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Karl Landsteiner (June 14, 1868 – June 26, 1943), an Austrian-born American physician was awarded the Nobel Prize in 1930 for Physiology or Medicine for detecting the major blood groups and creating the ABO system of blood typing. This discovery became the basis of modern transfusiology. His birthday is celebrated around the world as the World Blood Donor Day. The event serves to thank voluntary unpaid blood donors for their life-saving gifts of blood and to raise awareness of the need for regular blood donations to ensure availability of blood and blood products for patients in need. The theme of this year's campaign is "Thank you for saving my life".
In this issue of the *Vojnosanitetski Pregled* the article by Jovanović Srzentić S et al. (p. 489–494) is dealing with the program of voluntary blood donation promotion among students of the University of Belgrade.

Karl Landsteiner (14. jun 1868 – 26. jun 1943) američki lekar austrijskog porekla, dobitnik je Nobelove nagrade za medicinu 1930. godine za otkriće glavnih krvnih grupa (ABO) i uspostavljanje sistema za klasifikaciju krvi, što je predstavljalo osnov za razvoj savremene transfuziologije. Njegov rođendan (14. jun) slavi se širom sveta kao Svetski dan davalaca krvi u znak zahvalnosti dobrovoljnim davaocima krvi, i sa ciljem podizanja svesti o potrebi dobrovoljnog davanja krvi kao načina za njeno obezbeđenje svima kojima je to potrebno. Tema ovogodišnje manifestacije jeste: „Hvala za spašavanje mog života!"
U ovom broju „Vojnosanitetskog pregleda", članak Jovanović Srzentić S. i sar. (str. 489–494) donosi rezultate istraživanja promocije programa dobrovoljnog davanja krvi među studentima Beogradskog univerziteta.

ORIGINAL ARTICLES

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Serbian lymphoma study group: demografic characteristics of 257 patients with follicular lymphoma

Srpska limfomska grupa: demografske karakteristike 257 ispitanika sa folikularnim limfomom

Olivera Simonović*, Lana Mačukanović-Golubović*†, Boško Andjelić‡, Darko Antić‡§, Biljana Mihaljević‡§

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Abstract

Background/Aim. Follicular lymphoma (FL) is a B-cell tumor usually with indolent clinical course, yet in some cases the course of the disease can be very aggressive. The aim of the research was to determine distribution of patients into prognostic groups based on the International Prognostic Index (IPI) and Folicular Lymphoma International Prognostic Index (FLIPI) criteria, as well as to determine the importance of classifying patients into the prognostic groups, since this could potentially have the influence on selection of the treatment modality. **Methods.** The retrospective study was performed on 257 patients with follicular lymphoma diagnosed between January 2000 and April 2011. **Results.** Based on the IPI score, 153 (59.53%) patients had low risk, 57 (22.18%) low intermediate risk, 15 (5.84%) high intermediate risk, 9 (3.50%) high risk, whereas the classification of 23 patients diagnosed with FL remained with unknown risk according to the IPI. Based on the FLIPI prognostic index, 113 (43.97%) patients had low risk, 70 (27.24%) intermediate risk and 51 (19.84%) high risk, whereas the classification of 23 (8.95%) patients remained unknown. On the basis of the FLIPI 2 prognostic index, 48 (18.68%) patients had low risk, 145 (56.42%) intermediate risk and 41 (15.95%) high risk. The classification into prognostic groups for 23 (8.95%) patients remained unknown. According to the IPI, FLIPI and FLIPI 2 there were the patients that required treatment in all the risk groups. **Conclusion.** The FLIPI and FLIPI 2 effectively identify patients at high risk, thus helping in treatment decision for each single patient.

Key words:
lymphoma, follicular; serbia; predictive value of tests; combined modality therapy.

Apstrakt

Uvod/Cilj. Folikularni limfom (FL), B-ćelijski tumor obično je indolentnog kliničkog toka bolesti, ali u nekim slučajevima tok bolesti može biti veoma agresivan. Cilj istraživanja bio je da ce utvrdi raspodela bolesnika u prognostičke grupe u odnosu na internacionalni prognostički indeks (IPI) i folikularni limfom internacionalni prognostični indeks (FLIPI) kriterijume, kao i da se odredi značaj klasifikovanja bolesnika u prognostičke grupe koje bi mogle potencijalno uticati na izbor modaliteta lečenja. **Metode.** Retrospektivno istraživanje izvedeno je na 257 bolesnika sa FL koji su dijagnostikovani od januara 2000. do aprila 2011. **Rezultati.** Na osnovu IPI prognostičkog indeksa, nizak rizik imalo je 153 (59,53%) bolesnika, srednje nizak rizik 57 (22,18%), srednje visok rizik 15 (5,84%), visoki rizik 9 (3,50%) bolesnika, a nepoznato je bilo svrstavanje 23 bolesnika sa dijagnozom folikularnog limfoma prema IPI. Na osnovu FLIPI prognostičkogi indeksa, nizak rizik imalo je 113 (43,97%), srednji 70 (27,24%), visoki rizik 51 (19,84%), a nepoznato je bilo svrstavanje 23 (8,95%) bolesnika. Na osnovu FLIPI 2 prognostičkog indeksa, nizak rizik imalo je 48 (18,68%), srednji 145 (56,42%), a visoki 41 (15,95%) bolesnika sa FL. Nepoznato je bilo svrstavanje 23 (8,95%) bolesnika. Prema IPI, FLIPI i FLIPI 2 bilo je bolesnika koji zahtevaju lečenje u svim prognostičkim grupama. **Zaključak.** FLIPI i FLIPI 2 efikasno grupišu bolesnike u grupu visokog rizika i pomažu pri odluci o lečenju za svakog pojedinačnog bolesnika.

Ključne reči:
limfom, folikularni; srbija; testovi, prognostička vrednost; lečenje, kombinovano.

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Introduction

Follicular lymphoma (FL) is an indolent B-cell tumor making 22% of all B-non-Hodgkin's lymphoma and is the second most common type of lymphoma in adults in the countries in the western hemisphere [1]. The annual incidence of FL has increased since 1950 until today from 2 to 3 in 100,000 patients to 5 to 7 in 100,000 patients [2]. The median age of patients at diagnosis is 60 years and the disease is slightly more common in women [3]. It is usually diagnosed in advanced stages, III/IV. Clinical course of FL varies from the cases of spontaneous remission (15–20%), over indolent clinical course with present response to therapy, relapse, and median survival of 9–10 years, to the aggressive clinical cases [4]. Bearing in mind that patients diagnosed with non-Hodgkin's lymphoma can have large variations in clinical presentation, molecular profiles and clinical outcome of the disease, the choice of therapy could be potentially influenced by numerous parameters [5]. The most important prognostic factors that could influence the choice of therapy are: sex, age, factors pointing to the staging of the disease, laboratory (erythrocyte sedimentation rate, the level of serum albumin, hemoglobin, lactate dehydrogenase (LDH), beta 2 microglobulin), pathological (based on correlation of histological grade and clinical outcome of the disease), cytogenetic and other factors [6]. The problem with the current prognostic indices (IPI, FLIPI, FLIPI 2) lies in a limited number of parameters, so that the choice of therapy would require the use of existing indexes together with other parameters [7].

Gene expression pattern in immune response 1 (IR-1) corresponds to the mixed expression of T lymphocytes and macrophages, while the expression patterns in IR-2 corresponds predominantly to macrophage expression with the elements of follicular dendritic cell expression [8]. Examination of the correlation of gene expression and disease outcome pointed to a more favorable course of the disease and significantly longer survival in patients with IR-1. In addition, the predictive value of this model was not in correlation with the IPI.

Application of gene expression profile with 81 genes contributed to the classification of 100% of patients in low-risk and high-risk groups [9].

By the application of the International Prognostic Index – IPI [age, Ann Arbor clinical staging, Eastern Cooperative Oncology Group (ECOG) performance status, serum LDH, extranodal involvement] we classified a very small number of patients into the poor prognostic group, about 10–15% of them, so it could not precisely determine the group of patients in who could be eligible for intensive chemotherapy [4].

The first specific index for follicular lymphoma, ILI, was proposed by the Italian group for investigating lymphoma (Italian Lymphoma Intergroup) in 2000. This index involves demographic, clinical and biochemical factors affecting the prognosis [7].

The follicular lymphoma international prognostic index (FLIPI) (age, Ann Arbor clinical staging, hemoglobin level, serum LDH, the number of involved lymph regions), proposed by the International Cooperative Group in 2004, classified 27% of patients in the high risk group.

A new prognostic index, the Follicular lymphoma international prognostic index 2 (FLIPI 2) was defined at the end of 2009 by the International Cooperative Group headed by Federico et al. [10], after immunochemotherapy had become the standard of care in patients with FL. It includes age, elevated β2 microglobulin (ß2M), diameter of the largest affected lymph node of more than 6 cm, bone marrow infiltration and hemoglobin level as the most important predictive factors for the outcome of the disease. On the basis of this index, 20% of the patients were classified in the high-risk group [9]. In the last few years, the development of immunohistochemistry and molecular techniques contributed to the identification of a large number of new, potentially powerful prognostic markers for all types of non-Hodgkin's lymphomas (NHL) [11].

The aim of this study was to determine the incidence of FL, prognostic groups with respect to the sum of points based on the IPI and FLIPI criteria and to determine the importance of classifying patients into prognostic groups, which could potentially influence selection of treatment modality.

