadow 3 × 4 cm in size in the right upper pulmonary lobe

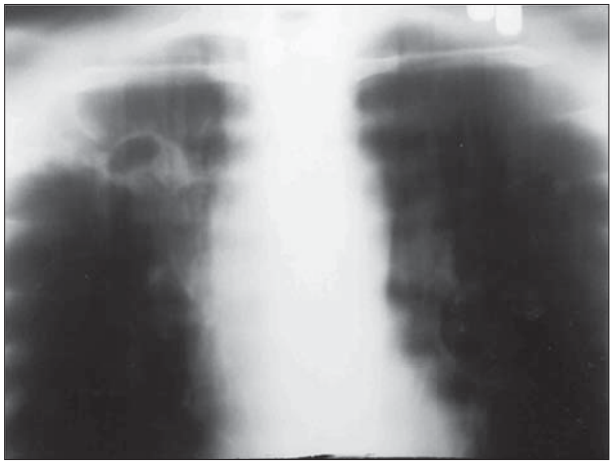

**Figure 6.** Frontal chest radio tomography (9 cm): typical slow regression of the right upper lobe cavity (Figure 5) two months after the commencement of the anti-tuberculosis treatment

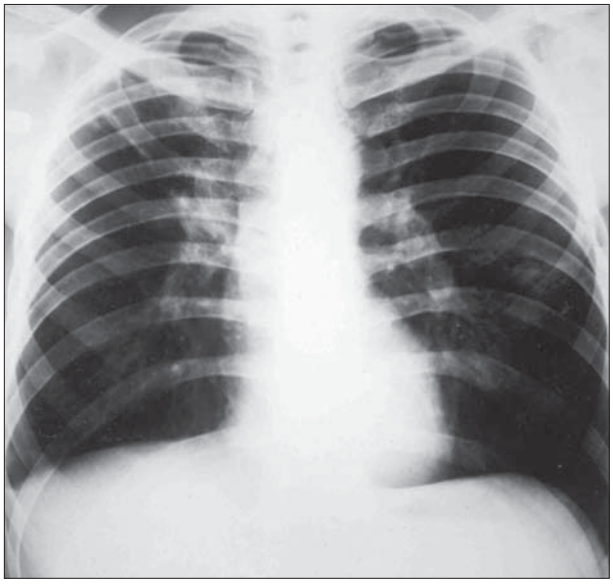

**Figure 7.** Standard chest radiography one year later: normally sized heart shadow; sequelae tuberculosis in the right upper lobe (second intercostal space) and obtuse right costophrenic sinus due to pleural adhesion

gression of myocarditis and left ventricle function recovery. Six weeks later, all radiographic pulmonary changes regressed except for one in the right upper lobe (Figure 5). Asymptomatic patient's stabile cardiac condition allowed continuation of the necessary diagnostic procedure. Bronchoscopy showed normal bronchial tree, and aspirate from the area of radiographic changes was acid fast bacilli positive on direct microscopy. Culture was positive for *Mycobacterium tuberculosis*.

Anti-tuberculosis treatment started following standardized short course regimen. Regression was evident on chest tomography two months later, at the end of the initial phase of treatment (Figure 6). The therapy was continued for the next four months until the end of the regimen.

The patient came for a medical examination one year later, having no respiratory complaints, at which time chest radiography was performed (Figure 7). Transient ventricular premature beats (bigeminy) were registered and further cardiologic follow-up and treatment were recommended.

## DISCUSSION

We presented a case of acute adenovirus myocarditis, which happened to be helpful in detection of advanced pulmonary TB. Atypically, our patient presented without fatigue, appetite or weight loss, sweating and cough. Cough is the most common symptom, and hemoptysis appears in about 20% of patients with pulmonary TB [2, 10]. Together with sneezing, singing or loud speaking, cough is considered crucial in the production of infectious droplet nuclei and spreading of the airborne infection [3]. Thus, absence of cough in patients with cavitary TB is especially important from the public health point of view. Moreover, on admission, the patient was acid-fast bacilli sputum smear negative on direct microscopy, which additionally diminished his presence as a serious source of infection.

Diagnosis of acute myocarditis is based on symptoms/signs, electrocardiography and echocardiography [11, 12]. The clinical picture may vary, ranging from asymptomatic courses to severe illness and the necessity of intensive care therapy [13]. Our patient had no symptoms related to an infectious disease or cardiac failure until rapidly growing low extremities' oedema appeared. These were the only reason of his concern and coming to see a doctor. We could not but to mention fulminant myocarditis as a distinct entity characterized by sudden onset of severe congestive heart failure or cardiogenic shock. Adenovirus is among its known causes. It usually develops following a flu-like illness, which missed in our patient, who had a stable hemodynamic state during the course of the disease, absence of symptoms such as dyspnea, chest pain, fever, cough or palpitations, and relatively fast recovery under diuretics, angiotensin-converting-enzyme inhibitors and bed rest.

Biopsy for diagnosis of myocarditis in patients who present clinically with congestive heart failure can be useful [14]. Although it remained the gold standard in diagno-

sis of viral myocarditis, it is used infrequently due to perceived risks and the lack of a widely accepted and sensitive histological standard [7, 15]. Serologic test alone is not considered valid for diagnosis, but together with clinical presentation, ECG, radiographic and echocardiographic findings it was adequate to suggest etiology of acute myocarditis and dilated cardiomyopathy in the presented case. The role of many viruses detected in cardiac tissue in causing myocarditis is controversial and even in forensic studies the issue remains challenging. One of the recent studies showed that adenovirus, enterovirus, and parvovirus B19 were found to be rare causes of myocarditis-related death [16]. It is suggested that noninvasive cardiac magnetic resonance imaging might provide an alternative method for diagnosis and its use is recommended as early as possible at onset of the disease when its validity is the highest. Recent research on patients in whom acute myocarditis was clinically suspected for the first time confirmed association of left ventricle transmural myocardial oedema evidenced by contrast-enhanced cardiac magnetic resonance and T wave inversion [17]. The latter has been registered in our patient's ECG (Figure 1) and also associated with myocardial oedema in the presence of minimal pericardial effusion on echocardiography. The pathophysiologic mechanisms of electrocardiographic T-wave inversion occurring in patients with acute myocarditis remain to be elucidated.

Causative differential diagnosis of myocarditis could include the other microbial or toxic agents. Although extrapulmonary TB is on the increase worldwide and in Serbia [1, 18], TB myocardial affection, usually detected on autopsy, was least possible in our patient due to prompt recovery, which is not a characteristic of the TB process [2]. History taking excluded possibility of alcohol-induced or other toxic heart disease.

Myocarditis may cause arrhythmias in its acute phase due to inflammatory infiltration and myocyte necrosis. Transient bigeminy, detected in our patient a year later in the chronic phase, could be attributed to an immune reaction, fibrosis, and resulting electric remodeling [19]. Patient follow-up is of crucial importance to abort complications such as sudden cardiac death [11, 20]. If antiarrhythmic drugs are not effective enough in patients with hemodynamically unstable ventricular tachycardia, an implantable cardioverter-defibrillator is taken into consideration [21].

While it is clear that high TB prevalence in our patient's country of origin might put him into higher risk of TB infection, we hardly succeeded to define any of risk factors for developing TB as an active disease except for one – prolonged emotional stress [3]. The young man had come from a post-war setting, familiar with some horrible local events that had happened.

Diagnostic delay is an important problem in TB control. It consists of patients' delay to seek medical care and the delay of the healthcare system [22]. Population education on TB usually results in better understanding of TB symptoms and earlier presentation of symptoms to a doctor, earlier detection and treatment with stopping the spread of the disease within a community. Moreover, continual medical education could strengthen the idea that patients and groups at higher risk for developing TB exist. The idea should always be present in physicians' minds to initiate an active approach in TB detection [23]. In the times of TB as global emergency, "Think TB!" principle should be a premise in both primary health care and differently profiled specialized institutions.

## ACKNOWLEDGMENT

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# Истовремено присуство кавернозне плућне туберкулозе и акутног вирусног миокардитиса имитирало је Вегенерову грануломатозу код 26-годишњег болесника – приказ случаја

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**КРАТАК САДРЖАЈ**

**Увод** Туберкулоза ретко протиче асимптоматски. Акутни вирусни миокардитис може да се испољи широком палетом тегоба од благе диспнеје или бола у грудима до кардиогеног шока и смрти.

**Приказ болесника** Непушач стар 26 година јавио се на преглед због брзог отицања ногу у последњих недељу дана. Три недеље раније имао је дводневну епизоду оскудних хемоптизија и субфебрилне температуре, због које се није обраћао лекару. Физикалним прегледом је нађен генерализован едем, а лабораторијским анализама знаци акутне реналне инсуфицијенције. Радиограм грудног коша показао је увећану силуету срца и хилуса плућа и обостране масивне промене у плућима, на томографији делом екскавиране, и плеурални излив. Микобактерије нису нађене у спутуму директном микроскопијом. ЕКГ и ехокардиографија су указивали на знаке акутног миокардитиса, тј. дилатативне кардиомиопатије као његове последице. Титар *IgM* на аденовирусе био је позитиван. Диуретици уз *ACE* инхибитор, блокатор калцијумових канала, мировање и антибиотик, довели су до брзог повлачења едема и компензације срца уз нормализовање реналне функције. Брзо се повукао и највећи део плућних промена, које су одговарале стази, али је заостала неправилна екскавација у десном горњем режњу. У бронхоаспирату из места рендгенски видљиве лезије микобактерије су нађене директном микроскопијом, а културом *M. tuberculosis*. И култура иницијалног узорка спутума била је позитивна. Примена антитуберкулотика према стандардном режиму довела је до излечења.

**Закључак** У реткој истовременој појави две болести, која је у почетку личила на Вегенерову грануломатозу, акутни вирусни миокардитис са дилатативном кардиомиопатијом допринео је откривању туберкулозе плућа. Да би се спречило кашњење у дијагнози туберкулозе, неопходни су додатни напори у просвећивању становништва и континуирана медицинска едукација.

**Кључне речи**: туберкулоза, плућна; миокардитис, вирусни; кардиомиопатија; дијагноза; едем; ренална инсуфицијенција

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# Atypical, polyarticular lipoma arborescens in a child

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**SUMMARY**

**Introduction** Lipoma arborescens is a rare, tumor-like lesion commonly involving synovial joints and less commonly bursae and synovial tendon sheaths.

**Case Outline** We report a case of a 12-year-old boy with symmetric involvement of the bicipitoradial bursae, synovial sheaths of extensor compartments of both hands and medial ankles. The diagnosis of polyarticular lipoma arborescens was proposed on magnetic resonance (MR) imaging and this diagnosis was histologically proven after biopsy of the bursae and later by open surgery of the synovial sheath of the right ankle tendons. Literature search was performed and twelve cases with polyarticular involvement were analyzed. Lipoma arborescens commonly involves suprapatellar recess of the knee and very rarely other joints or bursae. Histological analysis revealed an accompanying non-necrotizing granulomatous synovial inflammation.

**Conclusion** Polyarticular lipoma arborescens is a rare entity and symmetrical involvement of the joints other than the knees is exceedingly rare. MR imaging plays a significant role in the diagnostic protocol, and the characteristic fatty signal on MR imaging is highly suggestive of lipoma arborescens.

**Keywords:** lipoma arborescens; synovium; tendon; MR imaging

## INTRODUCTION

Lipoma arborescens (LA) is a rare, tumor-like lesion involving the synovial joints and less commonly bursae and synovial tendon sheaths. The suprapatellar recess of the knee is the most frequent location followed by the shoulder, elbow, hip, wrist, and hand [1–4]. Only twelve cases of multifocal polyarticular LA (not including bilateral knee involvement) have been reported in the English language literature [3–11]. We present a brief discussion on the polyarticular LA and the role of magnetic resonance (MR) imaging in the diagnostic algorithm of this rather infrequent tumor-like lesion.

## CASE REPORT

A 12-year-old boy presented with a four-year history of gradual-onset bilateral swelling of the bicipitoradial bursae, extensor compartments of hands and wrist, and ankles, involving flexor hallucis longus, flexor digitorum longus, and tibialis posterior tendons, bicipitoradial bursae and extensor compartments of the hand (Figure 1).

There was a mild pain in the wrists but no morning stiffness, and no history of trauma. Laboratory investigations were all within the normal ranges. Rheumatological examination revealed no abnormalities. MR imaging of both arms and ankles was performed (Figure 2, A–D).

A biopsy of the right ankle was performed and the histological changes were consistent with LA associated with a mild chronic synovial inflammation and several non-necrotizing sarcoid granulomas containing rare multinucleated giant cells (Figure 3, A–B). At follow-up, the lesions remained unchanged and there was neither pain nor any functional disability. Six months later, a partial synovectomy of the right ankle was attempted but on the opening of the tibialis posterior tendon sheath, fat tissue unusually firmly adherent to the tendon was found (Figure 4) and the surgeon decided against further radical excision.

A third surgical resection of the wrist LA on the right side was performed one year later, with successful outcome.

## DISCUSSION

English language literature search was performed using the subject term "Lipoma Arborescens" in the PubMed database in order to identify articles published from 1950 through July 2015. Among 137 articles yielded by the search criteria, all articles describing monoarticular involvement as well as 16 articles reporting bilateral knee involvement were excluded

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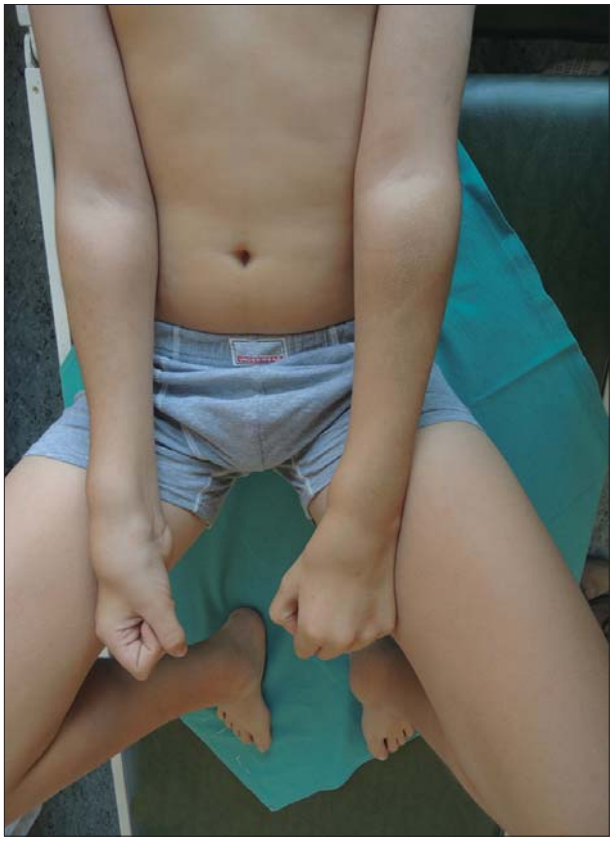

**Figure 1.** Photograph of the elbows, hands, and ankles showing bilateral soft-tissue swelling

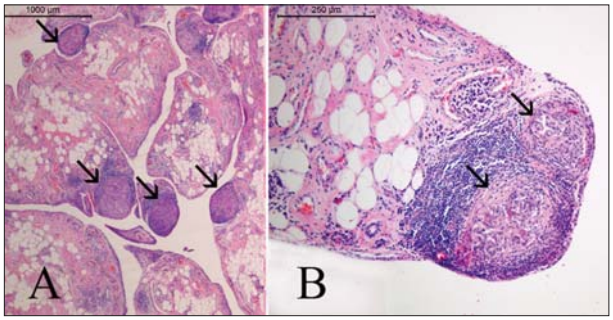


**Figure 3.** Photomicrographs of the specimen of the excised synovium shows elongated synovial folds containing small lobules of mature fat tissue typical for LA; several sarcoid granulomas and a mild lymphoplasmacytic infiltration are easily visible in the synovial folds (A – hematoxylin-eosin, ×25); the top of the fold contains two non-necrotizing epithelioid cell granulomas, partially surrounded by dense lymphocytic infiltration; there are also several foci of discrete, perivascular lymphoplasmacytic infiltration (B – hematoxylin-eosin, ×13.75)