Methods

The research was conducted on the basis of the database of the National Registry for Lymphoma LIRA and included the time period from 31 January 2000 to 25 April 2011.

Patients were classified into different risk groups, on the basis of age, prognostic indices IPI and FLIPI, the presence of unfavourable parameters that are not part of prognostic indices, such as voluminous tumor mass, biological parameters, such as Ki-67 protein, clinical characteristics of the disease and the risk of transformation of indolent into aggressive lymphoma.

The total number of patients diagnosed with FL during the above-mentioned time period was 257.

All the examined parameters were presented as frequencies and percentages.

χ^2 test was used for comparasion of frequencies.

Results

In the study group 151 (58.75%) patients were females and 106 (41.25%) males.

On the basis of the IPI score, most patients, 153 (59.53%) of them, were at low risk, statistically significantly more than the others (χ^2-test, $p < 0.001$), and the number in the other risk groups decreased with increase in the degree of risk.

In our research, the most prominent factor of the IPI was the clinical stage (CS) III or IV, 173 (67.32%) patients, which is statistically more prevalent than other IPI prognostic factors.

Figure 1 shows the number of patients with FL in risk groups on the basis of the FLIPI prognostic index. Most of them, 113 (43.97%) patients, were low-risk patients, statistically significantly more than the others (χ^2-test, $p < 0.001$) and the number of patients decreased in the other risk groups with increase in the degree of risk.

Clinical stage III or IV was again the most prominent factor of the FLIPI prognostic index in our research, and it was statistically more frequent in comparison to all the other FLIPI prognostic criteria.

Figure 2 shows the number of patients in risk groups on the basis of the FLIPI 2 prognostic index. Most patients, 145 (56.42%) of them, were at intermediate risk, statistically significantly more than in the other risk groups of patients (χ^2-test, $p < 0.001$).

Immunochemotherapy, immunotherapy, radiotherapy and the Watch and Wait strategy were used in treatment of all the stages of the disease.

Figure 3 shows the distribution of therapy on the basis of the IPI score. According to IPI, immunochemotherapy and chemotherapy were used in all the risk groups.

On the basis of the FLIPI score immunochemotherapy, chemotherapy and radiotherapy were used in treatment of all the risk groups.

On the basis of the FLIPI 2 score, immunochemotherapy, chemotherapy and radiotherapy were used in treatment of all the risk groups.

Discussion

Follicular lymphoma is a neoplasm built from follicular (germinal) center cells (centrocytes and centroblasts) which, at least in part, grow in follicular distribution [12].

It is formed by the transformation of germinal center B-

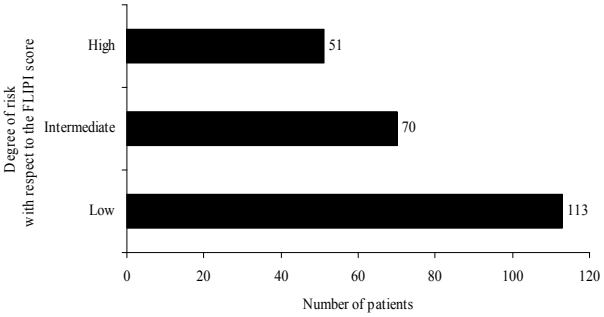

Fig. 1 – Patients with follicular lymphoma on the basis of the Follicular Lymphoma International Prognostic Index (FLIPI) score.

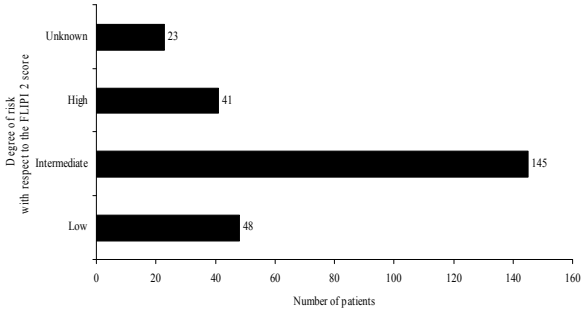

Fig. 2 – Patients with follicular lymphoma on the basis of the Follicular Lymphoma International Prognostic Index (FLIPI 2) score.

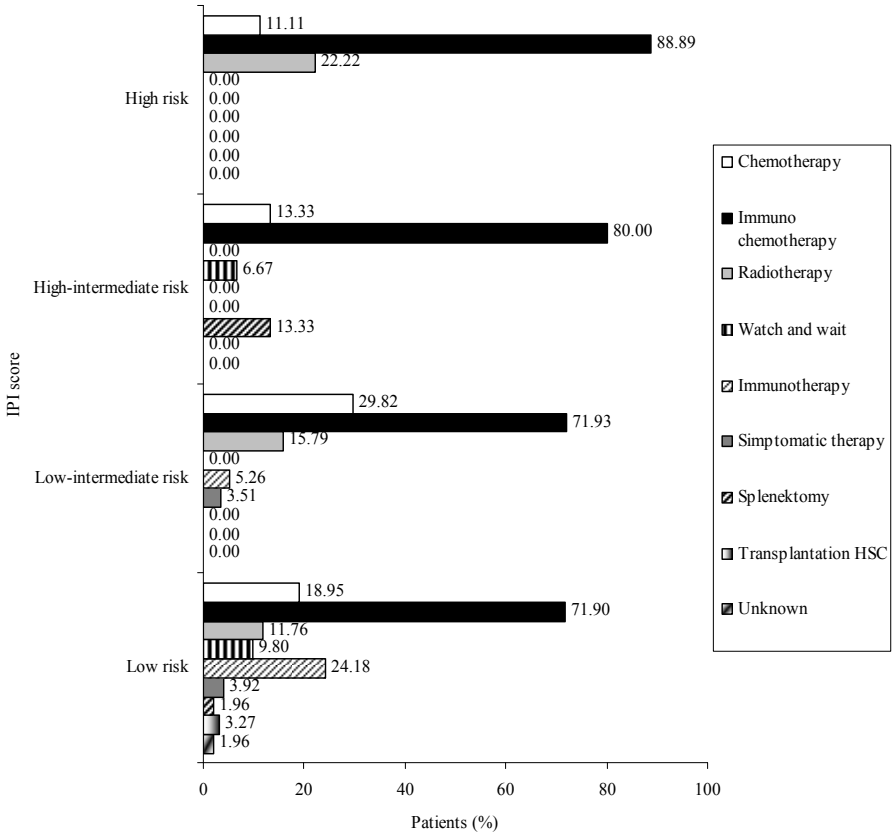

Fig. 3 – The distribution of therapy based on International Prognostic Index (IPI) score.

cells with the translocation t (14; 18) [13]. The hypothesis is that this early formed translocation causes difficulties in eradication of the neoplastic clone by chemotherapy because the IgH gene locus (14q32) gets replaced by proto-oncogene BCL2 (18q21).

The disease usually has indolent course, although relapse occurs in most patients after treatment [4]. The application of different therapeutic protocols starting in the 1980s and early 1990s did not have a significant impact on the survival of patients with FL [14]. The introduction of anti-CD20 monoclonal antibody significantly influenced the treatment of these patients [15]. The treatment could be potentially guided by prognostic indices such as the FLIPI and recently introduced FLIPI 2, what is one of the aims of ongoing clinical trials [16].

A study by Anderson et al. [17] showed a different distribution of certain types of non-Hodgkin lymphoma depending on geographic areas. The icidence of follicular lymphoma was higher in North America, London and Cape Town (31% in all cases) compared to other geographic areas (14%). The lowest icidence was recorded in Hong Kong, only 8% [17].

In our research, all the patients diagnosed with FL were, based on the IPI prognostic factors, divided into the following risk groups: 153 (59.53%) of the patients in the low-risk group, 57 (22.18%) in the low-intermediate risk group, 15 (5.84%) in the high-intermediate risk group, 9 (3.50%) patients were placed in the high-risk group, whereas the classification of 23 patients diagnosed with FL remained unknown. Solall-Céligny et al. [4] point out that, based on the IPI prognostic factors, 10–15% of patients could be classified as high-risk patients, which is a higher percentage than the one presented in our study.

The multicentre colaborative group designed the FLIPI prognostic index of follicular lymphoma registered in the period from 1985 and 1992 based on the database of 4167 registered patients. The FLIPI was able to classify patients older and younger than 60 years [4].

The testing of the FLIPI prognostic index was performed on 919 patients who were classified into low-risk, intermediate-risk and high-risk groups. On the basis of this index, 36% of the patients were placed in the low-risk group, 37% in the intermediate-risk group and 27% in the high-risk group. Thus, it was concluded that the FLIPI prognostic index classifies patients with aggressive FL in a better way compared to the IPI [4].

In our research, the patients with FL were, based on the FLIPI prognostic score, placed in the following risk groups: 113 (43.97%) of the patients were placed in the low-risk group, 70 (27.24%) in the intermediate-risk group, 51 (19.84%) in the high-risk group, whereas the classification of 23 (8.95%) of the patients remained unknown. This result indicates a lower percentage of high-risk patients as compared to the work by Solal-Céligny et al. [4] in which, based on the FLIPI prognostic factors, 27% of the patients belong to the high-risk group. As with the IPI prognostic index, most of the patients were placed in the low-risk group, 113 (43.97%), but unlike the IPI prognostic index, a higher percentage of patients, 51 (19.84%) were classified into the high-risk group, *versus* 9 (3.50%) of the patients according to the IPI [18].

Federico et al. [10] designed FLIPI 2 prognostic index. Different risk groups were made up: low-risk group, intermediate-risk group and high-risk group. Progression-free survival for the period of three years was 91%, 69% and 51%, respectively, depending on the risk group. The 3-year overall survival depending on prognostic was 99%, 96% and 84%, respectively.

In our research, bone marrow involvement was present in 83 (32.30%) of the patients with FL, which is a small percentage compared to the work of Federico et al. [19] who discuss about a larger proportion of bone marrow involvement in 52% of the patients and emphasize its association with poor survival. On the basis of the FLIPI 2, 48 (18.68%) of the patients were placed in the low-risk group, 145 (56.42%) in the intermediate-risk group and 41 (15.95%) of the patients with FL were placed in the high-risk group. Classification into prognostic groups remained unknown for 23 (8.95%) of the patients. Most of them, 145 (56.42%), were in the intermediate-risk group. Our results are in concordance with the work of Federico et al. [10] who proved that the application of the FLIPI 2 prognostic index contributed to the classification of 20% of the patients into the high-risk group.

The application of prognostic indices could be important for the timely treatment of patients diagnosed with follicular lymphoma and the proper selection of therapy [20]. Friedberg et al. [21] investigated the initial therapy in newly-diagnosed FL during the period from 2004 to 2007. In their work observation was performed in 17.7% of the patients; rituximab monotherapy in 13.9% of the patients; clinical trials in 6.1% patients; radiation therapy in 5.6% of the patients, only chemotherapy in 3.2% of the patients, chemotherapy with rituximab in 51.9% of the patients.

The literature shows that about 20% of patients with FL are characterized by localized disease with no bulky tumors. In case of these patients, application of radiotherapy as the target therapy contributes to a complete remission in 95% of them and to a 10-year survival in about 50% of them [22]. The probability of cure is very small and most patients relapse [22]. In our research, radiation therapy was administered in the treatment of 31 (12.06%) of the patients with FL, which is a smaller percentage compared to the previously mentioned literature data [22]. Radiation therapy was administered in the treatment of the patients with FL in all the prognostic groups based on the IPI, FLIPI and FLIPI 2 and the majority of the patients, 18 (11.76%) of them, which were treated with radiation therapy, were from the low-risk group based on the IPI prognostic index. The Watch and Wait strategy is applied if there is the lack of large tumor masses, the absence of B symptoms, tumor masses smaller than 7 cm in diameter, less than 3 nodules larger than 3 cm, no compressed organs or effusion, normal LDH and B2M. The Watch and Wait strategy was applied in the treatment of patients with follicular lymphoma belonging to the low-risk group and the high-intermediate group (IPI), the low-risk and the high-risk group (FLIPI), the low-risk and the intermediate-risk group (FLIPI 2), whereas the majority of patients, 15 (13.27%), were from the low-risk group based on the FLIPI index.

There is no universal first-line treatment for FL [20]. Until now, the treatment has included: alkylating agents (cyclophosphamide, chlorambucil), purine analogues (fludarabine), combination chemotherapy (CVP –cyclophoshamide, vincristine and prednisone, CHOP – cyclophosphamide, hydroxydaunorubicin (doxorubicin), Oncovin (vincristine) and prednisolone or FND – fludarabine, mitoxantrone, dexamethasone) and intensive chemotherapy with auto/allo transplantation of bone marrow or peripheral blood [21]. It is likely that early treatment with immunochemotherapy in individual patients encourages the delay of disease progression [15]. In our research, chemotherapy was applied in the treatment of the patients with FL in all the prognostic groups based on the IPI, FLIPI and FLIPI 2 indices, mostly in the patients belonging to the intermediate-risk group, 31 (21.38%), based on the FLIPI 2 prognostic index. Immunochemotherapy was most commonly used, and it was applied in 175 (68.09%) of the patients. Immunochemotherapy was applied in all prognostic groups of the IPI, FLIPI and FLIPI 2 index and the majority of patients, 110 (71.90%) were from the low-risk group based on the IPI index. Immunotherapy was applied in the low-risk and the low-intermediate risk group based on the IPI index, as well as in all the groups of other indices, the FLIPI and the FLIPI 2. Most of the patients, 37 (24.18%), were from the low-risk group based on the IPI index. Splenectomy was performed in 5 (1.95%) of the patients with FL. Hematopoietic stem cell transplantation was applied in 5 (1.95%) of the patients with FL. Coiffier [15] emphasizes that FLIPI prognostic factors do not identify patients with FL in whom the delay of treatment, the Watch and Wait strategy, stands for the best form of treatment. In addition, the aforementioned author points out that FLIPI prognostic index does not identify patients with poor prognosis who require intensive therapy [15]. These patients could have large tumor mass and high LDH values but belong to the low-risk group based on FLIPI. Therefore, some trials use the *Groupe d'Etude des Lymphomes Folliculaires* (GELF) criteria associated with large tumor mass (involvement of 3 or more regional lymph nodes, the presence of nodal or extranodal tumor mass greater than 7 cm, B symptomatology, splenomegaly, pleural effusion or ascites, cytopenia and leukemic phase, ECOG greater than 1, serum LDH or B2M above normal values) [23].

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Conclusion

The application of the IPI, FLIPI 1 and 2 contribute to the classification of a large number of patients in the clinical stage II–IV into risk groups, which potentially could be of help for treatment decision.

Immunochemotherapy was applied in all the clinical stages and all the prognostic groups

R E F E R E N C E S

1. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood 1997; 89(11): 3909−18.
2. *Dreyling M, Ghielmini M, Marcus R, Salles G, Vitolo U*. ESMO Guidelines Working Group. Newly diagnosed and relapsed follicular lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow up. Ann Oncol 2011; 22(Suppl 6): 59−63.
3. *Agarwal AM, Agarwal N, Glenn MJ, Lim MS*. Blastic Transformation of Low-Grade Follicular Lymphoma. J Clin Oncol 2007; 25(16): 2326−8.
4. *Solal-Céligny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez* R. Follicular Lymphoma International Prognostic Index. Blood 2004; 104(5): 1258−65.
5. *Sehn LH*. Optimal use of prognostic factors in non Hodgkin lymphoma. Hematology Am Soc Hematol Educ Program. 2006: 295−302.
6. *Federico M, Vitolo U, Zinzani PL, Chisesi T, Clò V, Bellesi G*, et al. Prognosis of follicular lymphoma: a predictive model based on a retrospective analysis of 987 cases. Blood 2000; 95(3): 783−9.
7. *Kersten MJ, Jong Dd, Raemaekers JM, Kluin PM, Hagenbeek A*. Beyond the International Prognostic Index: New prognostic factors in follicular lymphoma and diffuse large-cell lymphomaA meeting report of the Second International Lunenburg Lymphoma Workshop. Hematol J 2004; 5(3): 202−8.
8. *Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC*, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med 2004; 351(21): 2159−69.
9. *Glas AM, Kersten MJ, Delahaye LJ, Witteveen AT, Kibbelaar RE, Velds A*. Gene expression profiling in follicular lymphoma to assess clinical aggressiveness and to guide the choice of treatment. Blood 2005; 105(1): 301−7.
10. *Federico M, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, Vitolo U, et al.* Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. J Clin Oncol 2009; 27(27): 4555−62.
11. *Anđelić B, Mihaljević B*. Prognostic factors in follicular lymphoma. Acta Clin 2010; 10(3): 49−62.
12. *Katzenberger T, Ott G, Klein T, Kalla J, Muller-Hermelinek HK, Ott MM*. Cytogenetic alterations affecting bcl6 are predominantly found in follicular lymphomas grade 3B with a diffuse large B cell component. Am J Pathol 2004; 165(2): 481−90.
13. *Stamatopoulos K, Kosmas C, Belessi C, Stavroyianni N, Kyriazopoulos P, Papadaki T*. Molecular insights into the immunopathogenesis of follicular lymphoma. Immunol Today 2000; 21(6): 298−305.
14. *Gilles AS*. Clinical features, prognosis and treatment of follicular lymphoma. ASH Education Book 2007; 216−25.
15. *Coiffier B*. First line treatment of follicilar lymphoma in the era of monoclonal antibodies. Clin Adv Hematol Ocol 2005; 3(6): 484−91.

16. *Salles G, Seymour JF, Offner F, López-Guillermo A, Belada D, Xerri L*, et al. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet 2011; 377(9759): 42−51.
17. *Anderson JR, Armitage JO, Weisenburger DD*. Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. Ann Oncol 1998; 9(7): 717−20.
18. *Perea G, Altés A, Montoto S, López-Guillermo A, Domingo-Doménech E, Fernández-Sevilla A*. Prognostic indexes in follicular lymphoma: a comparison of different prognostic systems. Ann Oncol 2005; 16(9): 1508−13.
19. *Federico M, Vitolo U, Zinzani PL, Chisesi T, Clò V, Bellesi G*, et al. Prognosis of follicular lymphoma: a predictive model based on a retrospective analysis of 987 cases. Intergruppo Italiano Linfomi. Blood 2000; 95(3): 783−9.
20. *Horning SJ*. Natural history of and therapy for the indolent non-Hodgkin's lymphomas. Semin Oncol 1993; 20(5 Suppl 5): 75−88.
21. *Friedberg JW, Taylor MD, Cerhan JR, Flowers CR, Dillon H, Farber CM,* et al. Follicular lymphoma in the United States: first report on the national LymphoCare study. J Clin Oncol 2009; 27(8): 1202−8.
22. *Mac Manus MP, Hoppe RT*. Is radiotherapy curative for stage I and II low-grade follicular lymphoma? Results of a long-term follow-up study of patients treated at Stanford University. J Clin Oncol 1996; 14(4): 1282−90.
23. *Rosenbaum CA*. Evolving paradigms in follicular lymphoma: Re-evaluating prognostic factors and challenig treatments dogmas. Molec Oncol Rep 2007; 1(2):33-8.