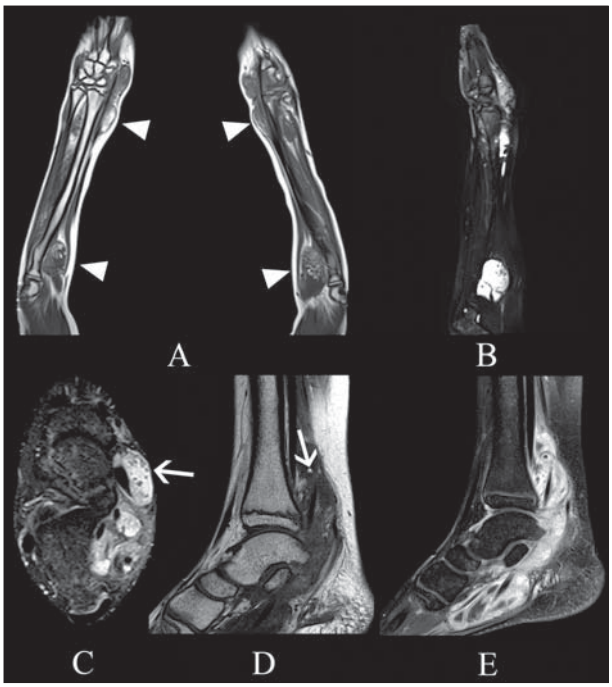


**Figure 2.** Coronal T1-weighted MR image of forearms shows fatty tumor-like lesions within bicipitoradial bursae and both wrists (A, arrowheads); sagittal fat saturated T2-weighted image – fluid-filled bicipitoradial bursa and frond-like fatty proliferations of the synovium; mild distension of the synovial sheaths of the extensor compartment tendons of the wrist (B); axial STIR image of the right ankle shows fluid within tendon sheaths of the FHL, FDL, and TP, with intralesional foci of fat (C, arrow; D, arrow); fat-saturated contrast enhanced T1-weighted sequence of the right ankle shows thickened synovium with significant contrast enhancement (E), consistent with tenosynovitis

FHL – flexor hallucis longus; FDL – flexor digitorum longus; TP – tibialis posterior

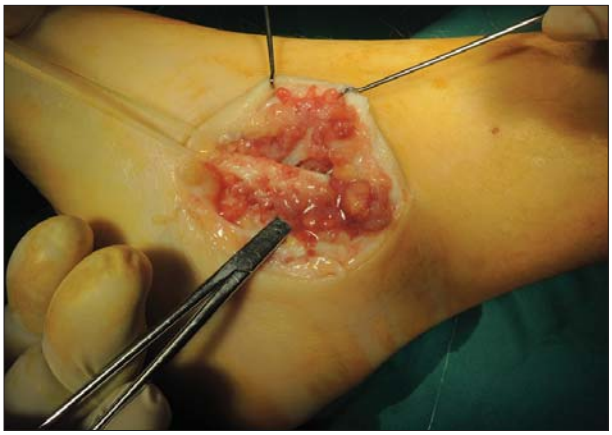

**Figure 4.** An intraoperative photograph of the right ankle with visible lipomatous mass after opening of the TP tendon sheath

because they were beyond the scope of this paper. Data of 12 patients reported in nine published articles [3–11] and the present report were analyzed (Table 1).

LA is not a true tumor but rather a villous synovial hyperplastic process that affects mostly men aged 40–60 years, and manifests itself as a slow-growing monoarticular painless swelling, usually in the suprapatellar pouch of the knee. Polyarticular involvement is rare and most frequently affects the knee, whereas bilateral involvement of other joints or a combination of the knee and another joint is far less common (Table 1). Our patient had several atypical features: 1) early age of disease onset; 2) involvement of joints that are otherwise infrequently affected by LA, in a combination that has not been reported to date; 3) symmetrical swelling without significant restriction of movement.

Etiology of LA is unknown. However, the majority of cases are primary, although some may represent a secondary reaction to chronic rheumatoid arthritis [12], psoriatic arthritis [13], sarcoidosis [14], joint trauma [15], and the proposed hypothesis is that subsynovial fatty infiltration may reflect a reaction to chronic inflammation [16].

Howe and Wenger [11] proposed a primary form of LA in younger patients without a detectable cause of chronic

**Table 1.** Multifocal LA, excluding bilateral knee involvement*

| Authors | Location | Onset age/sex | Bilateral involvement | Duration of symptoms | Associated diseases | Biopsy (+), synovectomy or excision |
|---|---|---|---|---|---|---|
| Bejia et al. [3] | Knee, hip | 24/M | + | 6 months | - | + |
| Siva et al. [4] | Wrist, hand, knee | 35/M | + | 20 years | Congenital short bowel syndrome | + |
| Gaede [5] | Knee, ankle | 14/M | - | 6 months | - | + |
| Dinauer et al. [6] | Bicipitoradial bursa | 37/M | + | 1.5 year | - | Synovectomy |
| Silva et al. [7] | Knee, ankle | 45/M | - | 9 years | Knee trauma | Excision of the ankle LA |
| Santiago et al. [8] | Knee, hip | 29/F | + | 12 years | Rheumatoid arthritis | + |
| Pandey and Alkhulaifi [9] | Subdeltoid bursa | 57/M | + | 3 years | Prior shoulder trauma, osteoarthritis | + |
| Martin et al. [10]** | Hip (iliopsoas bursa) | N/A | + | Not specified | Osteoarthritis, AVN of both femoral heads | Not specified |
| Howe et al. [11]** (3 patients) | - Knee and ankle<br>- Wrist extensor compartment<br>- Bilateral knee and bicipitoradial bursa | N/A<br>N/A<br>N/A | -<br>+<br>- | Not specified | Not specified | Not specified |
| Current report | - Bicipitoradial bursa, wrist, ankle | 10/M | + | 5 years | | Biopsy, 3 excisions later on |

* The table has been used and further modified with permission from Siva et al [4].

** Information about onset age, gender, symptom duration and surgical procedures from articles by Martin et al. [10] and Howe et al. [11] have not been included in the table due to lack of necessary data.

M – male; F – female; N/A – not applicable; AVN – avascular necrosis

joint inflammation [10]. This form can involve more multiple joints [3], tendon sheaths [17], and bursae [6, 9, 10, 11]. Other cases of LA with polyarticular involvement but without an identifiable cause are found in the literature [3].

Two groups of fatty lesions that affect joints, bursae, and tendon sheaths are described as follows: a solid fatty tumor (a synovial lipoma) and a "lipoma-like" lesion in the form of diffuse, hypertrophic synovial villi distended with mature fatty tissue [18]. The second form is present in our case.

Mild lymphoplasmacytic synovial infiltration with non-necrotizing sarcoid granulomas in our patient may suggest a previous chronic synovial inflammation as an expression of arthritis as the underlying joint pathology. A primary inflammatory synovial process was also identified in all five cases reported by Martin et al. [10]. Arthritis and periarthritis as joint manifestations of sarcoidosis may occur in 14–38% of patients with sarcoidosis. They may be a presenting feature or appear later in the course of the disease when multiple large joints are involved more frequently [19]. The histology in our patient was consistent with a chronic synovial granulomatous inflammation seen in sarcoidosis. However, we did not register any clinical, radiological or laboratory features of sarcoidosis. In addition, we found neither anamnestic, clinical and laboratory diagnostic elements of tuberculosis and fungal infection, nor trauma with potential impaction of foreign bodies in our patient – infections and disorders in which granulomas could appear along with chronic synovial inflammation [20]. Only in one 24-year-old patient with rheumatoid arthritis LA-associated scattered multinucleated giant cells were found in the accompanying lymphoplasmacytic synovial infiltration [12]. In our patient, we found for the first time entirely formed sarcoid granulomas in the synovial folds. Pathological entities with granulomas are classified into infections, vasculitis, immunological aberrations, leukocyte oxidase deficiency, hypersensitivity, chemicals, and neoplasia. However, histopathologists can detect granulomas in many different pathological disorders that are outside of this classification and that probably indicate a good defense and a satisfactory outcome against an unknown antigenic aggression [20]. We have no plausible explanation for sarcoid like granulomas occurring in the setting of LA.

In general, open synovectomy is suggested as curative treatment [10].

In conclusion, polyarticular LA is a rare condition and MR imaging has a pivotal role in the correct diagnosis, and more widespread use of MR imaging in the examination of joints will probably reveal more cases of polyarticular LA in the future.

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# Атипични, полиартикуларни *lipoma arborescens* код детета


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**КРАТАК САДРЖАЈ**

**Увод** *Lipoma arborescens* представља ретку, тумору сличну лезију која захвата синовијалне зглобове и ређе бурзе и овојнице тетива.

**Приказ болесника** У раду се приказује случај 12-годишњег дечака са симетричним захватањем биципторадијалних бурзи, синовијалних омотача екстензора обе шаке и медијалних аспеката оба скочна зглоба. Након начињеног прегледа магнетном резонанцом (МР) постављења је сумња на полиартикуларни *lipoma arborescens*, а дијагноза је доказана хистолошки након биопсије бурзе и касније након отворене хирургије синовијалних овојница десног скочног зглоба. Хистолошка анализа је додатно утврдила постојање не-некротизирајуће грануломатозне синовијалне упале. Извршена је анализа дванаест случајева из литературе са полиартикуларним *lipoma arborescens*.

**Закључак** *Lipoma arborescens* је ретка, тумору слична промена која захвата супрапателарни рецесус колена и врло ретко друге зглобове и бурзе. Полиартикуларни *lipoma arborescens* је редак ентитет, а симетрично захватање зглобова је изузетно ретко. МР игра врло важну улогу у дијагностичком протоколу код оваквих пацијената, а карактеристичан сигнал масне компоненте тумора сугерише постојање *lipoma arborescens* са високом сигурношћу.



**Кључне речи:** *lipoma arborescens*; синовијум; тетиве; магнетна резонанца

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# Fondaparinux monitoring in a patient with heparin-induced thrombocytopenia on hemodialysis

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**SUMMARY**

**Introduction** Heparin-induced thrombocytopenia associated to hemodialysis is rare. In case when citrate dialysis and/or non-heparin anticoagulants are not available, only possible medication to use for anticoagulation during hemodialysis is fondaparinux. However, laboratory monitoring of fondaparinux based on anti-Xa activity in dialysis patients has not been sufficiently documented yet.

**Case Outline** We created a local anti-factor Xa assay for measuring fondaparinux plasma concentration and efficacy in a patient with heparin-induced thrombocytopenia during hemodialysis. Fondaparinux given subcutaneously increases risk of adverse events due to its extended release and prolonged maintenance of toxic levels. When used intravenously fondaparinux remains safe, with reached steady-state level within dialysis and low risk of toxicity afterwards.

**Conclusion** Fondaparinux may be used as an alternative anticoagulant medication during hemodialysis in patients who develop heparin-induced thrombocytopenia. Adequate dose must be adjusted to patients' dry weight (0.03 mg/kg intravenously) and fondaparinux anti-coagulation monitoring must be provided.

**Keywords:** heparin-induced thrombocytopenia; fondaparinux specific monitoring; hemodialysis

## INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is a serious, potentially fatal, decline in platelet number due to use of unfractionated heparin, low-molecular weight heparin, and/or other polyanions [1–4]. It may present as a non-immune or immune-mediated form, with immune complexes formed between heparin and platelet factor 4 (PF4) [5, 6]. This form of thrombocytopenia may be followed by life-threatening thromboembolic events, such as deep vein thrombosis and pulmonary embolism [7, 8]. HIT associated with hemodialysis is rare, but even small amounts of heparin used for anticoagulation during hemodialysis treatment may provoke HIT [3, 4, 8]. In chronic dialysis patients, HIT frequency is low (3.9% in the literature) in newly diagnosed patients [4, 8]. Application of non-heparin anticoagulant preparations, including the specific anti-Xa inhibitor fondaparinux, is necessary [9]. Therefore, clinical management of HIT in patients on hemodialysis is difficult, in some countries often limited by the lack of drug supplies. Laboratory monitoring of fondaparinux based on anti-Xa activity in dialysis patients has not been sufficiently documented. This case report presents the application and dose adjustment of fondaparinux in dialysis patients with type II HIT.

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## CASE REPORT

A 56-year-old patient was admitted to our hospital with terminal stage of chronic kidney failure secondary to polycystic kidney disease. During the hospitalization, he was started on the chronic program of hemodialysis, through central venous catheter. Arteriovenous fistula was created three days after his first (urgent) hemodialysis, as he previously refused it. Fifteen days after hemodialysis was started, the patient developed a significant decline in the platelets count from $286 \times 10^9$/L to $41 \times 10^9$/L. Clinical suspicion for HIT II was confirmed by a highly positive result for HIT-Ab (20.6 IU/mL). The presence of HIT antibodies was detected with immunoturbidimetric assay (HIT-AbPF4-H HemosIL, IL ACL 300, Instrumentation Laboratory Company, Milan, Italy).

When diagnosis was confirmed, heparin was discontinued. As citrate dialysis is not available in Serbia, and non-heparin anticoagulants are not proposed by the healthcare system for the diagnosis of HIT, the only possible medication to use was fondaparinux (fondaparinux sodium; GlaxoSmithKline, Brentford, UK). However, official data for fondaparinux used in patients on hemodialysis was limited. Thus, after consultation with transfusion medicine specialist, it was decided to establish a local fondaparinux-specific anti-Xa assay for the fondaparinux monitoring. Anti-Xa assay was created with fondaparinux calibrator and performed with anti-Xa heparin chromogenic

method (anti-Xa heparin assay IL on ELITE PRO® IL, Instrumentation Laboratory Company, Milan, Italy).

Fondaparinux plasma level was measured at the beginning of dialysis, every hour during the dialysis and 24 hours after every dialysis treatment. The known mean steady state plasma concentration is in the range of 0.46 to 0.62 mg/l, which is regularly applied to patients with symptomatic deep vein thrombosis and pulmonary embolism undergoing treatment with fondaparinux [10]. As hemodialysis session itself requires effective dosage of anticoagulant to prevent potential thrombotic complications in blood and extracorporeal circuit, and as HIT additionally favors thrombosis, we have used the same steady state for coagulation monitoring in our patient [10].

In our patient, 0.03 mg/kg (of patient's estimated dry-weight) of fondaparinux was firstly administered subcutaneously at the beginning of dialysis treatment. During the third hour of dialysis, plasma level of fondaparinux reached toxic values (0.71 mg/L). Two days afterwards, at the second dialysis course, twice-reduced dose (0.015 mg/kg) was administered subcutaneously at the beginning of dialysis, when fondaparinux plasma level was within steady-state levels. However, toxic levels were reached during dialysis: 0.89 mg/L, 0.77 mg/L, and 0.84 mg/L at the first, second and third hour, respectively. At the start of the third hemodialysis, 0.03 mg/kg of fondaparinux was applied intravenously. During the entire aforementioned treatment, fondaparinux plasma levels were within the optimal range, with maximum of 0.61 mg/L at the third hour of hemodialysis (Table 1).

Comparing pharmacokinetics between the dialyses, while fondaparinux was administered subcutaneously, its plasma levels were reaching toxic values, 1.13 mg/L and 0.69 mg/L even 12 hours after first and second dialysis, respectively. However, when fondaparinux was administered intravenously, its plasma level remained within the reference interval during the follow-up (Table 2). Therefore, 0.03 mg/kg of fondaparinux was continued intravenously during further hemodialysis treatments of this patient. Coagulation monitoring was checked after every hemodialysis session, and remained within the effective and safe range.

Nevertheless, type of dialysis was changed during the hospitalization. Both low-flux bicarbonate hemodialysis and postdilutional hemodiafiltration were inappropriate. Postdilutional hemodiafiltration was effective for fondaparinux elimination and prevention of its accumulation, but in the arteriovenous system it produced high hemoconcentration, which led to coagulation in the dialysis set. However, high-flux bicarbonate hemodialysis showed the best performance for both dialysis effectiveness and low fondaparinux accumulation.

Soon after heparin discontinuation, platelet number returned within reference levels. After four months, heparin was re-introduced to hemodialysis in the same patients, and no thrombocytopenia occurred.

**Table 1.** Fondaparinux serum levels during hemodialysis (HD)

| Fondaparinux (mg/L) | HD I | HD II | HD III |
|---|---|---|---|
| Before HD | 0.51 | 0.40 | 0.24 |
| 1 hour of HD | 0.74 | 0.89 | 0.59 |
| 2 hours of HD | 0.65 | 0.77 | 0.57 |
| 3 hours of HD | 0.71 | 0.84 | 0.61 |

**Table 2.** Fondaparinux levels within hemodialysis

| Time (h) | Fondaparinux (mg/L) |
|---|---|
| 12 h after 1st HD | 1.13 |
| 12 h after 2nd HD | 0.69 |
| 12 h after 3rd HD | 0.16 |
| 12 h after 4th HD | 0.28 |
| 12 h after 5th HD | 0.36 |
| 12 h after 6th HD | 0.39 |

## DISCUSSION

This case illustrates the main obstacles in treatment management of HIT in hemodialysis. Current guidelines recommend immediate discontinuation of heparin and use of non-heparin anticoagulants, such as anti-Xa and direct thrombin inhibitors. Danaparoid and lepirudin are usually recommended, together with new oral anticoagulants [7, 11, 12, 13]. However, Serbia has limited access to these anticoagulants. Citrate-based dialysis, which is recommended in case of HIT development, is also unavailable in Serbia. When guideline-suggested non-heparin medications and anticoagulants are not available for treatment of this serious condition, especially in the resource-limited settings, fondaparinux may present as a possible alternative.