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The development of the program of voluntary blood donation promotion in students population of the University of Belgrade

Razvoj programa promocije dobrovoljnog davanja krvi u studenstkoj populaciji Univerziteta u Beogradu

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Abstract

Background/Aim. Given that in each country students represent the most progressive population group, as of 2001, the Blood Transfusion Institute of Serbia (BTIS) has been carrying the program of voluntary blood donation promotion and education of volunteers at the University of Belgrade (UB). In 2011, the BTIS intensified all activities at the UB. The aim of this study was to present activities performed from 2001 at the Blood Donors` Motivation Department (DMD) of the BTIS related with increasing the level of awareness on voluntary blood donation in the Belgrade students` population, enhancing their motivation to become voluntary blood donors (VBDs), increasing the number of blood donations at faculties of the UB, and increasing the number of blood donations in the UB students population compared with the total number of blood units collected by BTIS in Belgrade, with the emphasis on the year 2013. **Methods.** Initially, the applied methodology was based on encouraging students to donate blood through discussions and preparatory lectures, followed by organized blood drives. Appropriate selection of volunteers at each faculty was crucial. Besides their recognisable identity, they had to have remarkable communication skills and ability to positivly affect persons in their environment. The applied principle was based on retention of volunteers all through the final academic year, with the inclusion of new volunteers each year and 1,000 preparatory lectures on the annual basis. The activities were realized using two Facebook profiles, SMS messages and continuous notification of the public through the media. **Results.** There was an increase in the average number of students in blood drives at the faculties from 2011, when the average number of the students *per* blood drive was 39, followed by 43 in 2012 and 46 in 2013. The number of students who donated blood in 2013 increased by 21.3% compared with 2012 data. **Conclusion.** The applied concept highly contributed to generation and retention of future VBDs willing to regularly donate blood in the coming years, with a minimum risk of transmission of transfusion transmissible diseases markers.

Key words:
blood donors; students; altruism; motivation.

Apstrakt

Uvod/Cilj. Imajući u vidu da studenti predstavljaju najprogresivniji deo populacije u svakoj zemlji, Institut za transfuziju krvi Srbije (ITKS) od 2001. realizuje program promocije dobrovoljnog davanja krvi i edukacije volontera na fakultetima u Beogradu. Tokom 2011, ITKS je intenzivirao aktivnosti na Univerzitetu u Beogradu (UB). Cilj ovog rada bio je prikazivanje aktivnosti sprovedenih od 2001. u Odseku za motivaciju dobrovoljnih davalaca krvi, ITKS, koje se odnose na podizanje nivoa svesti o dobrovoljnom davanju krvi u populaciji studenata u Beogradu, motivisanje studenata da postanu dobrovoljni davaoci krvi, povećanje broja davanja krvi na fakultetima UB i povećanje broja davanja krvi u grupi studenata davalaca krvi u odnosu na ukupan broj jedinica krvi koje je ITKS prikupio u Beogradu, sa naglaskom na rezultate u 2013. **Metode.** Primenjena metodologija prvobitno je bila zasnovana na ohrabrivanju studenata da daju krv kroz diskusije i pripremna predavanja, posle kojih su organizovane akcije davanja krvi. Adekvatna selekcija volontera na fakultetima bila je od suštinskog značaja. Pored prepoznatljivog identiteta, trebalo je da poseduju izražene komunikacione veštine i sposobnost da pozitivno utiču na okruženje. Primenjeni princip zasnivao se na zadržavanju volontera do završetka studija, uključivanju novih volontera svake godine i 1 000 pripremnih predavanja godišnje. Aktivnosti su realizovane putem dva fejsbuk profila, SMS poruka i stalnog informisanja javnosti posredstvom medija. **Rezultati.** Povećanje prosečnog broja studenata u akcijama davanja krvi uočava se od 2011. kada je prosečan broj studenata u tim akcijama na fakultetima iznosio 39, zatim 43 u 2012. i 46 u 2013. Broj studenata koji su dali krv 2013. povećan je za 21,3 % u poređenju sa podacima iz 2012. **Zaključak.** Primenjeni program rada značajno je doprineo formiranju budućih dobrovoljnih davalaca koji će redovno davati krv tokom niza godina, a nosi mali rizik od prenošenja markera transfuzijom prenosivih bolesti.

Ključne reči:
krv, davaoci; studenti; altruizam; motivacija.

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Introduction

Supply of sufficient quantities of safe blood mostly depends on blood donors who donate blood on a regular basis throughout the years and who bear minimum risk of having transfusion-transmissible diseases markers [1–3] in particular in activities to create and keep regular the population of donors.

Since university students are considered to be the most progressive population group in each country, ever since 2001, Blood Transfusion Institute of Serbia (BTIS) has initiated realization of the program of blood donation promotion and education of volunteers in this particular field at the faculties of the University of Belgrade (UB).

Up to 2001 blood donation among the population of Belgrade students was supported by various benefits exceeding the usual small token of gratitude, occasionally affecting the autonomy of the University. Allowed absence from two to three gym classes for each blood donation was contrary to the promotion of healthy life style, including only the first year students who had mandatory sports classes in blood drives. Allowing additional exam terms to blood donors was an act of discrimination for students not eligible to donate blood due to various health conditions. A low number of blood donations regarding the number of the first year students compared with the older, senior students, as well as a small number of volunteers coming from Belgrade, encouraged initiation of some novel activities [4].

The aim of this study was to present a series of activities realized since 2001 at the Blood Donors Motivation Department (DMD) of the BTIS in the domain of increasing the degree of notification regarding voluntary blood donation in the population of Belgrade students, increasing the motivation of students to become voluntary blood donors, increasing the number of blood donations in the population of Belgrade students and increasing the number of blood donations among students – blood donors in relation to the total number of blood units collected by the BTIS in the city of Belgrade, with the emphasis on the year 2013.

Methods

The activities directed towards the development of blood donation culture in the population of Belgrade students implied intensified existing approaches to the work with the population of students` initiated in 2001 [5–7] along with the introduction of new elements of activities related with promotion and education [4]. The preliminary activities to implement new elements in promotion and education were realized from 2011 to 2012. The starting methodology consisted of stimulating the audience to donate blood through conversation and organization of blood drive introductory lectures, as well as the actual realization of blood drives at the faculties in Belgrade. A basic precondition for those blood drives was appropriate selection of volunteers at each faculty who, apart from recognisable identity, had to have remarkable communication skills and the ability to positively affect people [6, 7]. The activities combined principles of retention of volunteers all through their final academic year, along with the inclusion of new students each successive year. The establishment of the volunteers` network depended on the size of the faculty, i.e. the number of academic years and study groups, as well as the number of students. Well organized volunteers` network covering each study group on each academic year guarantees the successful work in the domain of blood donation [4, 6, 7].

A somewhat lower degree of the Belgrade students` involvement in voluntary activities initiated a novel methodological approach based on conducting a poll among graduate students at high schools [6]. Questions listed in the interview were created by the BTIS DMD staff, as the result of experience in work with blood donors and the published results [4–7]. Participation in the poll was voluntary and it was carried in 12 Belgrade high schools during the 2011/2012 academic year, within the regular blood drives performed in the first and in the second semester.

The first poll was conducted in the first semester, entitled "Tell us how you felt, evaluate our work and help us improve", within which the interviewed students provided their opinion on the very act of blood donation and their motives that encouraged them to become blood donors, as well as their evaluation of the preparatory lecture and their impression regarding the actual blood drive. Multiple choice questions referred to the very act of blood donation, offering possible answers to how they felt before, during and after blood donation. In regard to their motives for becoming blood donors, the 8 most frequent answers derived from the previous experience were offered, with the possibility of marking one or more offered answers and option to add personal view. The elements of preparatory lectures given by the associates employed at the BTIS DMD which precede every blood drive at high schools, such as the level of education, was the lecture interesting and to what extent, how influential it was regarding decision to become blood donor and the actual preparation of the blood donation procedure were rated from 1 to 5. Besides, blood drives performed at schools were also rated from 1 to 5 regarding the general atmosphere and the feeling of satisfaction which depended on the mobile medical team staff. Additional questions referred to the decision to become repeated blood donors, as well as to their attitude towards blood donation that would exclude two days absence from school following each blood donation, otherwise customary at all secondary schools in Serbia [4].

In the second semester, the second interview was conducted. Basically, it was an application poll for their future voluntary work at faculties [4]. Participants of this poll were the students whose previous polls carried in the first semester were considered valid. The questions referred to the decision to continue to donate blood in future, willingness to be blood donation promotion volunteers at the faculties, information about the faculty they intend to enrol, personal data and contacts (telephone and E-mail address).

Statistical methods were applied in polls reviewing according to the frequency determination in relation to the total number of interviewed subjects.

Continuous education of the UB students, organized by the BTIS and the Red Cross of Serbia in the form of annual

seminars taken over from the previous practice, was continued because of the remarkable results in the education of students volunteers, their readiness for peer education and organization of blood drives at the faculties [6]. Direct education at the faculties within the major study subjects in each academic year and all the study groups was realized by the associates employed at the BTIS DMD [4, 7, 8]. The primary intention of this form of education is the preparation for the first ever blood donation and support to the peer education realized by students volunteers [4–7].

Within the promotion activities and in the course of blood donation drives at the faculties we strongly encouraged students` creativity in the preparation and realization of blood drives (presentations in the faculty halls, remarks and evaluation boards), motivation of faculty teachers, assistants and other faculty staff members to donate blood, developing distinctive uniforms to be worn during blood drive, preparation of a small token of gratitude for colleagues who donated blood – badges and origami, thank you notes and acknowledgements, creation of interesting messages and verses, providing a mascot, making pleasant atmosphere during blood drive, appropriate music and light refreshments, inclusion and engagement of deans and the members of scientific teachers` boards in the activities of promotion and organisation of blood drives and providing support of various students` organisations [4, 6].

The BTIS has its regularly updated web page www.nbti.org.rs and two Facebook profiles *dobrovoljni davaoci krvi srbije* and *udruženje studenata dobrovoljnih davalaca krvi*. Information regarding blood drives are forwarded *via* SMS.

Results

The number of realized blood drives at Belgrade faculties ranged from 54 in 2001 to 85 in 2011. The number of blood donations by the students at Belgrade faculties blood drives ranged from 1,232 in 2001 to 3,386 in 2006, after which the number gradually decreased. From 2011, increasing tendency was notable at the faculties in Belgrade, reaching 3,684 in 2013. The average number of students *per* blood drive varied from 22.8 in 2001 to 55.5 in 2006. The following gradual decrease recorded from 2006 reached the lowest value in 2010 with the average number of 34.0 students *per* blood drive. Intensified activities initiated in 2011 resulted in the increased average number of students participating in blood drives, and in 2013, that the number was 46,1. The number of students who donated blood in blood drives outside their faculties ranged from 124 in 2001 to 4,181 in 2008, followed by the decrease reaching the lowest value of 3,017 in 2011. Activities applied in the motivation for blood donation led to the increased number of this number starting from 2011. The tendency of increase continued also in 2013 when the number of students who donated blood at their faculties was by 23.1% higher than the number recorded in 2012 (Table 1).

The results of interviewed high school students poll demonstrate the following: before blood donation, 58.5% high school students felt relaxed, 30% had jitters, and 11.5% was little scared; during blood donation, 92% felt quite well, while 8% felt anxious/uncomfortable; basic motives for donating blood for the first time ever, with the possibility of marking several offered answers, were the altruistic reasons (131 answers), feeling of social responsibility (167 responses) and the influence of the community and family tradition (165 answers), evaluation regarding the importance of the introductory lecture and proper preparation for the first blood donation was marked with 4.25; evaluation regarding new information, interesting lecture that influenced making decision to donate blood varied from 3.3 to 3.7, which was in accordance with the level of education of high school students, but also pointed to the personal maturity when making this decision; the most encouraging was the fact that even 96% of high school students wanted to donate blood again and did not consider two days off from school as a motivating factor.

Out of the total of 138 high school students interviewed in the first poll, 50 (36.2%) students, within the second poll carried in the second term of the academic 2011/2012 year, applied for volunteer work at the faculties they intended to enrol. All of them were contacted after the faculty enrollment, and, with best wishes for future studies, invited to continue the cooperation initiated at high school. Following the interview and introduction and meeting with the senior volunteers at their faculties, 10 freshmen volunteers from 8 different faculties were included into the volunteer network.

Improved cooperation of the BTIS with the Belgrade faculties resulted in new activities, such as 7 video spots directed by the students of the Drama Art Faculty, Department of Film and TV Directing, dedicated primarily to their colleagues from other faculties. As a result of the cooperation between the BTIS and the marketing agency GREY, a campaign was realized. The slogan was: `We all do bad things sometimes, now, let`s do something good! Save life, give blood!`. At the prestigious international festival Golden Hammer, the campaign won the silver medal in the category of printed material. In the past three years, a significant participation of media was noted in their assistance to announce blood drives, as well as in the follow up of activities of the BTIS and the Red Cross in regard to the promotion of voluntary blood donation. In particular, the Public National TV and Radio Broadcasting Service, Radio TV Serbia, showed a significant level of social responsibility having started to broadcast a telop announcing the daily plans of BTIS mobile teams, i.e. specially designed audio and visibly recognisable graphics before the prime time news, providing frequent live appearances and interviews, videos covering stories from blood drives presented in the most popular TV shows and news broadcasts, and broadcasting of promotion videos. The same support has also been provided by the RTV Studio B, the most popular Belgrade public service, as well as all other national, cable and local televisions all over Serbia. More intensified cooperation has been established with the national news agencies Tanjug, Beta, as well as with numerous print media, daily and weekly papers. In mobile teams in Belgrade as well as in the BTIS, in 2013 blood was donated by 7,685 students,

21.3% more than in the same period in 2012 (Table 1). The participation of students in the total number of blood donations in Belgrade ranged from 3.2% in 2001 to the highest level of 16% in 2008 after which the number gradually felt (Table 1). In 2013 the participation of students in the total number of blood donations in Belgrade was 16.3%, or 2,2% more than in the previous year (Table 1).

Table 1

Comparative survey of the results achieved in the population of students and students participation in the total number of blood donations in Belgrade from 2001 to 2013

Year	No. of blood drives at faculties	No. of blood donations by students at faculties	Average no. of voluntary students in blood drives at faculties	No. of blood donations by students outside faculties	Total no. of voluntary students	No. of blood donations in Belgrade	Participation of students in total no. of blood donations in Belgrade (%)
2001	54	1232	22.8	124	1356	42205	3.2
2002	61	1532	25.1	327	1859	46253	4.0
2003	62	2728	44.0	696	3424	44202	7.7
2004	62	2941	47.4	1047	3988	44247	9.0
2005	61	3151	51.7	2228	5379	43252	12.4
2006	61	3386	55.5	2120	5506	42281	13.0
2007	72	2967	41.2	4079	7046	44751	15.7
2008	68	2834	41.7	4181	7015	43940	16.0
2009	69	2576	37.3	3519	6095	42773	14.2
2010	81	2755	34.0	3138	5893	42206	14.0
2011	85	3298	38.8	3017	6315	43941	14.4
2012	76	3261	42.9	3077	6338	45063	14.1
2013	80	3684	46.1	4001	7685	47200	16.3

Discussion

The published data show that each year 24 million patients in the USA and around three million in Great Britain are treated with blood transfusion, which is over 10% of all hospitalized patients in those two countries [9].

Understanding the motives which inspire citizens to donate blood enable the staff in the blood transfusion service to help blood donors to create positive attitude towards blood donation and to encourage their determination to maintain regular blood donors [9]. Percentage of citizens who actively donate blood or blood components is relatively low in relation to the total population, so that blood transfusion employees depend on the small number of volunteers who provide sufficient quantities of blood required to satisfy the needs of a health care system [1–2].

Reality in the BTIS as well as in many other blood transfusion services is characterised by constant efforts to maintain the balance between the needs articulated by clinicians and to provide blood and blood components supply that would satisfy the actual needs for blood [9].

In order to better understand behaviour of blood donors' it is necessary to create a reliable system for analysis of the profile of blood donors', which would contribute to the understanding of the term "donor`s career". Investigations show that factors affecting people`s decision to donate blood for the first time can be completely different from the factors influencing all consecutive blood donations throughout the future donor`s career [10–12]. There have been discussions dealing with the qualitative difference between the groups of donors established on the basis of the number of blood donations [11, 12]. Persons who make decision to continue to donate blood gradually create their own identity of a blood donor by remaining active and repeating to donate blood [1, 11, 12]. Callero and Pilliavin [10] believe that after three to four blood donations donors overcome the psychological barrier and start to consider themselves as regular donors. The first three to four blood donations are necessary for making the habit of blood donation. Recent investigations show that the higher the number of blood donations the stronger the decision of persons to maintain the blood donor`s status, whereas the risk of quitting the career of a blood donor considerably decreases [11].

Studying behaviour and motivation of various groups of donors on the number of blood donations is the source of valuable information. Likewise, investigation of the frequency of blood donation can also be very useful in activities for motivation and preserving blood donors. A person with 20 blood donations in the course of five years requires different treatment from the person with the same number of blood units in a 20-year time interval [12]. A European Project dealing with blood donation entitled ′Donor Management in Europe′, known in its abbreviated form as DOMAINE, defined a new classification of blood donors using, as starting points, a total number of blood donations, different forms of donors` behaviour and frequency of blood donation [4]. This newly defined set of definitions is based on the previous definitions founded in the European Union, however, it is more detailed and as such more convenient for the use of blood donors data base [4, 9, 13].

Active donors are those who in the previous year donated blood at least once. They are divided to those who donated blood for the first time, regular donors and relapsing donors. Returning donors are those who donated blood at least two times, they donated blood only once in the previous 12 months, with the interval between that blood donation and the previous one which exceeds 24 months. Lapsing donors are

those who donated blood in the previous year, but were inactive donors within the previous 24 months. Inactive donors are persons who donated blood, but who did not donate blood in the past two years [4, 10, 13].