Although fondaparinux, as a coagulation factor Xa inhibitor, is noted to be used in several cases of HIT until now, there are still no officially established protocols for fondaparinux use [10, 14, 15]. This is particularly an inconvenience in a population which develops HIT during hemodialysis, because of its mainly renal route of excretion [9, 10]. Additionally, fondaparinux monitoring assays are not commercially available, which thoroughly disables its routine use [16].

The assay methodology for measuring fondaparinux plasma concentration and efficacy is very similar to the standard anti-factor Xa assay for low-molecular-weight heparin or unfractionated heparin, but still not equivalent [14, 15]. Thus, in our case, we created a fondaparinux-specific assay using fondaparinux calibrator with an anti-factor Xa heparin assay method (anti-factor Xa heparin IL).

As our case showed, fondaparinux had given subcutaneously increases risk of adverse events due to its extended-release and prolonged maintenance of toxic levels, even 12 hours after dialysis. However, when used intravenously, fondaparinux remains safe, with reached steady-state level within dialysis and low risk of toxicity afterwards.

Potential cross-reactivity of fondaparinux to previous use of heparin is acknowledged and may be provoked, but it nonetheless has lesser extent of immunogenicity than heparin [7]. Furthermore, dosage of fondaparinux must be adjusted to the rate of glomerular filtration due to its increased risk of accumulation in patients with renal failure [10, 14, 17]. When used in patients on hemodialysis, it is necessary to apply fondaparinux adjusted to patients' estimated dry weight. If possible, it is advisable to perform high-flux bicarbonate dialysis. Therefore, when fondaparinux is used in specific groups, such as patients with renal failure and/

or patients on chronic dialysis, a specific fondaparinux anti-coagulation assay must be developed.

In conclusion, fondaparinux may be used as an alternative anticoagulation during hemodialysis in patients who develop HIT. Adequate dose must be adjusted to patients' dry weight, and fondaparinux anti-coagulation monitoring must be provided.

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# Коагулациони мониторинг фондапарина код болесника са хепарином индукованом тромбоцитопенијом на хемодијализи

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**КРАТАК САДРЖАЈ**

**Увод** Хепарином индукована тромбоцитопенија је нежељена реакција на примену хепарина, а код болесника на хемодијази је ретка компликација. Код особа које развију хепарином индуковану тромбоцитопенију током хемодијализног третмана контраиндикована је примена хепарина за антикоагулацију, те се у свету у оваквим случајевима примењује цитратна дијализа и/или нехепаринска антикоагулантна терапија. Уколико су цитратна дијализа и/или нехепаринска антикоагулантна терапија недоступне, потенцијални начин антикоагулације током хемодијализе је примена фондапарина (инхибитор фактора коагулације *Xa*). Ипак, до сада није креиран рутински коагулациони мониторинг за процену ефикасности и безбедности примене фондапарина код болесника на хемодијализи.

**Приказ болесника** За потребе нашег болесника са хепарином индукованом тромбоцитопенијом који је на хроничном програму хемодијализе креирали смо локални специфични мониторинг анти-*Xa* активности за одређивање концентрације фондапарина у плазми и праћење његове ефикасности и безбедности. Како је наш приказ случаја доказао, примена фондапарина субкутано повећава ризик од нежељених ефеката због продуженог времена ослобађања и одржавања токсичних вредности. Када се фондапарин примењује интравенски (0,03 *mg/kg* суве телесне тежине), његова примена је безбедна, са малим ризиком од нежељених реакција.

**Закључак** Фондапарин се може применити као алтернативни антикоагуланс за болеснике на хроничном програму хемодијализе који су развили хепарином индуковану тромбоцитопенију. Адекватна доза се одређује према сувој телесној тежини болесника (0,03 *mg/kg* интравенски) уз примену специфичног антикоагулационог мониторинга.

**Кључне речи:** хепарином индукована тромбоцитопенија; специфични коагулациони мониторинг фондапарина; хемодијализа

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# Mucormycosis of the paranasal sinuses in a patient with acute myeloid leukemia

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**SUMMARY**

**Introduction** Invasive fungal infection is among the leading causes of morbidity, mortality, and economic burden for patients with acute leukemia after induction of chemotherapy. In the past few decades, the incidence of invasive fungal infection has increased dramatically. Its management has been further complicated by the increasing frequency of infection by non-Aspergillus molds (e.g. Mucorales). Neutropenic patients are at a high risk of developing an invasive mucormycosis with fulminant course and high mortality rate (35–100%).

**Case Outline** We are presenting the case of a 72-year-old male with an acute monoblastic leukemia. The patient was treated during five days with hydroxycarbamide 2 × 500 mg/day, followed by cytarabine 2 × 20 mg/sc over the next 10 days. He developed febrile neutropenia, headache, and edema of the right orbital region of the face. Computed tomography of the sinuses revealed shadow in sinuses with thickening of mucosa of the right paranasal sinuses. Lavage and aspirate from the sinuses revealed *Rhizopus oryzae*. Mucormycosis was successfully treated with amphotericin B (5 mg/kg/day) followed by ketoconazole (400 mg/day). Two months later the patient died from primary disease.

**Conclusion** In patients with acute leukemia who developed aplasia, febrile neutropenia, and pain in paranasal sinuses, fungal infection should be taken into consideration. New and non-invasive methods for taking samples from sinuses should be standardized in order to establish an early and accurate diagnosis of mucormycosis with the source in paranasal sinuses, and to start early treatment by a proper antifungal drug. Clear communication between physician and mycologist is critical to ensure proper and timely sampling of lavage and aspirate from sinuses and correct specimen processing when mucormycosis is suspected clinically.

**Keywords:** acute leukemia; neutropenia; mucormycosis; paranasal sinuses; invasive fungal infection

## INTRODUCTION

Patients with acute leukemia (AL) are considered a population at high risk for developing an invasive fungal infection (IFI) [1]. In the United States, in the past few decades, the incidence of IFI increased dramatically by approximately 200% between 1979 and 2000 [2]. In a recent study, the cumulative probability of developing IFI after a diagnosis of AL was 11.1% at 100 days [3]. Patients undergoing treatment for hematologic malignancies have estimated cause-specific mortality due to IFI of 35% [4]. The most common causes of IFI are *Candida* and *Aspergillus*, but most recent reports show an increasing frequency of infection by non-Aspergillus molds, especially Mucorales order.

Mucormycosis is an aggressive opportunistic fungal infection with fulminant course caused by various members of the Mucorales order. The disease-causing genera in humans include *Absidia*, *Rhizopus*, and *Mucor,* which are widespread in nature. They produce airborne spores that enter the body mostly through inhalation or ingestion; occasionally, infection may be through hematological dissemination from a different site, but sinuses and lungs are usually the entry points [5]. Fungi in sinuses cause fungal rhinosinusitis (FRS), which has a spectrum from noninvasive disease to acute fulminant FRS [6]. Predisposing factors for the development of acute fulminant FRS are numerous, but neutropenia is the leading one, especially when neutrophils are below $0.5 \times 10^9$/L. Invasive forms of FRS have very rapid course in neutropenic patients and because of that require early diagnosis, early induction of antifungal therapy, and sometimes surgery [7, 8].

We present a case of a patient with acute monoblastic leukemia (AML) and invasive mucormycosis of paranasal sinuses.

## CASE REPORT

A 72-year-old male patient with diagnosed AML and body mass index of 27.7 kg/m$^2$, Eastern Cooperative Oncology Group (ECOG) performance status of 1 and Sorror score of 2, was admitted to the Clinic for Hematology of the Clinical Centre of Serbia. At presentation, laboratory data were as follows: hemoglobin 95 g/l, white blood cell count $76.5 \times 10^9$/L, and platelets $45 \times 10^9$/L. In hypercellular bone marrow 92% of blasts predominantly myeloperoxidase positive were found. Morphologic finding

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was in accordance with AML M5a. Immunophenotyping with flow cytometry revealed a population of mononuclear cells with immunophenotype HLA-DR, cMPO, cLizozim, CD13, CD33, CD15, CD11b, CD11c, CD64, CD14, CD163, CD36 and CD56+, indicating the diagnosis of AML CD56+ with monocytic differentiation. Karyotype was normal 46XY, and molecular analyses for FLT3 and NPM were negative. The patient was ranged in intermediate group I according to Eureopean Leukemia Net classification. Hemostasis tests showed the following: fibrinogen 6.59 g/L, prothrombin time 70%, partial thromboplastin time 30.7 sec., and D dimer 2.1 μg/L. Biochemical analyses of blood showed elevation of lactate dehydrogenase 1,497 U/L, blood urea nitrogen 15.9 mmol/L, creatinin 388 μmol/L, and acidum uricum 917 μmol/L. Liver function tests were within normal limits. Echocardiography showed ejection fraction of 67%, while chest radiography was normal. Abdominal ultrasound showed normal size of the liver and splenomegaly of 195 mm. The patient was treated for five days with hydroxycarbamide 2 × 500 mg/day including rehydration and xanthine oxidase inhibitors in order to decrease the number of white blood cells. The therapy was continued with cytarabine 2 × 20 mg/sc during the next ten days. More aggressive treatment was not recommended because of the previous renal failure. After chemotherapy, the patient became neutropenic and febrile. He was treated with broad spectrum antibiotics without effects. Twenty days after the last dose of chemotherapy, the patient felt pain in the right maxillary and zygomatic region, as well as nasal congestion and headache. On the following day, the patient's right palpebrae started swelling and the pain was spread all over the right half of the face. The radiography of paranasal sinuses revealed opacification of the right maxillary sinus with central illumination. Fungal hyphae was detected in an inducted lavage and aspirate of the right maxillary sinus, while culture isolation showed growth of *Rhizopus oryzae* (Figure 1). X-ray of paranasal sinuses showed filled lumen of cavities with oedematous epithelium. Computed tomography (CT) revealed mucosal thickening, hypoattenuation, opacification of sinuses as soft tissue attenuation of the right maxillary, sphenoidal, and both ethmoidal sinuses, as well as partly of the right frontal sinus, but without signs of invasion of endocranium. In the right nasal cavity the thick mucin like material was also present (Figure 2). After positive CT finding and mycological identification of *Rhizopus oryzae*

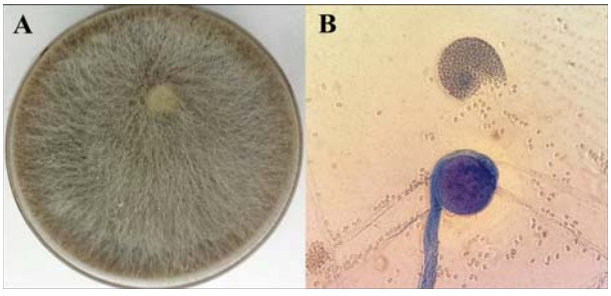


**Figure 1.** *Rhizopus oryzae*: A) culture on Sabouraud dextrose agar; B) microscopy (lactophenol cotton blue, 40× magnification)

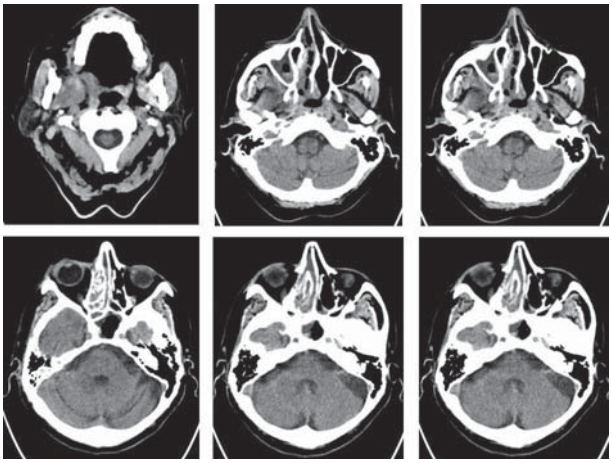

**Figure 2.** CT showing the presence of fluid-like content in the right maxillary, sphenoidal and both ethmoidal sinuses, predominantly in the right, as well as partly in the right frontal one, but without invasion of endocranium; in the right nasal cavity, the thick mucin-like material was also noted

in sinonasal lavage and aspirate, the diagnosis of mucormycosis of the nose and paranasal cavities was established. Invasive mucormycosis was successfully treated with amphotericin B (5 mg/kg/day), followed by ketoconazole 400 mg/day, according to the results of antimycogram. Symptoms, pain, and changes over the paranasal region were resolved after treatment with antifungal therapy. Control aspirate and lavage of sinuses after two weeks of the last dose of antimycotic drugs were negative. Unfortunately, control bone marrow aspirate again showed the presence of 87% of monoblasts. Two months later the patient died due to the primary disease.

## DISCUSSION

IFI is a major cause of morbidity and mortality in patients with AL. Despite increased number of diagnosed cases of IFI, due to development of prompt and accurate diagnostic approaches and continual education about fungal diseases, the death rate due to IFI has dropped nearly 50% in the past two decades, from 44% during the 1995–2000 period to 28% during the 2001–2004 period [4]. The most frequent causing agents are *Aspergillus, Dematiaceous* molds such as *Bipolaris*, *Curvularia*, and *Alternaria species* and the Mucormycetes *Rhizopus*, *Mucor*, and *Lichthemia* [6]. *Mucoraceae* are ubiquitous fungi that are commonly found in soil and particularly in decaying matter [5]. In a study of patients with hematologic malignancies, the most frequent sites of mucormycosis were the lungs (64%) and the orbito-sinus-facial structures (24%), while cerebral involvement and disseminated infection were observed in only 19% and 8% of the cases, respectively [9, 10]. Predisposing factors for these infections are neutropenia, especially when neutrophils are lower than $0.5 \times 10^9$/L, long-lasting glucocorticosteroid therapy, radiotherapy, malnutrition, diabetes, and other accompanied diseases that have impact on a patient's immune system [7, 8, 9, 11, 12]. Mucormycosis is an aggressive infection that can cause a significant disease in immunocompromised patients. Although many

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patients with rhinocerebral mucormycosis undergo similar treatment, pathogen speciation should not be underappreciated; it may be imperative to guide antifungal drug selection, as some mucoraceous fungi may exhibit variable resistance to conventional therapy [13].

Mucormycosis due to inhalation of fungal spores begins in the nose and paranasal sinuses with spreading to orbital or intracranial structures either by direct invasion or through blood vessels. The mortality rate is high, ranges 50–85%, while in disseminated and untreated forms it could be 100% [3, 4, 14]. An immediate CT scanning of the paranasal sinuses and an endoscopic examination of nasal passages with biopsies of any suggestive lesions should be performed. Samples from sinuses should be obtained by non-invasive methods, due to condition of patients with neutropenia. Proper samples include inducted sinonasal lavage and aspirate, and should be taken at the onset of the disease before deterioration of symptoms. Clinical samples should be cultured and examined by histology and direct microscopy. Delays in diagnosis and treatment lead to increased mortality [11, 12].

We suppose that our patient had previous fungal colonization of paranasal sinuses, without symptoms, before diagnosis of AL was established. After worsening of AL, developing aplasia of bone marrow and febrile neutropenia IFI had rapidly progressed due to immunocompromised state. Positive CT finding, absence of response to broad spectrum antibiotics, and deterioration of symptoms were indications that FRS should be considered. CT scan revealed spread of the disease over paranasal sinuses and aspirate and lavage of the sinuses were taken. *Rhizopus oryzae* was identified and according to antimycogram two antimycotic drugs, amphotericin B and ketoconazole, were successfully applied. Although the mucormycosis was successfully solved, AL was resistant to chemotherapy and the patient died as a more aggressive therapy could not have been applied because of the previous chronic renal failure.