The use of up to date principles contributed to the improvement in the work of the BTIS staff with the students` population. The stated data point to the fact that the blood donation culture in the students` population is on a rather high level and that blood donation is not only related with their faculties as blood donation sites. Students voluntarily donated blood in organized blood drives at their faculties, in students` dorms, abut also in bloodmobile located at the Republic Square, another bloodmobile and blood drives in local communities on various locations in Belgrade, as well as in the BTIS and their places of residence during holidays. A lower response of high school students to polls carried near the end of the final school year can be explained by their focus on filling the donors` questionnaire before the act of blood donation and the excitement during and after the actual blood donation procedure, when their priority was exchange of experience and the lack of concentration needed to fill in the poll questions. That is why the number of valid polls were smaller than the number of applied students.

Analysis of the achieved results in the population of students from 2001 till nowadays points to several significant facts. The increased number of blood drives at faculties and the existing resources show that the optimal number during the year should be 75 to 80, as was the case in 2013. It should be pointed to the fact that the implementation of the Bologna Declaration for the European Higher Education at the faculties in Belgrade considerably increased the time needed for attending the classes and reduced breaks, thus providing shorter intervals for blood donation at faculties, resulting in a lower response of students in one period. The number of blood donations in organized blood drives at faculties from 2007 to 2011 was below 3,000 per year. As of 2011, intensified activities led to the increase of the number of blood donations in the same circumstances. In the organized blood drives in 2013, even 3,684 students donated blood, which has been the highest recorded number from 2001, and by 8.8% higher than with the highest number achieved and recorded in 2006. The activities applied in 2011 also influenced the increase of the number of blood donations by students outside their faculties. The number of those donations ranged from 124 in 2001 to 4181 in 2008. A significant increase recorded in 2007 and 2008 was the result of the campaign "Make yourself and others a beautiful day, give blood!" realized during the summer of 2007 with the RTV B92. This campaign contributed to the general trend of the blood donation number increase, also within the population of students outside their own faculties. The activities applied from 2011 contributed to the increase of the number of blood donations by students in Belgrade outside their own faculties in 2013 in the total amount of 4,001, i.e. 30.0% more than in 2012. Considering the presented results, the intensive growing tendency is obvious, and in 2013, a total of 7,685 blood donations in the population of students was recorded, thus making that year the most successful one from 2001. A total of 7,685 blood donations in the population of students in 2013 makes up for 16.3% of 47,200 blood donations in Belgrade in that year (Table 1).

The population of healthy, active and receptive student is an enormous potential corpus of blood donors who meet safe blood requirements. However, there is a paucity of studies on awareness and attitude of health science students on voluntary blood donation. A paper by Sabu et al. [14] deals with the knowledge, attitude and practice on blood givinig among students of health science in the University Campus in South India. The objective of this study was to determine the knowledge and attitude towards blood donation of health science students. The highest knowledge level was found among allied health science students (53.1%) and the lowest among pharmacy students (20.7%). This study elicits the importance of adopting effective measures in the University Campuses to motivate students for voluntary blood donation.

Blood donation in the Kingdom of Saudi Arabia remains largely involuntary (50–60%), where relatives, colleagues, and friends of patients provide a considerable portion of required donations, while the rest is donated voluntarily [15]. There is an urgent need to move towards an entirely voluntary donor system. This was an observational study based on a cross-sectional design of different colleges in the King Saud University (KSU). Students were recruited at random from 6 colleges: Medicine, Dentistry, Applied Medical Sciences (Science), Engineering, and Arts. This selection was based on the assumption of presumed medical and scientific knowledge of students. The total number from each college was 600 students: 300 donors and 300 non-donors. The two questionnaires were prepared: one for donors and another one for non-donors. The first part of the questionnaires was related to the general knowledge about blood and blood donation. The second part was related to the factors that encouraged donors to donate blood and non-donors not to donate. The third part concerned incentives provided in return for donating blood and whether it would encourage non-donors to donate and donors to continue donating. The study findings highlight the importance of investing awareness and motivational campaigns in university students and youth in general that aim to correct misconceptions about blood donation and address issues that will increase donor flow. In particular, the provision of tokens or gifts and allowing donor teams to operate in proximity to workplaces and residences should be addressed. The overwhelming positive attitude exhibited by both donors and non-donors is a source of optimism, indicating that the move by blood banks towards a donor system that depends wholly on voluntary, non-remunerated donors is attainable. Finally, establishing a good communication system is significant for developing long-lasting relationships with blood donors [16].

Altruism is mentioned as the most frequent motive for blood donation in all subjects. Some studies show that altruistic motivation can have varying importance in a donor`s career, especially in young donors [8, 9].

Conclusion

Better understanding of blood donors` behaviour enables direction of activities towards the retention of donors`, and taking steps for encouraging new donors. The activities performed in the Blood Transfusion Institute of Serbia in order to increase the level of information availability to the

students, education of the population students` on voluntary blood donation and increasing their motivation to become blood donors, resulted in the increased number of blood donations in the population of the Belgrade University students, as well as to the increase of the number of blood donations by the students regarding the total number of blood donations given by all the other citizens of Belgrade in 2013. The applied concept of work will highly contribute to the creation of future generations of blood donors guided by the students, future leaders in all the significant aspects of social life. The acquired experience will be applied in the next period along with the continuous follow-up of the designed and realized results of work.

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R E F E R E N C E S

1. *Weidmann C, Kluter H.* Blood collection and donor motivation in Germany. In Vox Sanguines ISBT Science Series. 23rd Regional Congress of the ISBT; 2013 June 2-5; Amsterdam, the Netherlands. Wiley-Blackwell; 2013. 8: p 238-41.
2. *Bednall TC, Bove LL.* Donating blood: a meta-analytic review of self reported-reported motivators and deterrents. Transf Med Rev 2011; 25(4): 317−34.
3. *Vučetić D.* Selection of blood and haemoproducts donors. In: *Balint B*, editor. Transfusion Medicine. Belgrade: Zavod za udžbenike i nastavna sredstva; 2004. p.223−40. (Serbian)
4. *Kort de W, Veldhuizen I.* Donor base. In: *Kort de W*, editor. Donor Management Manual. Nijmegen, Netherlands: DOMAINE project; 2010. p.56−80.
5. *Belić B.* Blood Donor Guide. 2nd ed. Novi Sad: Assembly of the Atonomous Province of Vojvodina; 2013.
6. *Georgijev Milošević A, Jocić D, Rodić I, Knežević M.* Motivation of students blood donors at the Faculty of Pharmacy, University of Belgrade. Bilt Transfuziol 2013; 59(12): 66−71.
7. *Belić B.* Become a blood donor. Voluntary Blood Donors Manual. Novi Sad: Assembly of the Atonomous Province of Vojvodina; 2008.
8. *Goodnough LT, Levy HJ, Murphy M.* Concepts of blood transfusion in adults. Lancet 2013; 381(9880): 1845−54.
9. *Veidhuizen IJ.* Blood donor profiling using donation patterns.In Vox Sanguinis ISBT Science Series. 23rd Regional Congress of the ISBT; 2013 June 2-5; Amsterdam, Netherlands: Wiley-Blackwell; 2013; 8: p. 233−37.
10. *Callero PL, Pilliavin JA.* Developing a Commitment to Blood Donation: the Impact of Ones First Experience. J Appl Soc Psychol 1983; 13: 1−16.
11. *Pilliavin JA.* Why do they give the gift of life? A review of research on blood donors since 1977. Transfusion 1990; 30(5): 444−59.
12. *Pilliavin JA, Callero PL.* Giving blood: The development of an altruistic identity. Baltimore: John Hopkins University Press; 1991.
13. *Council of the European Union.* Council Recommendation of 29 June 1998 on the suitability of blood and plasma donors and the screening of donated blood in the European Community. Offic J Eur Comm 1998; L 203: pp.14−26.
14. *Sabu KM, Remya A, Binu VS, Vivek R.* Knowledge, Attitude and Practice on Blood Donation among Health Science Students in a University Campus, South India. Online J Health Allied Scs 2011;10(2): 6. Available from: http://www.ojhas.org/issue38/2011-2-6.htm.
15. *Abdullah KA, Aban SB, Abdullah AA, Mohammed SA, Mohannad KA, Tariq AA*, et al. Attitude to Blood Donation among Male Students at King Saud University. J Appl Hematol 2013; 4(2): 70−7.
16. *Gader AM, Al Momen AK, Osman A, Al-Hori I.* Blood donor potential in Saudi Arabia. The "War and "Peace" experience. Transf Today 2003; 54: 4−6.

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Patients' expectation and satisfaction with complete denture before and after the therapy

Očekivanje i zadovoljstvo pacijenata totalnom zubnom protezom pre i posle terapije

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Abstract

Background/Aim. Difficulties in the acceptance of dentures are multifactorial including psychosocial ones. It is questionable whether the patients' satisfaction depends only on the complete denture therapy characteristics. The aim of the study was to evaluated patients' expectation and satisfaction with complete dentures before and after the treatment concerning phonetics, chewing, comfort of use and aesthetics. **Methods.** Forty complete edentulous patients rated their expectation before and satisfaction after the treatment based on a questionnaire scores. Patient-related variables regarding age, gender and previous experience (whether worn complete denture or not) were also recorded. **Results.** Patients' rating for expectations were higher than the satisfaction after treatment regarding phonetics, chewing, comfort of use and aesthetics. A negative significant correlation was shown between the items before and after the treatment rating for phonetics, chewing, comfort of use and aesthetics. No statistical correlation was found between all the evaluated aspects' (i.e. phonetics, chewing, comfort of use and aesthetics) of expectation and satisfaction, and age, gender, and previous experience except a weak negative correlation noticed between age and comfort of denture use. **Conclusion.** Patients' expectations ratings significantly exceeded their satisfactions. Expectations and satisfaction ratings were irrespective of gender and patients previous experience.

Key words:
denture, complete; patient satisfaction; questionnaires; mastication; speech production measurement; esthetic, dental.

Apstrakt

Uvod/Cilj. Teškoće u prihvatanju zubne proteze su multifaktorijalne, a uključuju i psihosocijalne faktore. Pitanje je da li zadovoljstvo pacijenata protezom zavisi samo od karakteristika terapije kompletnom zubnom protezom. Cilj rada bio je da se procene očekivanja i zadovoljstvo pacijenata kompletnom zubnom protezom pre i posle lečenja, u pogledu izgovora glasova, žvakanja, osećaja komfora i estetike. **Metode.** Pomoću upitnika, ukupno 40 bezubih pacijenata ocenilo je svoja očekivanja pre lečenja i zadovoljstvo posle lečenja. Varijable povezane sa pacijentom, u pogledu životnog doba, pola i prethodnog iskustva (da li nosi ili ne nosi kompletnu protezu), takođe su zabeležene. **Rezultati.** Ocene pacijenata za očekivanja bile su više nego za zadovoljstvo posle lečenja u pogledu izgovora glasova, žvakanja, komfora pri korišćenju proteze i estetike. Negativno značajna korelacija nađena je između ocena pre i posle lečenja, za fonetiku, žvakanje, komfor pri korišćenju i estetiku. Nije nađena statistički značajna korelacija između svih procenjivanih aspekata (kao što su izgovor glasova, žvakanja, komfor pri korišćenju i estetika) očekivanja i zadovoljstva i životnog doba, pola i prethodnog iskustva, izuzev slabo negativne korelacije utvrđene između životnog doba i komfora pri korišćenju proteza. **Zaključak.** Procena očekivanja pacijenata značajno je prevazišla njihova zadovoljstva. Stepen očekivanja i zadovoljstva nisu bili u korelaciji sa životnim dobom i prethodnim iskustvom pacijenata.

Ključne reči:
zubna proteza, totalna; bolesnik, zadovoljstvo; upitnici; žvakanje; govor, produkcija, merenje; zub, estetika.

Introduction

Despite the decline in edentulism rates documented by Marcus et al. [1], due to ageing of the society, it is estimated that the number of edentulous patients will not decrease. Complete dentures are a favoured treatment option for edentulous patients. It deserves to be noted that the complete dentures are commonly accepted as they provide a pleasing appearance and maintain normal speech, as well as supply occlusal support and adequate means for mastication of food. Additionally, these dentures should be comfortable and should generally make a patient satisfied. Previous studies indicate that the great majority of completely edentulous patients are satisfied with their complete dentures [2, 3], while great emphasis is placed on the pa-

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tients who remain dissatisfied despite the clinical perfection of their oral rehabilitation, as patients' satisfaction with their dentures seems to be associated with their quality of life [3–6].

Difficulties in the acceptance of dentures are multifactorial, therefore, the risk of the dissatisfaction should be considered. Satisfaction with complete dentures has been associated with several factors as confirmed by several studies [7]. Among these factors, general health, age, gender, personality traits, experiences with previous dentures and patient expectation regarding treatment were evaluated in previous studies [8–14]. Although some studies found associations between those factors and complete denture satisfaction [10, 11], some did not [8, 9, 12]. Furthermore, some studies [14–16], albeit not all [17–19], have revealed that patient satisfaction is unrelated to denture quality and to sophisticated techniques for the treatment [4].

There remains a widespread acknowledgement that psychological factors may influence the outcome of denture treatments. For instance, some authors [15, 19] have found that psychological factors may play an important role in those patients who experience difficulty in adapting to new dentures. Furthermore, the majority of studies have evaluated the expectation as strongly associated with patients' satisfaction after treatment, yet not all the samples are observed [20].

The aim of the study was to evaluated patients' expectation and satisfaction with complete dentures before and after the treatment concerning phonetics, chewing, comfort of use and aesthetics.

Methods

The sample consisted of 40 individuals (22 women and 18 men), generally healthy, who attended the China Medical University and went through the completely new dentures therapy from February 2012 to December 2013. The selected patients were all completely toothless, in good health (to avoid the influence of their medical problems on their satisfaction with dentures), enjoying adequate cognitive ability for understanding and capacity to answer questions and to complete a form. The patients who met the eligibility criteria mentioned above received a written informed consent to participate. The study was approved by the China Medical University Ethical Review Board.

The dentures were made by graduate students under the supervision of professors, using a standardized technique compromising the following procedure: complete initial clinical examination; making of preliminary impression using a stock tray and irreversible hydrocolloid (alginate); and final impression using custom trays and light-bodied silicone impression material, after border moulding. The denture bases were polymerised with heat-cured acrylic resin (Vipi Cril Plus) at 72°C for 12 h. Occlusal wax rims were made over the denture bases and adjusted as necessary. The bases were mounted in centric relation at a predetermined occlusal vertical dimension in semi-adjustable articulators. The artificial acrylic resin teeth were arranged in balanced occlusion and tried in, and the dentures were flasked and polymerized (72°C/12 h) and then inserted and adjusted.

Assessment of patient expectation before and satisfaction after the therapy

To rate expectations before and satisfactions after the therapy, a Patient Denture Rating Questionnaire, covering four items relating to denture esthetics, speech, mastication and comfort, was used. The answers for each item could range from 0 (the worst result) to 10 (the best result). All the questions were explained to the patients, as to enable them to understand meanings of each question. All the patients were asked to choose the numbers according to their expectation. After completing the treatment (a week after the insertion), the patients were again asked to choose the numbers according to their satisfaction.

A copy of the self-completed denture rating questionnaire is presented in Table 1.

Assessment of patient-related variables

Patient-related variables including gender, age and previous experience (whether worn complete dentures or not) were noted.

Statistical analysis

The statistical Package for the Social Sciences (SPSS version16.0, SPSS Inc., IBM) was used for statistical analysis. The Wilcoxon Signed Rank test was used to compare the patients' expectation before and satisfaction after the treat-

Table 1

The questionnaire sent to all the patients. The questionnaire was proposed at the same patients before and a week after the treatment. Answers to each question range from 0 (worst possible outcome) to 10 (best possible outcome)

Questions	On this scale of 0–10, how would you score the following aspects?
How do you rate the appearance of your denture?	0 1 2 3 4 5 6 7 8 9 10
How do you rate the quality of expression and phonetics?	0 1 2 3 4 5 6 7 8 9 10
How do you rate the quality of your mastication?	0 1 2 3 4 5 6 7 8 9 10
How do you rate the removal and insertion of your denture?	0 1 2 3 4 5 6 7 8 9 10
How comfortable is your denture?	0 1 2 3 4 5 6 7 8 9 10

ment, while the Spearman's correlation test was used to determine whether correlations existed between the patients' expectation before and satisfaction after the treatment and to check for possible correlations between age and the abovementioned scores. The Mann-Whitney test was used to test associations between the scores and gender, as well as to check possible associations between the scores and the previous experience. A *p*-value less than 0.05 were accepted as a statistically significant.

Results

Of the 40 participants who completed all stages and filled in the questionnaires, 56% were female.

The results on correlations between the investigated parameters before and after the treatment a given in Table 2. A negative correlation was found between age and comfort of use expectation ($p = 0.0041$, -44.41%). No correlation was found between phonetics, chewing and aesthetics, respectively to age ($p = 0.608$, 8.63%; $p = 0.651$, 7.39%; $p = 0.517$, 10.55%). ($p = 0.0017$, -48.16%), comfort of use ($p = 0.0054$, -43.19%) and aesthetics ($p = 0.001$, -50.01%).

Discussion

The subjects in this study had higher expectations regarding complete denture treatment compared to satisfaction for phonetics, chewing, comfort of use and aesthetics. Our results corroborate the findings of de Siqueira et al. [12], while other studies [9, 12] contradict our findings. A possible explanation for the divergence is that the satisfaction with complete denture is a complex phenomenon [7, 18] which could be fulfilled by previous comprehensive awareness of anatomical features and, particularly, by an insight into patients' physiological and psychological capacities [21–23].