In hematologic patients with prolonged febrile neutropenia, headache, painful paranasal sinuses, fungal infection should be seriously taken into consideration. An early diagnosis of fungal infections of paranasal sinuses could be relatively easy established nowadays based on the radiography, CT scan visualization, and endoscopy with the invasive sampling as biopsy and histology of suspected lesion. New and non-invasive methods for taking samples from sinuses should be developed in order to make early and accurate diagnosis of mucormycosis with the source in the paranasal sinuses and to start early treatment by proper antifungal drug in patients with neutropenia. Clear communication between physician and laboratory is critical to ensure correct specimen processing when mucormycosis is suspected clinically.

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# Инвазивна мукормикоза код пацијента са акутном мијелоидном леукемијом

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**КРАТАК САДРЖАЈ**

**Увод** Инвазивне гљивичне инфекције (*IFI*) водећи су узрок морбидитета, морталитета и финансијског оптерећења за пацијенте са акутном леукемијом после примене индукционе хемотерапије. Неколико последњих деценија инциденца *IFI* се драматично повећала. Лечење *IFI* је додатно отежано и због повећане учесталости инфекција изазваних не-*Aspergillus* плеснима (нпр. *Mucorales*). Пацијенти са неутропенијом су под високим ризиком за развој инвазивне мукормикозе која има фулминантни ток и високу стопу морталитета (35–100%).

**Приказ болесника** Приказан је случај пацијента старог 72 године, мушкарца, са акутном монобластном леукемијом, који је лечен током пет дана хидроксикарбамидом у дози од 2 × 500 *mg*/дан, праћено цитарабином у дози од 2 × 20 *mg*/*sc* током наредних 10 дана. Пацијенту се појављују симптоми главобоље, неутропенија и едем десне половине лица. Компјутеризована томографија (*CT*) параназалних синуса је открила засенчење синуса, са задебљањем мукозе десних параназалних синуса и са деструкцијом кости. У лавату и аспирату синуса је показано присуство гљивице *Rhizopus oryzae*. Инвазиовна мукормикоза је успешно излечена амфотерицином Б (5 *mg*/*kg*/дан) праћено кетоконазолом (400 *mg*/дан). Два месеца касније пацијент је егзитирао због примарне болести.

**Закључак** Код хематолошких пацијената са акутном леукемијом код којих постоји присуство аплазије, пролонгиране фебрилне неутропеније и бола у параназалним синусима, потребно је размотрити могућност присуства гљивичне инфекције. Требало би стандардизовати нове неинвазивне методе за узорковање клиничког материјала из синуса, са циљем да се правовремено и тачно успостави дијагноза мукормикозе, чији извор се налази у параназалним синусима и да се започне рана терапија са одговарајућим антигљивичним леком. Добра комуникација између клиничара и миколога је неопходна како би се осигурало правилно и правовремено узорковање лавата и аспирата синуса код сумње на мукормикозу.

**Кључне речи:** акутна леукемија; неутропенија; мукормикоза; параназални синуси; коинвазивна гљивична инфекција

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# Hepatocellular carcinoma and impact of aflatoxin difuranocoumarin derivative system – A case report

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**SUMMARY**

**Introduction** Hepatocellular carcinoma (HCC) is the most frequent type of liver malignancy. As a carcinogen, aflatoxin B1 (AFB1) causes HCC by inducing deoxyribonucleic acid adducts that lead to genetic changes in liver cells and may be the cause of HCC in up to 30% of cases. The incidence of HCC has been on the rise and is an issue in the countries of the Western Balkans.

**Case Outline** This paper presents a case of a 37-year-old woman who was diagnosed with HCC, without hepatitis B, hepatitis C, or liver cirrhosis. The patient consumed milk and dairy products in quantities of over two liters per day over the course of 20 years, which indicates the impact of aflatoxin in milk on HCC. A positive signal for the presence of AFB1 was detected by ELISA (enzyme-linked immunosorbent assay) in-house using immunoperoxidase screening test.

**Conclusion** As carcinogenic difuranocoumarin derivative, aflatoxin B1 is the most likely cause of malignant transformation of hepatocytes, which resulted in hepatocellular carcinoma in this patient.

**Keywords:** aflatoxin molecule; hepatocarcinogenic; hepatocellular carcinoma; Vojvodina

## INTRODUCTION

Hepatocellular cancer (HCC) is the most frequent liver cancer, causing the mortality in men and women with ≈500,000 new cases per year and >600,000 deaths annually [1]. Hepatitis B virus and hepatitis C virus infection, aflatoxin B1 exposure, iron, and arsenic are the most frequent of all registered chemical stressors. Regions of developing countries that have increased exposure to aflatoxin correspond with regions where HCC incidence is most evident. There are four major aflatoxins, namely B1, B2, G1, and G2, and two additional monohydroxilated metabolic products, M1 and M2, which are found in milk and dairy products. AFB1 is the emerging mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The fungal mycotoxin, AFB1, can be found in agricultural products such as maize, cereals, rice, and dried fruits that are stored in hot and humid conditions [2, 3, 4]. Reports from epidemiological studies have demonstrated that AFB1 is the most hepatocarcinogenic mycotoxin especially for HCC [5].

The aim of this paper is to present a patient with HCC and positive anamnesis of a milk lover, absence of viral etiological factors for HCC, and evidence of AFB1 as the most significant factor in etiopathogenesis of hepatocellular carcinoma in Vojvodina, an agricultural region where the presence of AFB1 has been proven in both animal feed and human bodily fluids.

## CASE REPORT

A Caucasian woman, age 37, graphic designer, married, with two children, with no previous surgery, had a traffic trauma, which is why she was referred for upper abdomen ultrasound. The ultrasound registered a tumour mass in the right liver lobe, which had over 10 cm in diameter. After that, computed tomography scan and magnetic resonance (MR) imaging gave the diagnosis of primary liver tumour (Figure 1), which was compressing the right and middle hepatic veins. Other laboratory parameters showed slightly elevated hepatic enzymes with normal bilirubin levels. There were no hepatitis antibodies or elevated alpha-fetoprotein (AFP). The patient was in good physical condition, bicycle driver and hiker. The only suggestion she gave in medical interview before the operation was that she consumed over two liters of milk and other dairy products (yoghurt and white cheese) per day over the course of 20 years. Simplified methods of ELISA in-house testing using immunoperoxidase screening test detected a positive signal for the presence of AFB1 in the patient's serum in 12 repetitions. In addition, the same simplified screening methodology of ELISA did not show a positive signal for the viral hepatitis B and C. The patient was operated on in January 2012, when right hepatectomy was performed on non-cirrhotic liver. Pathology revealed a hepatocellular carcinoma and immunohistochemistry confirmed with CD34 that liver sinusoids in the tumour mass

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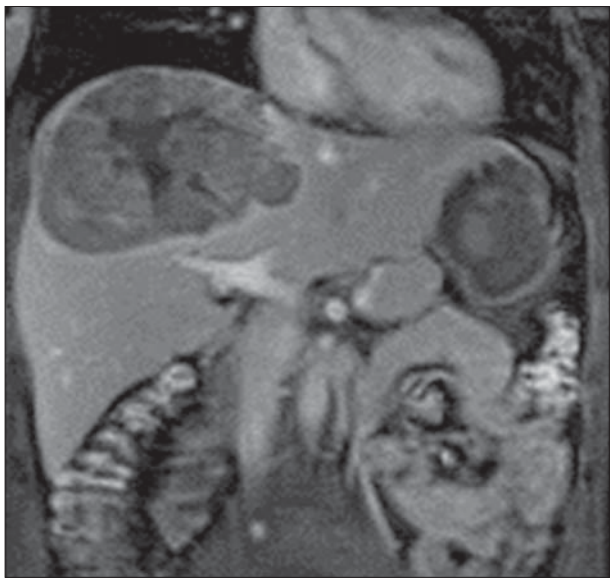

**Figure 1.** Initial MR imaging, performed on a 3T machine: coronal post-contrast T1W image shows a hypovascular mass that is dominantly hypointense with low signal intensity in the central portion of the tumour, which correlates to necrosis

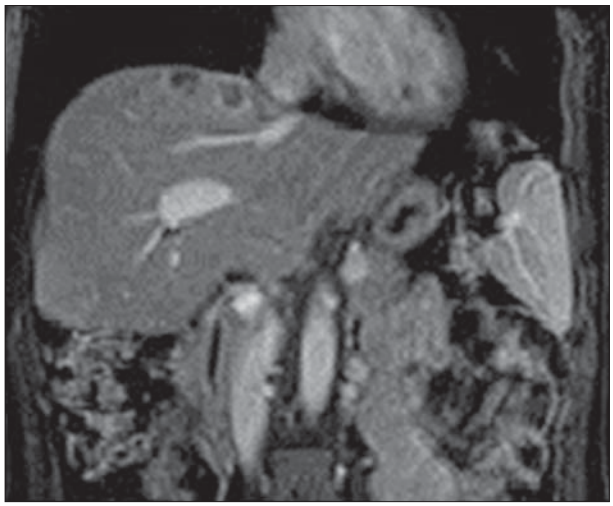

**Figure 2.** Liver MR imaging performed 13 months after the surgery (on the same 3T scanner): coronal post-contrast T1W image demonstrates multifocal tumour recurrence, two hypointense nodes with rim enhancement

were positive, whereas in the non-tumorous liver tissue (on the resection margin) they were negative. Nine months after the operation there were no new HCC in the rest of the liver. Thirteen months later, there were two new liver nodules on MR imaging (Figure 2), and the second operation was done with resection of the HCC metastases, as well as one diaphragm metastasis. In the second year after this operation, a new tumour mass developed in the rest of the liver, and radiofrequency ablation was performed. The patient died two years after the second operation.

## DISCUSSION

The International Agency for Research on Cancer (IARC) classifies AFB1 as a carcinogenic substance within the first group of carcinogens for HCC. In April 2004, Lewis et al. [6] studied contamination of maize on the market and found that 55% of the maize was contaminated by AFB1. The association between aflatoxin levels in the maize found on the market and an aflatoxicosis outbreak was confirmed. The study focused on detecting AFM1 in cow, goat, and donkey milk, different dairy products, complete feed mixtures for dairy cattle, as well as meat and eggs [7].

Depending on the induction of cancer, mutagens are classified as genotoxic and non-genotoxic molecules. Genotoxic molecules are microcomponents of nutrition, such as aflatoxins, which cause genetic alterations. AFB1 may induce cancer, and the target organ that metabolizes food contaminated with aflatoxins is the liver. Aflatoxins, especially AFB1, may be metabolized by cytochrome-P450 enzymes to a highly nucleophilic-reactive genotoxic intermediate (AFBO) or hydroxylated to two isomers (AFM1 and AFQ1) and a demethylated (AFP1) metabolite. Unstable reactive intermediate AFBO forms adduct to DNA with strong covalent bonds, resulting in AFB1-guanine molecular system or in forming of AFB1-albumin and other protein adducts. AFB1-guanine adducts induce the p53 gene mutation, which is responsible for carcinogenic effects. Another way for causing the aflatoxicosis is binding the AFBO to amino acids [8].

Approximately more than four billion of the world's population is exposed to environment contaminated by aflatoxins through the food chain, particularly in developing countries [1]. Kew [9] has published data on the daily intake of maize (g/person/day) in Eastern Europe. In Vojvodina, there is no systematic or regular monitoring of aflatoxin in agricultural products, milk, and dairy products. There is also no biomonitoring of human milk, serum, and liver tissue. This region has no data on the impact of AFB1 and AFM1 on HCC. This paper presents a case report of a possible carcinogenic effect of AFB1 on the development of HCC in the agricultural region of Vojvodina. The patient was a woman, age 37, who suffered from HCC, without hepatitis B, hepatitis C, or liver cirrhosis, but who consumed milk and dairy products in quantities of over two liters per day over the course of 20 years.

The results obtained by Spirić et al. [3] show a weak contamination of 54 analyzed cheese samples from the market. In 13% of all analyzed samples, the level of AFM1 contamination was above the adopted limit of 0.25 mg/kg [4]. Tomašević et al. [8] detected AFM1 in milk and dairy products from the same market and found that in more than 30% of analyzed samples, the levels exceeded the EU's maximum residue limit. In order to assess carcinogenic effects of AFB1, the authors suggest routine analyses of aflatoxin metabolites, DNA and protein adducts in the blood, tissues, and urine in patients with HCC [9]. There are two methods for detecting only AFB1 with sophisticated equipment (ultra-high pressure liquid chromatography tandem mass spectrometry – UHPLC-MS/MS) or common detection by an in-house ELISA and in-house immunoperoxidase test. AFB1 detection in serum specimens was performed using an in-house ELISA, developed

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as per standard procedures with slight modifications, in which the peroxidase-conjugated anti-AFB1 was used at a concentration of 1:1,000 [10].

The impact of aflatoxin alone on HCC needs to be examined in patients who have neither hepatitis B, hepatitis C, nor liver cirrhosis, with the aim of finding new antagonists for preventing and minimizing the genotoxic effect of aflatoxin in liver tissue.

The described case of a patient with diagnosed and surgically treated HCC, as well as the outcome characteristic for this illness where, aside from the presence of AFB1 in the blood, no viral etiological factors had been proven, indicates that in rural regions where the exposure to carcinogens is increased, all patients with HCC need to be tested for AFB1 in bodily fluids. Legislation must follow clinical observations.

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# Хепатоцелуларни карцином и утицај дифуранокумаринског система афлатоксина – приказ случаја


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**КРАТАК САДРЖАЈ**

**Увод** Хепатоцелуларни карцином (ХЦК) најчешћи је тип малигнитета јетре. Афлатоксин (АФБ1), као канцерогено једињење, изазива ХЦК индуковањем дезоксирибонуклеинских продуката, који доводе до генетских промена у ћелијама јетре, и може изазвати до 30% случаја ХЦК. Учесталост хепатоцелуларног карцинома расте и представља проблем у земљама Западног Балкана.

**Приказ болесника** У овом раду је приказан случај младе жене, старости 37 година, која је оболела од ХЦК, без присуства хепатитиса Б, хепатитиса Ц и без цирозе јетре. Пацијенткиња је конзумирала млеко и млечне производе у количинама преко два литра дневно током 20 година, што указује на утицај афлатоксина у млеку на ХЦК. Позитиван сигнал на присуство АФБ1 је детектован помоћу *ELISA* (имуноензимски тест високе осетљивости) применом имунопероксидаза „скрининг" процедуре.

**Закључак** Афлатоксин, као канцерогени дифуранокумаринski дериват, највероватније је проузроковао малигну трансформацију хепатоцита и довео до настанка хепатоцелуларног карцинома јетре код ове пацијенткиње.



**Кључне речи:** молекул афлатоксина; хепатокарциногени; хепатоцелуларни карцином; Војводина

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# Shiga toxin-producing *Escherichia coli* hemolytic uremic syndrome

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**SUMMARY**
The hemolytic–uremic syndrome (HUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI). The major cause of HUS in childhood (>90%) is infection with verocytotoxin (Shiga-like toxin – "Stx")-producing bacteria, usually enterohemorrhagic *Escherichia coli* (VTEC/STEC). The infection may be transmitted by the consumption of undercooked meat, pasteurized dairy products, contaminated vegetables, fruits and water, or by contact with STEC diarrhea. After an incubation period of three to eight days, patients commonly develop bloody diarrhea followed in 5–22% by HUS that may be complicated by central nervous system, pancreatic, skeletal, and myocardial involvement. HUS is one of the main causes of AKI in children in Europe. The management of HUS includes the usual treatment of children with AKI. Transfusion with packed red blood cells is needed in case of a severe anemia, while platelet transfusions are limited to the need for a surgical procedure or in active bleeding. Currently, there is no consensus on the use of antibiotic therapy. Treatment with plasma and/or plasma exchange has not been proven beneficial in STEC-HUS. Eculizumab has been used for the treatment of STEC-HUS, but the value of this treatment remains to be determined. The mortality of HUS is reported to be 3–5%. About 12% of patients will progress to end-stage renal failure within four years and about 25% will have long-term complications, including hypertension, proteinuria, renal insufficiency, and insulin-dependent diabetes mellitus. Transplantation can be performed without increased risk for the recurrence of the disease.
**Keywords:** acute kidney injury; children; D-HUS

## INTRODUCTION

The leading clinical features of hemolytic uremic syndrome (HUS) are non-immune microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI) [1]. HUS is one of the main causes of AKI in children in Europe [2]. Histopathology of HUS is characterized by thrombotic microangiopathy consisting of endothelial cell injury, activation of coagulation with consequent microvascular thromboembolic occlusion, and organ failure, which is transient in the majority of cases [3, 4, 5]. HUS includes diseases that have similar histopathologic and clinical features, but different pathogenesis. The initial, historical classification of HUS distinguished two of its types: typical or post-diarrheal (D+) HUS due to Shiga toxin (Stx)-producing *Escherichia coli* (STEC) and atypical HUS (aHUS) or diarrheal negative (D-) HUS that included any HUS not due to STEC [6]. This classification of HUS based on pre-diarrheal syndrome proved to be misleading as post-diarrheal onset does not eliminate the diagnosis of aHUS. With increasing the knowledge of HUS, especially with better understanding of its molecular basis, the European Pediatric Research Group for HUS proposed a new classification of HUS and related diseases into two sections. The first section includes diseases with known etiology, and the second one covers diseases with unclear causes (Table 1) [7]. According to this HUS classification, the previous name – diarrhea-positive (D+HUS) or typical HUS – has been replaced by the more accurate term STEC-HUS to underline the essential role of Stx in the pathogenesis of the disorder.

This paper addresses current knowledge about HUS caused by STEC. It reviews the historical features, etiopathogenesis, clinical aspects, and treatment of STEC-associated HUS.

## HISTORY

The term HUS was first reported by Gasser et al. [1] who described five fatal patients with hemolytic anemia, renal failure, and thrombocytopenia. After about 30 years, Karmali et al. [8] discovered the cause of this disease and very soon Riley et al [9] described epidemics with painful bloody diarrhea linked by the consumption of undercooked hamburgers contaminated by *E. coli* O157:H7 [9]. O'Brien et al. [10] pointed to the link between the toxic properties of *E. coli* O157:H7 and that of *Shigella dysenteriae* serotype 1.

## ETIOLOGY

The major cause of STEC-HUS in childhood is infection with enterohemorrhagic *E. coli* and in some tropical regions *Shigella dysenteriae* type 1 [8, 11]. Verocytotoxin–producing *Citrobacter*

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**Table 1.** Classification of HUS and related disorders proposed by the European Pediatric Research Group for HUS [7]

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| Part 1: Etiology advanced |
| Infection induced<br>(a) Shiga and verocytotoxin (Shiga-like toxin)-producing bacteria (enterohemorrhagic *Escherichia coli*, *Shigella dysenteriae* type 1, *Citrobacter*)<br>(b) *Streptococcus pneumoniae*, neuraminidase, and T-antigen exposure |
| Disorders of complement regulation<br>(a) Genetic disorders of complement regulation<br>(b) Acquired disorders of complement regulation, for example anti-FH antibody |
| von Willebrand proteinase, ADAMTS13 deficiency<br>(a) Genetic disorders of ADAMTS13<br>(b) Acquired von Willebrand proteinase deficiency; autoimmune, drug induced |
| Defective cobalamin metabolism |
| Quinine induced |
| Part 2: Clinical associations or etiology unknown |
| HIV |
| Malignancy, cancer chemotherapy, and ionizing radiation |
| Calcineurin inhibitors and transplantation |
| Pregnancy, HELLP syndrome, and oral contraceptive pill |
| Systemic lupus erythematosus and antiphospholipid antibody syndrome |
| Glomerulopathy |
| Familial, not included in Part 1 |
| Unclassified |

HUS – hemolytic uremic syndrome; FH – factor H; HELLP – hemolytic anemia, elevated liver enzymes, and low platelets; HIV – human immunodeficiency virus; ADAMTS – a disintegrin-like and metalloprotease with thrombospondin type I repeats

**Table 2.** Annual incidence of hemolytic uremic syndrome (HUS) in children

| Region | Study time period | Cases of HUS/100,000 children (age, years) | STEC-HUS (%) | Reference |
|---|---|---|---|---|
| British Isles | 1985–88 | 0.65–0.91 (<16) | 95 | 21 |
| Argentina | 2002 | 12.2 (<5) | >90 | 22 |
| France | 1998 | 0.7 (<15) | 83 | 23 |
| Germany | 1997–2000 | 0.7 (<15) | 83 | 24 |
| Norway | 1999–2008 | 0.5 (<16) | 81 | 25 |
| USA | 2000–2007 | 0.78 (<18) | 90 | 26 |
| Australia | 1994–1998 | 0.64 (<15) | 84 | 27 |
| Austria | 1997–2000 | 0.37 (<15) | 83 | 24 |
| Italy | 1988–2000 | 0.28 (≤15) | 73 | 28 |
| Serbia | 2011–2014 (all)<br>1985–2014 (D ± PE) | 0.28 (≤15)<br>0.11 | 70 | / |

D – dialysis; PE – plasma exchange; STEC-HUS – Shiga toxin-producing *Escherichia coli* hemolytic uremic syndrome

*freundii* has also been reported [12]. Synonymous for enterohemorrhagic *E. coli* (EHEC) are verotoxigenic, verocytoxin-producing, or verocytotoxigenic *E. coli* (VTEC/STEC). STEC expressing somatic (O) antigen 157 and flagellar (H) antigen 7 are the serotype most frequently associated with HUS, but at least during certain periods, non-O157:H7 STEC appears to be more common [13–17].

*E. coli* produces Shiga toxins 1 (Stx1) and 2 (Stx2). These toxins consist of two A and five B subunits. B subunits have affinity to globotriaosylceramide (Gb3Cer) receptors located in the membranes of glomerular, colonic, and cerebral epithelial or microvascular endothelial cells, as well as in renal mesangial and tubular cells, monocytes, and platelets. The A subunit causes cell death by its N-glycosidase activity, which inhibits protein synthesis at the level of 28S ribosomal RNA [18].

## EPIDEMIOLOGY

Most STEC infections are asymptomatic, or manifested by non-bloody diarrhea, while minority have bloody diarrhea and HUS. The majority of STEC-HUS cases are sporadic, although large outbreaks have been reported [19]. Cattle are a major reservoir of STEC infection, which does not cause disease in them because their vascular endothelial cells have no Gb3Cer receptors. The infection may be transmitted by the consumption of undercooked meat, pasteurized dairy products, contaminated vegetables, fruits, and water, or by contact with STEC diarrhea. The risk of HUS development is higher in patients infected with EHEC O157:H-, in children younger than five years of age, those who use antimotility agents while use of antibiotics remains controversial [20]. Bloody diarrhea, fever, vomiting, leukocytosis, and not receiving intravenous hydration and volume expansion were also recognized as risk factors for HUS development [21].

An average annual incidence for HUS in children under 15 years of age is about 0.7 per 100,000 [21–28]. The highest HUS incidence worldwide occurs in Argentina (12.2 cases/100,000 children aged < 5 years) [22], and the lowest incidence was reported in Italy (0.28 cases/100,000 children ≤15 years old [28]. The incidence rate in Serbia is also low (Table 2). The reasons for variable incidence in different regions are unclear, but it may be related to exposure to STEC, and potential genetic susceptibilities of specific populations.

STEC-HUS is a dominant form of HUS in children with the participation of 72–95% of all cases of HUS. It is more common in younger children, with most cases occurring in summer's months. At the beginning of May 2011, a large outbreak of STEC infections started in northern Germany [19]. More than 3,800 cases of *E. coli*-positive infections were reported, including 845 patients with HUS. Majority (88%) of the patients with HUS were adults, with an overrepresentation of women. This may be explained by ingestion of infected sprouts, which are rarely consumed by young children.

## PATHOGENESIS

A major event in the pathogenesis of HUS is the EHEC colonization of the colon. EHEC strains express adhesins by which they adhere to the intestinal epithelial cells. [18]. They may cause colitis with bloody diarrhea due to intestinal cell death and vascular damage. Infected intestinal cells secrete cytokines in the intestine and into the circulation. It is proposed that Stx is transported in circulation by

leukocytes and platelets, which also transport Stx to the microvasculature of the target organs that possess Gb3Cer receptors [18]. After B subunit of Stx binds to the Gb3Cer receptor, A subunit is transported to the Golgi apparatus and releases a protease which inhibits ribosomal function, leading to cell death. This process can also activate signaling pathways which induce an inflammatory response in affected cells. In fact, binding of Stx to its receptors has at least dual effect – the first one stimulating the release of cytokines including TNF-a, IL-1, and IL-6, and the second effect being cytotoxic, directly killing the cells. [18]. The released cytokines induce an inflammatory reaction, which amplifies further secretion of cytokines, and in association with Stx, lipopolysacharide, and other virulence factors increase the cytotoxic damage. Thus, Stx is capable of stimulating cells to release cytokines as well as inducing cell death by inhibition of protein synthesis or by apoptosis. In contrast to the brain, kidneys and other Gb3Cer-endowed organs prone to inflammatory and cytotoxic EHEC effects, monocytes, polymorphonuclear cells, and platelets are resistant to the Stx cytotoxic effects [18]. Stx provokes direct endothelial damage by a ribotoxic stress response and by increasing inflammation stimulating expression of cytokines and chemokines. These lead to the formation of microthrombi, followed by injury of the target organs, especially the kidney and the brain. In addition, damaged endothelium causes fragmentation of erythrocytes with consequent hemolytic anemia while platelet consumption results in thrombocytopenia. Glomerular thrombi and damage to tubular epithelial cells decrease glomerular filtration and cause AKI.

The role of complement activation in STEC-HUS is still a subject of debates. *In vitro* studies of endothelial cells have demonstrated that exposure to Stx increases expression of P-selectin, which binds and activates C3 via the complement alternative pathway [29]. The C3a that is produced following the cleavage of C3 potentates the activation of the alternative pathway of complement, reduces the expression of thrombomodulin, and promotes thrombus formation [29]. Reports of low serum levels of C3 in children with STEC-HUS date back to the early 1970s [30]. More recent studies have confirmed C3 reductions in severe cases of STEC-HUS [31].

## CLINICAL ASPECTS

The interval between ingestion of *E. coli* and the onset of diarrhoea is usually three days. About 50% of these patients develop nausea and vomiting. Only 30% have fever. Bloody diarrhea occurs in about 90% of cases. The colon can be quite severely affected. Severe colitis can result in transmural necrosis with perforation and/or later development of colonic stricture. HUS is typically attained about a week after the onset of diarrhoea in 5–15% of cases, but during outbreaks it may increase up to 30%. Thrombocytopenia is the first abnormality in most patients. Patients suffer from hemolytic anemia, thrombocytopenia, and renal failure [6]. Elevation of pancreatic enzymes is common, and edema of the pancreas, indicative of pancreatitis, can be detected by ultrasound or computed tomography scan. Hematological manifestations include the following: acute decay of hemoglobin, fragmented erythrocytes, low or undetectable levels of haptoglobin, thrombocytopenia, and massive increase of lactate dehydrogenase (LDH). By definition, all patients with STEC-HUS manifest some degree of renal insufficiency. Approximately 30–40% of the patients will require dialysis therapy for an average duration of 10 days.

The majority of patients show a complete restitution. Poor prognostic factors for chronic renal disease include neutrophilia >20,000/ml, shock during the acute phase, anuria for more than two weeks, central nervous system involvement, severe colitis and/or rectal prolapse, renal cortical necrosis, extensive renal thrombo-microangiopathic lesions (50% glomeruli), and proteinuria at one year [20, 32–35]. The multivariate model which included all markers for severe acute disease showed that the association with poor long-term outcome was stronger for the use of plasma treatment than for other markers of severe acute disease such as neurological symptoms, hypertension, use of dialysis, and longer duration of dialysis [34]. Neurological complication occurs in 20–25% of patients with STEC-HUS [34]. They represent a combined effect of Stx-induced vascular injury, endothelial dysfunction, hypertension, and electrolyte disorders. Their manifestations may be seizures, loss of consciousness, thrombotic strokes, and cortical blindness, but also sudden death [34]. Skeletal muscle involvement and myocardial lesions are rare clinical manifestations.

Mortality rates in pediatric STEC-HUS range from 1–5% in sporadic cases, but in some outbreaks may be even up to 11%. Acute mortality rate declined dramatically with the introduction of early dialysis for severely affected oligo-anuric patients. Brain involvement is the most common cause of death, and less frequent causes are congestive heart failure, pulmonary hemorrhage, hyperkalemia/arrhythmia, and bowel perforation/hemorrhagic colitis. Having in mind long term complications, it is important to follow up patients who had HUS for at least five years, and over longer periods if indicated. Strict blood pressure regulation and normalization of proteinuria is very important for control of chronic kidney disease progression [36].

## DIAGNOSIS

The tests required for a diagnosis of STEC-HUS are presented in Table 3. Main clinical diagnostic criteria include combination of microangiopathic anemia, thrombocytopenia and AKI. The criterion for anemia is age-dependent and sex-dependent and the confirmation of the microangiopathic character is based on microscopic review of the peripheral smear and detection of schistocytes. In addition to monitoring the hemoglobin, hemolytic parameters (LDH, haptoglobin), hematocrit, and platelet count, parameters of level of renal function, amylase, lipase, glucose, and liver function studies should be performed during the

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**Table 3.** Tests for diagnosing hemolytic uremic syndrome

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| General investigations: |
| Chemistry including renal and liver function, LDH, glucose, urate, lipase, amylase |
| Full blood count, microscopic review of the peripheral smear and detection of schistocytes |
| Clotting screen |
| Urine examination, proteinuria, albuminuria |
| Stool microscopy and culture |
| VTEC serology (ELISA and western blot) |
| Molecular diagnosis of STEC in stool directly with ELISA or indirectly by VT PCR |
| Selective investigations: |
| Direct Coombs test |
| Thorax and abdominal X-ray |
| Renal and abdominal ultrasound |
| Abdominal CT |
| ECG |
| EEG |
| MRI (or CT) of the brain |
| Renal biopsy if required |

LDH – lactate dehydrogenase; VTEC – verocytotoxigenic *Escherichia coli*; ELISA – enzyme-linked immunosorbent assay; STEC – Shiga toxin-producing *Escherichia coli*; VT PCR – verotoxin polymerase chain reaction; ECG – electrocardiogram; EEG – electroencephalogram; MRI – magnetic resonance image; CT – computed tomography

acute phase of the disease. Screening for and isolation of EHEC from stool specimens has the highest likelihood of positive culture on the third day. Molecular diagnosis of STEC can be done in stool directly with the detection of Stx with enzyme-linked immunosorbent assay (ELISA) or indirectly (Stx genes) with polymerase chain reaction (PCR). Specific serogroup identification can be achieved using ELISA and western blot with specific antibodies detection, both in the acute and convalescent periods. *E. coli* O157: H7 can best be detected by plating of fresh feces on sorbitol-MacConkey agar. This agar has sorbitol, not lactose, as a carbon source. Unlike most human fecal *E. coli*, O157:H7 strains cannot ferment sorbitol after overnight incubation on sorbitol-MacConkey agar, and they therefore appear as colorless colonies.

Selective investigations include Coombs test, which is negative in HUS, thorax and abdominal X-ray, renal and abdominal ultrasound. In case of pancreatitis or suspect collection, it is necessary to perform abdominal computed tomography. Electrocardiogram should be done in case of presented cardiac failure and/or severe electrolyte disturbance. Neurological examination screens for central nervous system involvement, electroencephalogram and magnetic resonance imaging / radiographic imaging is needed in symptomatic patients. Renal biopsy is very rarely required. In the renal histology, the arteriolar afferentes – and more rarely the arteriolar efferentes – show swelling of the endothelial cell, subendothelial deposits of fibrinoid substances, and thrombosis of the arterioles. In the glomerulus, swelling of capillary endothelial cells and capillary dilatation are found. The deposits of fibrin in the capillaries, including thrombosis and hyalinosis, are also described. Tubular damage, focal or segmental, with necrosis and atrophy is of significance for long-term outcome.

## THERAPY

There is no specific therapy for STEC-HUS. The management of HUS includes the usual treatment of children with AKI [37]. Early diagnosis and supportive care are of major importance. Maintaining an appropriate renal perfusion is very important in preventing HUS. Studies show that adequate pre-HUS volume expansion is associated with lesser AKI in HUS [35]. Packed red cell transfusion is indicated for Hb <6 g/dl, or in case of Hb <7 g/dl, but symptomatic. It may worsen hyperkalemia and cause volume overload. Platelet transfusion is rarely indicated unless invasive surgery is planned or there is intracranial hemorrhage. Preferred treatment for hypertension is with vasodilators [38]. Renal replacement therapy should be implemented according to the usual indications in AKI [39]. Medication that could elevate the risk or worsen AKI (diuretics, NSAIDs, ACE inhibitors, and nephrotoxic agents) should be avoided. Currently, much controversy revolves around antibiotic treatment of EHEC. They are not a part of routine management. However, recent data suggest that fluoroquinolones and azithromycin might actually be beneficial in preventing HUS or decreasing the severity of symptoms in STEC-HUS patients [40, 41]. Well-designed studies are needed to establish the role of antibiotics in this setting.

Plasma treatment is currently the treatment of choice for atypical HUS patients and is at times used in severe pediatric patients with STEC-HUS without proven beneficial effects [19, 20]. The assumption that plasmapheresis can remove the Stx from the circulation is not based on hard evidence [42]. Unlike the usual practice for the limited application of plasmapheresis in pediatric patients with STEC-HUS, in adult patients plasma therapy has until recently been "the cornerstone of treatment" [43, 44]. Multicenter retrospective case-control study which included 298 adult patients with STEC-HUS, in which plasmapheresis was carried out in 84% of the patients, questioned this traditional therapeutic approach as no benefit or only marginal benefit was found [42]. Neutralizing monoclonal antibody of Stx is promising amongst new therapies in the development of Stx [41]. The Synsorb® Pk (Synsorb Biotech Inc., Calgary, Canada) was not found to be beneficial, but Starfish has been shown to bind to Stx 1,000 times more efficiently than Synsorb® Pk [41]. Eculizumab is a recombinant, humanized, monoclonal immunoglobulin G antibody directed against C5 protein, inhibiting activation of the terminal complement pathway. It is clearly indicated in some children with atypical HUS, but its value in patients with STEC-HUS remains to be determined [19, 45]. Anti-motility agents, anticoagulation, antiaggregation and fibrinolysis are not associated with better outcome. Corticosteroids, plasmapheresis, and plasma infusion are not superior to supportive therapy. Renal transplantation in "classic" HUS is rare and recurrence of STEC-HUS is the absolute exception [21]. Therefore, in patients with Stx-associated HUS, transplantation can be performed without increased risk for transplant failure.

## PREVENTION

Preventive measures and effective therapies remain important goals. They can be summarized into three categories: (1) infection prevention (exposure reduction, active immunization against 'protective' bacterial antigens and Stx), (2) primary or causal therapies targeting Stx directly, and (3) secondary therapies, aiming at downstream molecular and pathological events (inhibitors of stress-activated protein kinases, apoptosis, the thrombotic cascade) [41]. More recently, advanced, multibranched Stx receptor analogs and polymers, and peptide-based intracellular toxin inhibitors have been developed with therapeutic potential for oral and intravenous application [41]. However, hygienic measures and education remain the basic management in reducing the incidence of STEC infection and HUS.

## CONCLUSION

Most commonly (70–90%), HUS is due to STEC infection. STEC-HUS usually affect children <5 years of age occurring in the summer months. Usually, it is a self-limiting disease with current mortality rates less than 4% during the acute illness. Due to long term renal and non-renal sequelae, it is important to follow up these patients for at least five years, and over longer periods if indicated.

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# Хемолитички уремијски синдром изазван ешерихијом коли која продукује токсин шига

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**КРАТАК САДРЖАЈ**

Хемолитички уремијски синдром (ХУС) карактерише микроангиопатска хемолизна анемија, тромбоцитопенија и акутно оштећење бубрега (АОБ). Главни узрок ХУС (>90%) у детињству је инфекција изазвана бактеријама које продукују вероцитотоксин (сличан токсину шигела – „*stx*"), најчешће ентерохеморагијском ешерихијом коли (*VTEC*/*STEC*). Инфекција се може пренети употребом некуваног меса, пастеризованих млечних производа, контаминираног поврћа, воћа и воде, или контактом са особама које имају дијареју. После инкубационог периода од три до осам дана болесници обично добију хеморагијски колитис, после којег се код 5–22% оболелих развија ХУС који може бити компликован екстрареналним поремећајима укључујући поремећаје централног нервног система, панкреаса, скелетног мишића и срца. *STEC* ХУС је један од главних узрока АОБ код деце у Европи. Лечење ХУС код деце обухвата уобичајени третман АОБ. Трансфузија еритроцита је потребна код тешке анемије, а трансфузија тромбоцита само пре хируршке интервенције или код активног крварења. Употреба антибиотика није јасно дефинисана. Није доказано корисно дејство инфузија плазме или плазмаферезе. Ецулизумаб је примењен код оболелих од *STEC* ХУС-а, али корист овог третмана остаје тек да се тачније сагледа. Стопа морталитета је 3–5%. Код око 12% болесника ХУС проградира у терминалну бубрежну инсуфицијенцију у току четири године, а у 25% ће имати дуготрајне компликације укључујући хипертензију, повећану протеинурију, бубрежну инсуфицијенцију и инсулин-зависни дијабетес мелитус. Трансплантација бубрега се може применити без ризика од повратка болести.

**Кључне речи:** акутно оштећење бубрега; деца; Д-ХУС

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# Development of cardiopulmonary bypass – A historical review

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**SUMMARY**
The idea of isolated organ perfusion, a precursor of cardiopulmonary bypass, came by Legalois in 1812. First isolated organ perfusion was described by Loebell in 1849. The first closed system for oxygenation and returning the blood through arteries was created by Frey and Gruber in 1885. Gibbon Jr. is considered the father of extracorporeal circulation. In spring of 1934 he began constructing a machine for extracorporeal circulation in Boston. He published the first description of this system in 1937. Gibbon won the grant of the International Business Machines Corporation for developing the machine in 1947. Together they developed Model I in 1949 and Model II in 1951. After a few unsuccessful attempts in 1952, the first successful surgical intervention on the heart (closure of atrial septal defect) using cardiopulmonary bypass was performed on May 6, 1953. In 1945, Kirklin and his working group reported on a series of eight successfully treated patients in a row who underwent surgery with extracorporeal circulation. First successful valve surgery under the direct vision was performed by Dodrill in 1952, using his "Michigan Heart" machine as a right heart bypass. Using cardiopulmonary bypass, cardiac surgeons can deal with the complex cardiac pathology and save millions of lives.
**Keywords:** invention; history; cardiopulmonary bypass; extracorporeal circulation; Gibbon

## INTRODUCTION

Cardiac surgery, a radical-reconstructive surgical discipline like it is today, could not have been developed without the interaction between medicine and engineering. For most reconstructive surgical procedures on the heart it is necessary to stop and empty the heart to acquire clear operative field, especially for the surgery of cardiac chambers. The only possible way to achieve this is to use cardiopulmonary bypass in cardiac arrest induced by cardioplegic solution. Discovery of heparin in 1917 by William H. Howell and Jay McLean led to the idea of developing extracorporeal circulation [1]. Our objective is a historical review of the cardiopulmonary bypass development.

## HEART SURGERY BEFORE THE DEVELOPMENT OF EXTRACORPOREAL CIRCULATION

Before the invention of cardiopulmonary bypass, cardiac surgery had a high rate of perioperative complications, often resulting in fatality. The surgery of a 24-year-old patient, performed by Daniel Williams Halle in Chicago on July 10, 1899, is considered to be the first successful operation of a stab wound on the heart, although the patient had only suffered pericardial trauma with no heart involvement [2]. The first attempts of pulmonary artery thromboembolectomy were described by Trendelenburg in 1908 and 1909. Both of them were unsuccessful [3]. The first successful pulmonary artery thromboembolectomy was performed in 1924 by Kirschner [4]. Poor outcomes of patients with attempted pulmonary thromboembolectomy were noted by Gibbon in 1937, inspiring him to develop a machine for extracorporeal circulation. Specifically, at that time, only nine out of 142 patients worldwide in whom pulmonary embolectomy was attempted survived this procedure [4]. At that time, heart valve surgery and congenital heart disease surgery were mainly at palliative level.

## ISOLATED ORGAN PERFUSION AS A PRECURSOR OF CARDIOPULMONARY BYPASS

The idea of the isolated organ perfusion came from the reports of Julien Jean César Legalois in his monography *Expériences sur le principe de la vie* in 1812. He argued that if it were possible to make an artificial device that could use injectors to deliver arterial blood to the device, then it could be possible, using this device, to keep life of any part of the body, as well as throughout the body, indefinitely, thus ensuring resurrection.

The first isolated organ perfusion (artificial blood supply to a dog's kidney) was described by Karl Eduard Loebell in his dissertation in Marburg in 1849. Alexander Ludwig and Carl Schmit presented a method for continuous artificial blood supply to a dog's kidney with venous blood under the effect of hydrostatic pressure in 1867.

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At the Institute of Pharmacology in Strasbourg, Waldemar von Schroeder developed a device that could oxygenate the blood during perfusion (a primitive bubble oxygenator) in 1882. [5].

The real precursor of extracorporeal circulation was constructed at the Institute of Physiology in Leipzig in 1885 by Max von Frey and Max Gruber. The system consisted of the venous blood supply, oxygenation cylinder that used film oxygenation principle and pumps that pumped the blood in continuing fashion under the influence of hydrostatic pressure. Their system consisted of devices that affected the blood temperature, a thermometer, and pressure gauges. It was the first machine that could perform blood oxygenation without any interruption of the blood flow [6]. This device seemed too complicated for Carl Jacobj, from the Institute in Strasbourg, and in 1890 he developed his perfusion system that he called "Hematizator." He used a pump with pulsatile blood flow, and, unlike before, he developed systems that had pumps with continuous blood flow [7]. The pump with pulsatile flow dynamics was developed by Hamel at the Institute of Physiology of the University of Bern [5]. Oxygenation of the blood was performed in the tank, by direct air contact (bubble oxygenation). Since air embolism was a concern, Jacobj abandoned this technique in 1985. He developed a machine that he named "Double Hematizator." The machine consisted of two pumps. Isolated lungs of an experimental animal were inserted between the pumps performing blood oxygenation [8].

At the beginning of the twentieth century, Zeller in Berlin created a link between the earlier experiments of artificial perfusion of organs and eventual use in clinical practice. In 1908 he declared that the use of artificial perfusion should be considered in the surgery of heart wounds, pulmonary thromboembolism, as well as in bleeding to death [5].

## JOHN HEYSHAM GIBBON JR. – THE FATHER OF EXTRACORPOREAL CIRCULATION

Gibbon Jr. was born on September 29, 1903 in Philadelphia. He attended Princeton University in New Jersey from 1919 to 1924. In 1927 he completed his studies at the Jefferson Medical College in Hillsboro, Missouri. He was the fifth generation of physicians in his family, and the third generation of surgeons. His father was a professor of surgery at the Jefferson Medical College and a nationally recognized surgeon [9]. After graduation, he spent two years on internship at a hospital in Pennsylvania [10]. After that he went to Harvard and carried a one-year research fellowship under the guidance of surgeon Edward Dundrige Churchill [11]. During that time, Gibbon got an idea that changed his life and profoundly influenced the history of cardiac surgery.

Gibbon's wife, Mary Hopkinson, who was Gibbon's technical assistant in the research, said that October 3, 1930 was a day that changed the history of cardiac surgery. On that day, in the afternoon, Dr. Churchill and his colleague James White were called by a patient, who was recovering from a gallbladder surgery, and complained about the problems

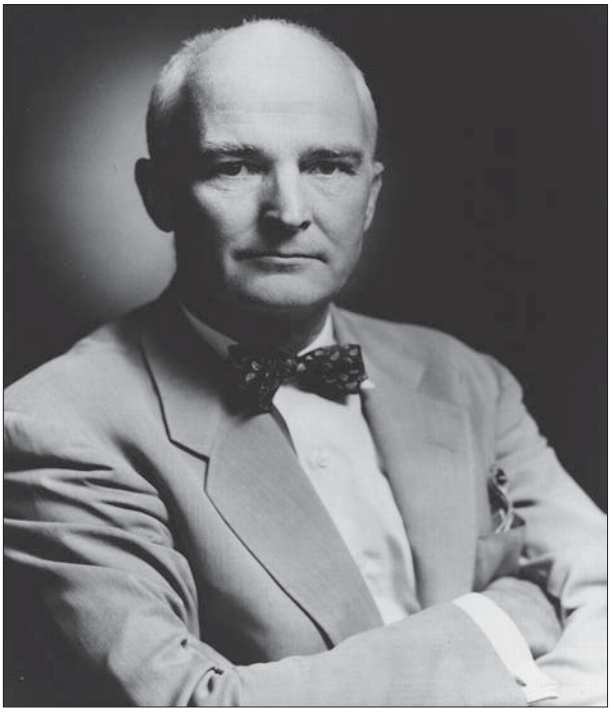

**Figure 1.** Dr. John Heysham Gibbon Jr.

regarding shortness of breath. Based on the clinical symptoms and signs, they quickly diagnosed acute pulmonary thromboembolism [12]. It was obvious that the patient needed surgical intervention. However, since pulmonary thromboembolectomy was successfully performed only in Europe, Churchill decided to postpone surgical intervention, and said that the intervention could be performed only if the patient's condition became so bad that there was no other solution. A day later, the patient's condition deteriorated, and Churchill performed the surgery. The patient did not survive [12, 13]. Gibbon, who was responsible for monitoring the patient's condition prior to the surgery, in order not to miss the moment when the operation was necessary, got an idea. Several years later, Gibbon recounted his thoughts, "... during the 17 hours by this patient's side, the thought constantly recurred that the patient's hazardous condition could be improved if some of the blue blood in the patient's distended veins could be continuously withdrawn into an apparatus where the blood could pick up oxygen and discharge carbon dioxide, and then pump this blood back into the patient's arteries. Such a procedure would also lend support to the patient's circulation while the embolectomy was being performed" [14].

At that time, Gibbon was interested in the study of pulmonary thromboembolism. On October 15, 1930, he began experimenting on cats, examining the correlation between the degree of artificially created stenosis of pulmonary artery and drop of blood pressure [15]. In March 1931, Mary Hopkins and John Gibbon were married, and shortly thereafter they returned to Philadelphia. Gibbon talked about his idea for the next three years, but it was generally thought impossible to implement this idea in practice. Meanwhile, Gibbon worked with Eugene M. Landis on other experiments, but it was Landis that encouraged his intent to make invention [16]. In the spring

of 1934, Gibbon returned to Churchill in Boston, and convinced him to provide a laboratory for his research. Churchill accepted it, but without much enthusiasm [13].

Gibbon thought that the biggest problem was to provide adequate oxygenation of the blood, although at that time several methods for blood oxygenation had already been discovered. He published the first description of this system in 1937. His perfusion system was able to provide the blood oxygenation for the flow of 500 ml per minute. Oxygenation was conducted in a rotating vertical cylinder under the principles of film oxygenation. The system consisted of two pumps that were securing a continuous flow under the influence of hydrostatic pressure, one that pumped the blood from the venous cannula to the oxygenator, and the other that pumped the blood from the oxygenator to the arterial circulation [17]. Shortly afterwards, Dale and Schuster proposed a modification, according to which the pump worked in pulsatile fashion [18].

Gibbon had been using cats as experimental animals since 1934. He wanted to prove that it was possible to maintain life without the blood flow in the lungs. After another year spent in Boston, he returned to Philadelphia. After solving various technical problems of the pump, on May 10, 1935, he was able to open and close the pulmonary artery of a cat. During that time (39 minutes), the function of cardiopulmonary system was replaced by extracorporeal circulation, and the cat survived. He continued his research, with an interruption from 1941 to 1944 due to World War II [17].

After World War II, Gibbon recognized that there was a problem in the capacity of blood oxygenation. Such a low capacity was insufficient for larger animals. He became aware that he was unable to improve the machine by himself, and decided to seek help from engineers. Dean of the University of Philadelphia advised Gibbon to meet with heads of IBM (International Business Machines Corporation) in 1946, and they referred him to the director of IBM, Thomas J. Watson, with whom he met in 1947 [11]. After listening to Gibbon, Watson promised him financial and technical support for the development of the extracorporeal circulation over the next seven years [12].

## THE RESULT OF COMMON WORK

IBM and Gibbon produced Model I extracorporeal circulation in 1949. This model had improved the capacity of oxygenation of the blood, while the pulsatile pump was used instead of the double-roller pump. Michael DeBakey modified the pump with the pulsatile flow [19]. However, during the experimental use, a flaw was spotted – the capacity of blood oxygenation was still insufficient for the animals larger then cats. In addition, there was a problem with hemolysis and repeated air embolisms. They decided to stop experiments on animals until they found a solution to these problems [20].

The result of further common research was Model II, which was significantly different from the previous model. Instead of the cylindrical oxygenator based on the principle of film oxygenation, Model II used a system of grids placed in an oxygen atmosphere. This system combined the principles of film oxygenation and turbulent blood flow through the grid. The grid had openings of different diameters, depending on the required capacity of blood oxygenation. It still had the double-roller pump, whereby the venous pump had to provide recirculation of blood in the oxygenator, and to maintain constant thin film of the blood on the grid [21]. The machine was capable of measuring blood pH, blood oxygen saturation, blood flow rate, and temperature, and possessed a mechanism for regulating the temperature and the pH level [20]. This model was much more effective than the previous ones. In April 1951, at the Jefferson Institute in Missouri, a dog weighing 10 kg survived a 96-minute-long total cardiopulmonary bypass. After the development of Model II, the mortality of experimental animals reduced considerably, compared to the period before 1949 [22]. Despite a significant reduction in mortality, there was still a problem of gas embolism after opening the heart cavities. Attempts to solve this problem were made in different ways, and the optimal solution was found by Bernard J. Miller in 1952. He described the need for an artificial tube, which would be introduced into the heart chambers to draw out the air. Miller's advice was that this kind of tubes, called vents, could be introduced through the heart apex into the left ventricle [23]. After that, Albritten reported the introduction of the vent through the left atrium [24].

## FIRST ATTEMPTS AT CLINICAL USE OF EXTRACORPOREAL CIRCULATION

After the encouraging results of experimental studies on animals, the conditions for the clinical use of extracorporeal circulation were met. The first clinical use of the Model II machine took place in February 1952 by Gibbon. It was a 15-month-old baby girl with the suspected presence of atrial septal defect (ASD). After placing the patient on cardiopulmonary bypass and opening the right atrium, there was no sign of ASD. The patient died on the table, and a large patent ductus arteriosus was found on autopsy.

In an adult patient, Model II was first used on March 7, 1952. It was a 41-year-old patient with heart failure, with suspicion of myxoma or thrombus in the right atrium. Frank Albritten performed the surgery, using the machine as a right heart bypass. Cardiopulmonary bypass was overseen by Miller. After opening the right atrium, neither a tumor nor a thrombus was found. After terminating cardiopulmonary bypass, cardiac contraction began to fail and finally stopped. Resuscitation methods briefly restored the heart function, and they were able to close the chest, but during immediate postoperative course, the patient developed pulmonary edema and died. The autopsy report verified that the preoperative diagnosis was misplaced. The patient was suffering from dilated cardiomyopathy due to interstitial myocarditis [25].

On April 17, 1952 Gibbon used his machine to support the circulation in an older anuric patient with central

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**Figure 2.** Heart–lung machine dating from 1958 (design first appeared in 1955), used in London's Middlesex Hospital

cyanosis and edema. For angioaccess, he used the right internal jugular vein and the right radial artery. He set the flow rate at 300 ml/min. for a period of 45 minutes. During the circulatory support, the patient's face turned pink. The patient died three hours after the termination of assisted circulation [17].

## FIRST SUCCESSFUL SURGICAL INTERVENTION USING CARDIOPULMONARY BYPASS

Cecilia Bavolek, an 18-year-old girl, had symptoms of the right heart failure. Donald Burns Lewis initially thought that she had mitral stenosis, and introduced Gibbon to the case under that diagnosis [25]. After the cardiac catheterization, Gibbon and co-workers found that she most likely had ASD, and decided to perform the exploratory surgical intervention.

On May 6, 1953 Gibbon and associates started with the surgery, for which he had prepared the Model II machine, filled with heparinized blood of adequate blood group. Through the bilateral thoracotomy in the fourth intercostal space, they accessed the mediastinum and identified the dilated right ventricle and the pulmonary artery. After opening the pericardium, they saw that the right atrium was also dilated. By palpation, Gibbon found that the patient had ASD. It was decided that the patient should be placed on total cardiopulmonary bypass, during which they would cannulate the left subclavian artery and both venae cave. After the onset of cardiopulmonary bypass, they spotted a blood clot in the oxygenator, due to the insufficient dose of heparin applied, but they continued with the operation. Opening the right atrium, Gibbon verified a small ASD. At the initiative of his assistant Frank Albrichten, the defect was closed by the direct suture instead of the pericardial patch, as was done in experimental animals. After 26 minutes of total cardiopulmonary bypass, they removed the ligature from both venae cave, proceeded as a partial cardiopulmonary bypass, removed the vent from the left ventricle, and after 45 minutes stopped extracorporeal circulation. An hour after the surgery the patient woke up without any neurological deficit. After the surgery, Gibbon personally informed Alfred Blalock and Clarence Crawford by telephone that the operation was successful [25].

Subsequently, in July 1953, Gibbon operated on two more patients with congenital heart diseases, but none of them survived [26]. In 1954, Gibbon closed the ventricular septal defect in a 38-year-old patient with four interrupted sutures. The patient survived the operation, but died a few hours later [27]. Gibbon was so disappointed that he announced that he would not operate on heart for one year and would deal with liver surgery [26].

IBM discarded Model II, and presented Model III, which was submitted to Jefferson College in July of 1954. IBM presented its plans to produce it for about ten institutions around the world [28].

From March 22, 1954, modified Model II, called Mayo Gibbon Pump Oxygenator, was used in Rochester by a working group led by John Webster Kirklin. They first published a report on a series of eight consecutive patients successfully treated with the use of extracorporeal circulation (Figure 2).

## OTHER WORKING GROUPS

While Gibbon was developing his machine, there were attempts from other working groups as well. Clarence Denice started to develop his machine for extracorporeal circulation in Minneapolis in 1947, on the principles of Gibbon's experiments. In April and May of 1952, they already clinically tested their pumps in two patients. The first patient, who instead of his preoperative diagnosis of ASD actually had atrioventricular canal defect, died in the operating room, while the other patient, despite successfully closed ASD, died of a massive gas embolism [29, 30].

Forest Dewey Dodrill and General Motors developed their machine, which initially had no oxygenator and did not exclude lung function. The machine changed the function of one or both chambers separately. They started the trials in 1951. The first use of their machine called "Michigan Heart" was on July 3, 1952. The machine was used as the left heart bypass (cannulation of the left subclavian artery and the left superior pulmonary vein, after which the cannula was moved in the left atrium) in a 41-year-old patient with mitral stenosis. Initially, it was planned to perform the operation of the mitral valve under the direct vision; however, Dodrill failed to execute the operation due to excessive bleeding, and performed closed commissurotomy with finger. The patient survived after a 50-minute-long left heart bypass [31]. Dodrill performed the first operation of the pulmonary valve under the direct vision on October 21, 1952. It is considered the first

successful operation of valves with the application of extracorporeal circulation. The operation was performed on 16-year-old Charles Moses, with the diagnosis of congenital pulmonary stenosis. The operation was carried out using the right heart bypass (cannulation sites were the right atria and the pulmonary artery) with the "Michigan Heart" machine, with blood flow rate of 5.4 L/min., and by total clamping of the pulmonary artery. They performed valvuloplasty of pulmonary valve, and the clamp was taken off after 25 minutes. The patient was discharged after one month [32]. After the successful use of different types of oxygenators by Gibbon and Kirklin, Dodrill changed his mind about the uselessness of the oxygenator. In 1955, he developed a machine with an integrated oxygenator, and in 1956 reported the successful closure of VSD in six patients and a successful operation of tetralogy of Fallot [33]. However, Dodrill's machine was replaced by the modified Mayo–Gibbon machine shortly thereafter, because Dodrill's machine required complicated cannulation.

## CONCLUSION

The machine for extracorporeal circulation, and John Gibbon as its inventor, radically changed cardiac surgery and the prognosis of patients with cardiac diseases. With this invention, cardiac surgeons can deal with complex cardiac pathology and save millions of lives.

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# Проналазак кардиопулмоналног бајпаса – историјски преглед

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**КРАТАК САДРЖАЈ**

Идеја о изолованој перфузији органа, која је претеча кардиопулмоналног бајпаса, долази од Легалое 1812. године. Прва изолована перфузија органа је описана од стране Лебела 1849. године. Први затворен систем за оксигенацију и враћање крви кроз артерије је конструисан од стране Фреја и Грубера 1885. године. Гибон млађи се сматра оцем екстракорпоралне циркулације. Он је почео да конструише машину за екстракорпоралну циркулацију на пролеће 1934. године. Први опис овог система даје 1937. године, а 1947. године добија подршку за развој машине од *International Business Machines Corporation*. Они развијају Модел I 1949. и Модел II 1951. године. После неколико неуспешних покушаја 1952. године, 6. маја 1953. године је први пут успела операција на срцу (затварање атријалног септалног дефекта) уз помоћ кардиопулмоналног бајпаса. Године 1945. Киркlин и његова радна група публикују прву серију од осам успешно третираних пацијената уз помоћ екстракорпоралне циркулације. Додрил је извео прву успешну операцију залистака под контролом ока 1952. године, коришћењем машине *Michigan heart* као бајпаса десног срца. Уз помоћ машине за екстракорпоралну циркулацију кардиохирурзи су у могућности да решавају комплексне срчане болести и да спасу милионе живота.

**Кључне речи:** проналазак; историја; кардиопулмонални бајпас; екстракорпорална циркулација; Гибон

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# Postponed diagnosis of alpha-1 antitrypsin deficiency

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Dear Editor,

Alpha-1 antitrypsin (A1AT) is a part of serine protease inhibitor family of proteins encoded by the SERPINA1 gene. A1AT function is inactivation of neutrophil-derived proteases, predominantly neutrophil elastase through destroying its structural integrity [1]. Alpha-1 antitrypsin deficiency (AATD) may lead to emphysema, liver disease, C-ANCA vasculitis, and panniculitis. Manifestation of the disease varies from asymptomatic to severe lung or liver disease.

The patient is a 65-year-old male with a major complaint of dyspnea, cough, and haemoptysis, who was for the first time hospitalized in our clinic. His dyspnea had been persistent and worsening in nature since he was diagnosed with chronic obstructive pulmonary disease (COPD) at age 50. Treatment with salmeterol/fluticasone 250/50 mcg 2 puffs twice a day via a hydrofluoroalkane inhaler did not provide benefit, and he required the use of salbutamol as a rescue inhaler multiple times per week. He had a 10 pack-year history of cigarette-smoking, but stopped five years ago. Evaluation of other systems was unremarkable. There was no prior family history of lung or liver disease. Medications at presentation included claritromicine 500 mg, two times per day, salmeterol/fluticasone 250/50 mcg 2 puffs twice a day, salbutamol as needed. Physical examination revealed bilateral wheezing, while the rest of the physical examination was unremarkable. He previously had numerous blood tests performed, including complete blood count, hepatitis serologies, HIV screening, TSH, Free T4, immunological tests (ANA, ANCA, ASMA, ANA HEp2, and AMA), which were all within normal limits. His ALT and AST levels at examination were slightly elevated for the first time in his medical history. Pulmonary function testing revealed an FEV1 (volume that has been exhaled at the end of the first second of the forced expiration) of 2.01 L (60% of predicted) with an FEV1/FVC ratio of 72%. He failed to demonstrate significant reversibility with salbutamol treatment (post-bronchodilator increase in FEV1 of 8%). He was found to have a reduced diffusing capacity of the lungs of 17.73 ml/min./mmHg (62% of predicted), and evidence of air trapping with a residual volume of 3.26 L (127% of predicted). The latter may have led to an artifactually low forced vital capacity (FVC) resulting in an FEV1/FVC ratio that was only mildly decreased. His pulmonary function tests over the past five years were also reviewed, and showed a 20.6% decrease in FEV1 over that time period, despite treatment with inhaled corticosteroids and long-acting bronchodilators. Abdominal ultrasound revealed inhomogeneous liver with otherwise unremarkable organs. Given the poor response to treatment and the early onset of COPD with a minimal smoking history, the diagnosis of A1ATD was considered. Serum testing for A1AT revealed a low level of 30 mg/dL (normal range is 100–190 mg/dL). A genotype analysis, performed at a commercial laboratory, was reported as ZZ genotype.

A1ATD is relatively common autosomaly recessive genetic disorder, caused by mutations of SERPINA1 gene. M alleles are normal, and have six subtypes: M1–M6. M1 is the most frequent subtype and is present in 95% of the population. Alleles with most severe clinical manifestations are Z alleles [1]. Diagnosing A1ATD can be demanding due to low recognition of the disease, genetic heterogeneity, and complexity of diagnosis. Its association with asthma or COPD is common, but even then the diagnosis is usually established late [2]. The American Thoracic Society (ATS) and the European Respiratory Society (ERS) recommend screening of A1ATD to all symptomatic adults with COPD or asthma with an airflow obstruction that is not fully reversible after a bronchodilatators treatment. Also, children with a bleeding disorder or prolonged neonatal jaundice should be screened for A1ATD, as well as patients with cryptogenic liver cirrhosis [3]. At present, treatment of A1ATD is very limited. Only in the terminal stage of the lung or liver

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disease transplantation is a solution. Like with emphysema, patients with A1ATD can improve their symptoms with prolonged bronchodilators and corticosteroids. Once diagnosed, the prognosis of these patients is variable. It has been showed that FEV1 is the most important predictor of survival in patients with emphysema [4, 5, 6]. Patients without prior history of smoking, who had been diagnosed in family screening, have expected prognosis the same as healthy individuals [5].

We presented the case of a 65-year-old men whose serum levels of A1AT were below the low range, and ZZ genotype was confirmed. The delayed diagnosis of our patient seems to emphasize the need to remind the doctors about AATD, frequently associated with asthma or COPD symptoms. The low estimated prevalence of AATD prompted the establishment of a registry with the aim of learning more about the medical history and the quality of care of these patients.

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DOI: 10.2298/SARH1612678E

# ERRATUM

## Streptococcus pneumoniae serotype distribution in Vojvodina before the introduction of pneumococcal conjugate vaccines into the National Immunization Program: Erratum

In the article that appeared on pages 521–526 of the September–October 2016 issue of the Serbian Archives of Medicine (Srpski arhiv za celokupno lekarstvo), an incorrect figure – an MRI scan unrelated to the article in question – was published on page 523 instead of Graph 1. The publisher regrets this error. Below is the correct graph, as it should have appeared in the original publication of the article.

### REFERENCE

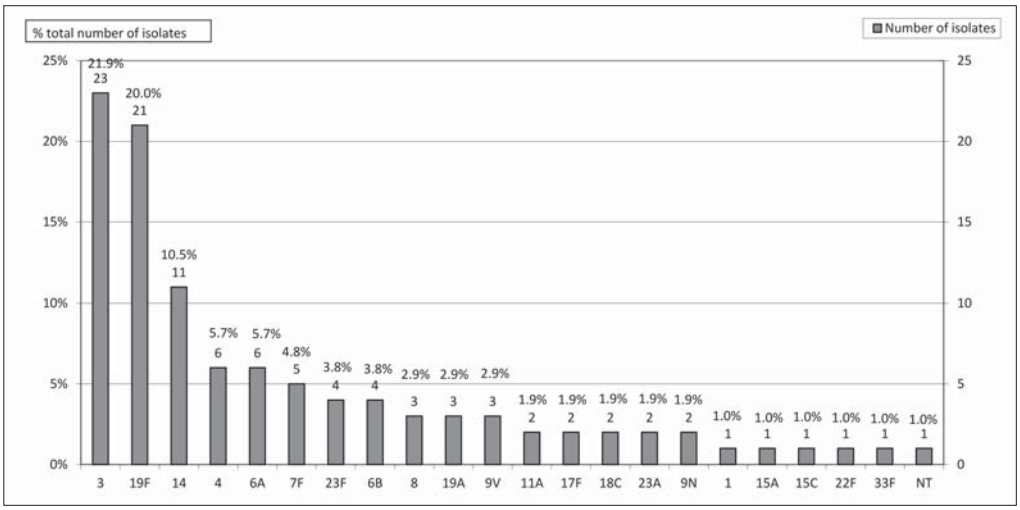
Petrović V, Šeguljev Z, Ristić M, Djekić-Malbaša J, Radosavljević B, Medić D, Mihajlović-Ukropina M, Hadnadjev M, Gajić I, Opavski N. Streptococcus pneumoniae serotype distribution in Vojvodina before the introduction of pneumococcal conjugate vaccines into the National Immunization Program. Srp Arh Celok Lek. 2016 Sep-Oct; 144(9-10):521–526. (doi: 10.2298/SARH1610521P)

## Дистрибуција серотипова *Streptococcus pneumoniae* у Војводини у периоду пре увођења конјуговане вакцине у Национални програм имунизације: *Erratum*

У чланку који је објављен на странама 521–526 у септембарско-октобарској свесци из 2016. године часописа „Српски архив за целокупно лекарство“ грешком је на страни 523 уместо Графикона 1 објављен снимак магнетном резонанцом који није у вези са наведеним чланком. Издавач искрено жали због овог ненамерног пропуста. Овом приликом приказујемо графикон који је требало да буде објављен у оригиналној публикацији чланка.

### РЕФЕРЕНЦА

Petrović V, Šeguljev Z, Ristić M, Djekić-Malbaša J, Radosavljević B, Medić D, Mihajlović-Ukropina M, Hadnadjev M, Gajić I, Opavski N. Streptococcus pneumoniae serotype distribution in Vojvodina before the introduction of pneumococcal conjugate vaccines into the National Immunization Program. Srp Arh Celok Lek. 2016 Sep-Oct; 144(9-10):521–526. (doi: 10.2298/SARH1610521P)




**Graph 1.** Circulating serotypes of Streptococcus pneumoniae in Vojvodina in the period from January 2009 to April 2016

# Списак рецензената који су рецензије радова доставили крајем 2015. и у 2016. години (закључно са 21. децембром 2016)

Поштовани рецензенти, чланови Уређивачког одбора, лектори и сарадници,

Захваљујем вам на ангажовању у реализацији 144. волумена и прилога објављених у „Српском архиву за целокупно лекарство". Значајно смо напредовали у увођењу стандарда за међународне часописе. Квалитет рецензија и укупна обрада рада до публиковања су побољшани и убрзани. Достигли смо ниво да у истој години када је предат Уредништву прихваћени рад буде и објављен, што до сада, сем изузетно, није био случај. Очекујемо да ће преуређењем и усавршавањем сајта часописа комуникација с Уредништвом бити још више олакшана и убрзана. Верујем да ће даље ангажовање Уређивачког одбора, ентузијаста у стручном и научном раду, допринети подизању квалитета и статуса часописа у оквиру домаће и међународне медицинске публицистике, уз жељу да нам наступајућа 2017. године донесе још више успеха.

1. Алемпијевић Ђорђе
2. Аткинсон Хенри Душан Едвард (*Atkinson, Henry Dushan Edward*)
3. Аћимовић Слободан
4. Бабић Раде
5. Башчаревић Зоран
6. Белеслин Бранко
7. Бељић-Живковић Теодора
8. Бјеловић Милош
9. Божић Милена
10. Бошковић Ксенија
11. Брдар Радивој
12. Бреберина Милан
13. Бркић Злата
14. Брковић Божидар
15. Брмболић Бранко
16. Бумбаширевић Марко
17. Васиљевић Зорана
18. Вејновић Тихомир
19. Велимировић Душан
20. Велисављев-Филиповић Гордана
21. Величковић Зоран
22. Вељковић Снежана
23. Вера Јерант-Патић
24. Веселиновић Драган
25. Видаковић Снежана
26. Вићентић Сретен
27. Војводић Никола
28. Војиновић Јелена
29. Врбић Светислав
30. Вујић Драгана
31. Вујичић Исидора
32. Вујотић Љиљана
33. Вуковић Дејана
34. Вукомановић Владислав
35. Вучетић Чедомир
36. Вучинић Предраг
37. Вучинић Славица
38. Вучковић Чедо
39. Гига Војислав
40. Глишић Бранислав
41. Голубовић Емилија
42. Грга Ђурица
43. Грујичић Даница
44. Давидовић Лазар
45. Дамјановић Светозар
46. Дачић Драгица
47. Де Лука Силвио (*de Luka, Silvio*)
48. Деклева Милица
49. Делић Драган
50. Димитријевић Јован
51. Добрић Милан
52. Драча Петар
53. Дучић Синиша
54. Ђорђевић Бобан
55. Ђорђевић Видосава
56. Ђорђевић-Дикић Ана
57. Ђукановић Љубица
58. Ђукић Александар
59. Ђуровић Александар
60. Ђуровић Марина
61. Живковић Зорица
62. Живковић Славољуб
63. Жунић Гордана
64. Златковић-Швенда Мирјана
65. Игњатовић Миле
66. Илијевски Ненад
67. Илић Драган
68. Јаковљевић Бранко
69. Јакшић Весна
70. Јанковић Борко
71. Јанковић Горан
72. Јанковић Љиљана
73. Јанковић Срђа
74. Јаношевић Љиљана
75. Јаношевић Мирјана

76. Јашовић-Гашић Мирослава
77. Јелић Светислав
78. Јовановић Марина
79. Јовановић-Симић Јелена
80. Јовић Небојша
81. Јовић-Стошић Јасмина
82. Калезић Невена
83. Каменов Борислав
84. Кањух Жељко
85. Карамарковић Александар
86. Кашиковић-Лечић Светлана
87. Кисић-Тепавчевић Дарија
88. Ковачев-Завишић Бранка
89. Ковачевић Драган
90. Константиновић Витомир
91. Константиновић Љубица
92. Контић-Вучинић Оливера
93. Костић Гордана
94. Костић Мирјана
95. Костић Томислав
96. Крављанац Ђорђе
97. Кривокапић Зоран
98. Крстић Зоран
99. Крстовски Нада
100. Кршљак Елена
101. Лазић Војкан
102. Лазовић Милица
103. Латковић Зоран
104. Лековић Зоран
105. Лепосавић Љубица
106. Лечић-Тошевски Душица
107. Лешић Александар
108. Максимовић Наташа
109. Максић Ђоко
110. Маликовић Александар
111. Мандарић Драган
112. Манојловић Небојша
113. Марић-Бојовић Нађа
114. Марковић Велимир
115. Марковић Дејан
116. Мацут Ђуро
117. Микић Драган
118. Миков Момир
119. Миковић Жељко
120. Милашин Јелена
121. Милашиновић Горан
122. Миленковић Бранислава
123. Миленковић Светислав
124. Милетић Ивана
125. Милић-Лемић Александра
126. Милошевић Зоран
127. Мирковић Дарко
128. Мирковић Љиљана
129. Мирковић Синиша
130. Мировић Вељко
131. Митковић Милорад
132. Митровић Радивоје
133. Михајловић-Укропина Мира
134. Мицев Марјан
135. Младеновић Марија
136. Мусић Љиља
137. Недељковић Ивана
138. Ненадић Дане
139. Николић Живорад
140. Николић Предраг
141. Николић Татјана
142. Новаковић Маријан
143. Обрадовић Косовка
144. Обрадовић Слободан
145. Павловић Милан
146. Павловић Милорад
147. Павловић Синиша
148. Палибрк Иван
149. Палибрк Томислав
150. Парезановић Војислав
151. Пауновић Иван
152. Пејновић Нада
153. Пејчић Татјана
154. Пекмезовић Татјана
155. Пелемиш Мијомир
156. Перић Момчило
157. Петаков Милан
158. Петровић Љубомир
159. Петровић-Ођано Гордана (*Petrović-Oggiano, Gordana*)
160. Пешић Срђан
161. Плавец Горан
162. Плешинац-Карапанџић Весна
163. Половина Снежана
164. Рабреновић Виолета
165. Радловић Недељко
166. Радовановић Зоран
167. Радовановић Небојша
168. Радовић Милан
169. Радојковић Милица
170. Раичевић Ранко
171. Ракић Снежана
172. Ранђеловић Томислав
173. Ребић Предраг
174. Ресан Мирко
175. Ристић Арсен
176. Ристић-Медић Данијела
177. Савић Ђорђе
178. Савић Слободан
179. Сагић Драган
180. Сајић Силвија
181. Самарџић Мира
182. Семниц Марија
183. Симић Драган
184. Спремовић-Рађеновић Светлана
185. Срдић Биљана
186. Стајевић-Поповић Мила
187. Стаменковић Драгослав
188. Станковић Небојша
189. Станојевић Маја
190. Станојловић Светлана
191. Стевановић Предраг
192. Степановић Јелена

193. Стефанова Елка
194. Стефановић Бранислав
195. Стојановић Весна
196. Стојановић Мирослав
197. Стојановић Роксанда
198. Стојић Синиша
199. Стојковић Миодраг
200. Стојковић Синиша
201. Стокић Едита
202. Столић Радојица
203. Стошовић Милан
204. Суботић Драган
205. Тарабар Дино
206. Татић Светислав
207. Тешић Драган
208. Тимотијевић-Марковић Ивана
209. Тихачек-Шојић Љиљана
210. Тодоровић Љубомир
211. Томић Александар
212. Томић Сергеј
213. Трбојевић Божо
214. Тричковић-Јањић Оливера
215. Ћук Владимир
216. Угљешић Миленко
217. Хајдуковић Зоран
218. Царевић Момир
219. Цветковић Добросав
220. Цвијановић Радован
221. Церовић Снежана
222. Чакић Саша
223. Чеканац Радован
224. Чинара Илијас
225. Човичковић-Штернић Надежда
226. Чоловић Наташа
227. Чоловић Радоје
228. Џодић Радан
229. Шегрт Зоран
230. Шешлија Игор

*Проф. др Павле Миленковић*
*главни и одговорни уредник*

**Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање.**
**Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.**

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални (научни и стручни) радови, метаанализе, прегледни радови, претходна и кратка саопштења, прикази болесника и случајева, слике из клиничке медицине, видео-чланци, радови за праксу, актуелне теме, радови из историје медицине и језика медицине, лични ставови, наручени коментари, писма уреднику, прикази књига и други прилози. Оригинални радови, претходна и кратка саопштења и прикази болесника и случајева публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. $^{99}$Tc, IL-6, $O_2$, $Б_{12}$, CD8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

**КЛИНИЧКА ИСТРАЖИВАЊА.** Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

**ЕТИЧКА САГЛАСНОСТ.** Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

**ИЗЈАВА О СУКОБУ ИНТЕРЕСА.** Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

**АУТОРСТВО**. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално

надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

**НАСЛОВНА СТРАНА.** На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

**САЖЕТАК**. Уз оригинални рад, претходно и кратко саопштење, метаанализу, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење, метаанализе и прегледне радове, сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

**КЉУЧНЕ РЕЧИ**. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh).*

**ПРЕВОД НА СРПСКИ ЈЕЗИК**. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом.

Уколико је рад у целости на српском језику (нпр. рад из историје медицине, језика медицине и др.), потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

**СТРУКТУРА РАДА.** Сви поднаслови се пишу великим масним словима (болд). Оригинални рад, метаанализа, претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навесстu као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор метаанализе и прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

**СКРАЋЕНИЦЕ.** Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

**ДЕЦИМАЛНИ БРОЈЕВИ.** У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

**ЈЕДИНИЦЕ МЕРА.** Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

**ОБИМ РАДОВА**. Целокупни рукопис рада – који чине насловна страна, сажетак, текст рада, списак литера-

туре, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, претходно и кратко саопштење, рад из историје медицине и преглед литературе до 5.000 речи, а за приказ болесника, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3.000 речи; радови за остале рубрике могу имати највише 1.500 речи.
**Видео-радови** могу трајати 5–7 минута и бити у формату *flv*. У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

**ПРИЛОЗИ РАДУ** су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

**ТАБЕЛЕ.** Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле.

Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

**СЛИКЕ**. Слике су сви облици графичких прилога и као „слике“ у СА се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).

Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *flv*. Уз видео доставити посебно слику која би била илустрација видео-приказа у *e*-издању и објављена у штампаном издању.

Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

**ГРАФИКОНИ**. Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета.

Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

**СХЕМЕ (ЦРТЕЖИ)**. Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме.

Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

**ЗАХВАЛНИЦА**. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

**ЛИТЕРАТУРА**. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE.*

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, а у метаанализи до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци,

односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html.* Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

**ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*).** Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

**ЧЛАНАРИНА И ПРЕТПЛАТА.** Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и сви аутори и коаутори морају бити претплатници на часопис за годину у којој се рад предаје Уредништву. Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату, као доказ о уплатама, уколико издавач нема евиденцију о томе. Аутори из иностранства нису дужни да буду чланови Српског лекарског друштва, а морају бити претплатници на часопис за текућу годину (односи се на е-верзију часописа). Додатне информације о чланарини и претплати могу се добити на телефоне 011/3245-149 и 011/3346-963, односно имејлом (*srparhiv@bvcom.net*) и на интернет-страници часописа (*http://www.srpskiarhiv.rs*).

**СЛАЊЕ РУКОПИСА**. Рукопис рада и сви прилози уз рад могу се доставити имејлом (*srparhiv@bvcom.net*), електронски преко система за пријављивање на интернет-страници часописа (*http://www.srpskiarhiv.rs*), препорученом пошиљком или лично, доласком у Уредништво. Уколико се рад шаље поштом или доноси у Уредништво, рукопис се доставља одштампан у три примерка и нарезан на *CD* (снимљени материјал треба да је истоветан оном на папиру).

**НАПОМЕНА.** Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доленаведене адресе и број телефона.

**АДРЕСА:**
Српско лекарско друштво
Уредништво часописа „Српски архив за целокупно лекарство“
Ул. краљице Наталије 1
11000 Београд
Србија

**ТЕЛЕФОН:** 011/409-2776
**E-MAIL:** *srparhiv@bvcom.net*
**ИНТЕРНЕТ АДРЕСА:** *http://www.srpskiarhiv.rs*

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When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., $^{99}$Tc, IL-6, $O_2$, $B_{12}$, CD8). If something is commonly written in italics, such as genes (e.g. *BRCA1*), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

**CLINICAL TRIALS.** Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

**ETHICAL APPROVAL.** Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

**CONFLICT OF INTEREST STATEMENT.** The manuscript must be accompanied by a disclosure statement from all authors (contained within the Submission Letter) declaring any potential interest or stating that the authors have no conflict of interest. For additional information on different types of conflict of interest, please see World Association of Medical Editors (WAME, *www.wame.org*) policy statement on conflict of interest.

**AUTHORSHIP.** All individuals listed as authors should be qualified for authorship. Every author should have participated sufficiently in writing the article in order to take responsibility for the whole article and results presented in the text. Authorship is based only on: crucial contribution to the article conception, obtaining of results or analysis and interpretation of results; design of manuscript or its critical review of significant intellectual value; final revision of the manuscript being prepared for publication.

The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

**TITLE PAGE.** The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed

by numbers; official name, place and country of the institution in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

**SUMMARY.** Along with the original article, preliminary and short communication, meta-analysis, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

**KEYWORDS.** Below the summary, 3 to 6 keywords or frases should be typed. The keywords need not repeat words in the title and should be relevant or descriptive. *Medical Subject Headings – MeSH* (*http://www.nlm.nih.gov/mesh*) are to be used for selection of the keywords.

**TRANSLATION INTO SERBIAN.** The third page of the manuscript should include: title of the paper in the Serbian language; each author's full name and family name (no titles), indexed by numbers; official name, place and country of the institution in which authors work. On the fourth page of the manuscript the summary (100–250 words) and keywords (3–6) should be typed, but this refers only to papers in which a summary and keywords are compulsory. The terms taken from foreign literature should be translated into comprehensible Serbian. All foreign words or syntagms that have a corresponding term in Serbian should be replaced by that term.

If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine", etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) – if any – should be translated into English as well.

**STRUCTURE OF THE MANUSCRIPT.** All section headings should be in capital letters using boldface. Original articles, meta-analyses and preliminary and short communications should have the following section headings: Introduction (objective is to be stated in the final paragraph of the Introduction), Methods, Results, Discussion, Conclusion, References. A review article includes: Introduction, corresponding section headings, Conclusion, References. The firstly named author of a meta-analysis or a review article should cite at least five auto-citations (as the author or co-author of the paper) of papers published in peer-reviewed journals. Co-authors, if any, should cite at least one auto-citation of papers also published in peer-reviewed journals. A case report should consist of: Introduction (objective is to be stated in the final paragraph of the Introduction), Case Report, Discussion, References. No names of patients, initials or numbers of medical records, particularly in illustrations, should be mentioned. Case reports cannot have more than five authors. Letters to the editor need to refer to papers published in the *Serbian Archives of Medicine* within previous six months; their form is to be comment, critique, or stating own experiences. Publication of articles unrelated to previously published papers will be permitted only when the journal's Editorial Office finds it beneficial.

All enclosures (tables, graphs, photographs, etc.) should be placed at the end of the manuscript, while in the body of the text a particular enclosure should only be mentioned and its preferred place indicated. The final arrangement (position) of the enclosures will depend on page layout.

**ABBREVIATIONS.** To be used only if appropriate, for very long names of chemical compounds, or as well-known abbreviations (standard abbreviations such as DNA, AIDS, HIV, ATP, etc.). Full meaning of each abbreviation should be indicated when it is first mentioned in the text unless it is a standard unit of measure. No abbreviations are allowed in the title. Abbreviations in the summary should be avoided, but if they have to be used, each of them should be explained when first mentioned in the text of the paper.

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