Furthermore a negative significant correlation was determined between expectation and satisfaction for all the evaluated criteria (i.e. phonetics, chewing, comfort of use and aesthetics). As edentulous patients generally expect that new complete dentures fit and function equal to or even better than their

Table2

The results on correlations between the investigated parameters before (expectation) and after the treatment (satisfaction)

Parameter	Phonetics		Chewing		Comfort		Aesthetics	
	before	after	before	after	before	after	before	after
Age	0.608 (8.63%)	0.572 (9.22%)	0.651 (7.39%)	0.079 (28.06%)	0.0041* (-44.41%)	0.076 (28.37%)	0.517 (10.55%)	0.917 (1.70%)
Gender	0.625	0.593	0.638	0.325	0.947	0.882	0.795	0.195
Previous experience,	0.196	0.231	0.333	0.472	0.573	0.611	0.195	0.818

***A statistically significant difference (Spearman's correlation test and Mann-Whitney test).**

No significant association was found between the four aspects of expectations, and gender ($p = 0.625$, $p = 0.638$, $p = 0.947$, $p = 0.795$), and previous experience ($p = 0.196$, $p = 0.333$, $p = 0.573$, $p = 0.195$). The same holds true for the association between the four aspects of satisfactions, and gender ($p = 0.593$, $p = 0.325$, $p = 0.882$, $p = 0.195$), age ($p = 0.572$, 9.22%; $p = 0.079$, 28.06%, $p = 0.076$, 28.37%; $p = 0.917$, 1.70%), and previous experience ($p = 0.231$, $p = 0.472$, $p = 0.611$, $p = 0.818$).

The mean scores for the four aspects before and after the therapy are given in Table 3. The analysis showed a significantly decreased in the mean scores on phonetics ($p = 0.000136$), chewing ($p = 0.000007$), comfort of use ($p = 0.000002$) and aesthetics ($p = 0.000006$). Moreover, there was a negative significant correlation between before and after treatment rating for phonetics ($p = 0.026$, -35.14%), chewing natural teeth, despite the presence of resorbed ridges, collapsed muscles and other physical changes that occurred. Considering patients' baseline physical condition regarding harmony, smile appearance, denture comfort and masticatory and phonic ability, the new dentures provide an adequate solution from dentist's point of view. However, patients do not take into account their own baseline considerations. Sensation of foreign object, nausea, phonetic problems and difficulty in chewing, are among common complaints of edentulous patients during the first few days after insertion of their complete dentures. Since complete dentures do not generally match the patients' expectations, patients are no longer willing to wear their dentures, developing a feeling of mistrust toward their dentist and his/her treatment plan and demand that dentists make numerous and sometimes technically unnecessary adjustments. However, patients should understand that compromises may be necessary.

Table 3

Comparison of expectation ratings before and satisfaction ratings after, presented as means ± standard deviations

Parameter	Before	After	*p*-value
Phonetics	9.53 ± 0.85	7.85 ± 1.72	0.000136*
Chewing	9.28 ± 0.88	7.53 ± 1.15	0.000007*
Comfort	9.6 ± 0.67	6.75 ± 2.12	0.000002*
Aesthetics	9.34 ± 0.87	7.58 ± 1.08	0.000006*

***A statistically significant difference (Wilcoxon test).**

Dentists ought to be fully aware of patients' expectations before treatment and provide a patient with detailed introduction into the problem, which not aims to explain the limitations and possibilities of complete denture treatment *per se*, but rather to help a patient to learn how to cope with the complete dentures. Therefore, the rate of satisfaction may be raised.

Other variables (previous experience, age and gender) were also tested to assess patients' expectations before and satisfaction after the therapy. However, the majority of these possible associations proved not to be statistically significant in this sample, with the exception of a weak negative correlation between age and comfort of use. These results are incongruent with previous studies [24, 25], but suggested by other studies [20, 23].

The limitations of the present study involve a relatively small number of patients, which limits possible evaluation of causal relationship among the evaluated variables and some other factors, such as denture quality, personality traits and patient/professional relationship [12, 13, 23].

Conclusion

Patients' expectations ratings significantly exceeded their satisfactions. Expectations and satisfaction ratings were irrespective of gender and previous experience.

Hence, pondering the necessity of gaining a deeper comprehensive of patients' psychosomatic phenomena, it seems that more extensive, clinically and patient-based research should be carried out to gain more knowledge about patients' expectations and final evaluation after complete denture treatment.

R E F E R E N C E S

1. *Marcus PA, Joshi A, Jones JA, Morgano SM.* Complete edentulism and denture use for elders in New England. J Prosthet Dent 1996; 76(3): 260−6.
2. *Carlsson GE.* Facts and fallacies: an evidence base for complete dentures. Dent Update 2006; 33(3:) 134−6, 138−40, 142.
3. *Yoshida M, Sato Y, Akagawa Y, Hiasa K.* Correlation between quality of life and denture satisfaction in elderly complete denture wearers. Int J Prosthodont 2001; 14(1): 77−80.
4. *Ellis JS, Pelekis ND, Thomason JM.* Conventional rehabilitation of edentulous patients: the impact on oral health-related quality of life and patient satisfaction. J Prosthodont 2007; 16(1): 37−42.
5. *Carlsson GE, Omar R.* The future of complete dentures in oral rehabilitation: A critical review. J Oral Rehabil 2010; 37(2): 143−56.
6. *Brković-Popović S, Poštić S, Ilić D.* Satisfaction were also high in ptients who kept their remained teeth, specifically prepared for overdentue. Stom Glas Srb 2011; 58(2): 90−6. (Serbian)
7. *Kovac Z, Troskot Z, Uhac I, Cabov T, Lajnert V, Pavicić DK,* et al. Multivariate analysis of different factors affecting the patient general satisfaction with complete dentures. Coll Antropol 2012; 36(3): 791−4.
8. *Marachlioglou CR, Dos Santos JF, Cunha VP, Marchini L.* Expectations and final evaluation of complete dentures by patients, dentist and dental technician. J Oral Rehabil 2010; 37(7): 518−24.
9. *Bellini D, Dos Santos MB, De Paula Prisco da Cunha V, Marchini L.* Patients' expectations and satisfaction of complete denture therapy and correlation with locus of control. J Oral Rehabil 2009; 36(9): 682−6.
10. *Baracat LF, Teixeira AM, dos Santos MB, da Cunha VD, Marchini L.* Patients' expectations before and evaluation after dental implant therapy. Clin Implant Dent Relat Res 2011; 13(2): 141−5.
11. *de Lima EA, dos Santos MB, Marchini L.* Patients' expectations of and satisfaction with implant-supported fixed partial dentures and single crowns. Int J Prosthodont 2012; 25(5): 484−90.
12. *de Siqueira GP, dos Santos MB, dos Santos JF, Marchini L.* Patients' expectation and satisfaction with removable dental prosthesis therapy and correlation with patients' evaluation of the dentists. Acta Odontol Scand 2013; 71(1): 210−4.
13. *Fenlon MR, Sherriff M, Newton JT.* The influence of personality on patients' satisfaction with existing and new complete dentures. J Dent 2007; 35(9): 744−8.
14. *Gaspar MG, Dos Santos MB, Dos Santos JF, Marchini L.* Correlation of previous experience, patient expectation and the number of post-delivery adjustments of complete dentures with patient satisfaction in a Brazilian population. J Oral Rehabil 2013; 40(8): 590−4.
15. *Wolff A, Gadre A, Begleiter A, Moskona D, Cardash H.* Correlation between patient satisfaction with complete dentures and denture quality, oral condition, and flow rate of submandibular/sublingual salivary glands. Int J Prosthodont 2003; 16(1): 45−8.
16. *Fenlon MR, Sherriff M.* Investigation of new complete denture quality and patients' satisfaction with and use of dentures after two years. J Dent 2004; 32(4): 327−33.
17. *Celebic A, Knezovic-Zlataric D, Papic M, Carek V, Baucic I, Stipetic J.* et al. Factors related to patient satisfaction with complete denture therapy. J Gerontol A Biol Sci Med Sci 2003; 58(10): M948−53.
18. *van Waas MA.* The influence of clinical variables on patients' satisfaction with complete dentures. J Prosthet Dent 1990; 63(3): 307−10.
19. *al Quran F, Clifford T, Cooper C, Lamey PJ.* Influence of psychological factors on the acceptance of complete dentures. Gerodontology 2001; 18(1): 35−40.
20. *Jonkman RE, van Waas MA, van Hof MA, Kalk W.* An analysis of satisfaction with complete immediate (over)dentures. J Dent 1997; 25(2): 107−11.
21. *Bolender CL, Swoope CC, Smith DE.* The Cornell Medical Index as a prognostic aid for complete denture patients. J Prosthet Dent 1969; 22(1): 20−9.
22. *Freeman HL.* Quantifying quality. Neuro Endocrinol Lett 1999; 20(5): 263.
23. *Yamaga E, Sato Y, Minakuchi S.* A structural equation model relating oral condition, denture quality, chewing ability, satisfaction, and oral health-related quality of life in complete denture wearers. J Dent 2013; 41(8): 710−7.
24. *Siadat H, Alikhasi M, Mirfazaelian A, Geramipanah F, Zaery F.* Patient satisfaction with implant-retained mandibular overdentures: a retrospective study. Clin Implant Dent Relat Res 2008; 10(2): 93−8.
25. *Pan S, Awad M, Thomason J, Dufresne E, Kobayashi T, Kimoto S,* et al. Sex differences in denture satisfaction. J Dent 2008; 36(5): 301−8.

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Surgical site infection surveillance in orthopedic patients in the Military Medical Academy, Belgrade

Nadzor infekcije operativnog mesta kod ortopedskih bolesnika u Vojnomedicinskoj akademiji, Beograd

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Abstract

Background/Aim. Active surveillance is an important component of surgical site infection (SSI) reduction strat-egy. The aim of this study was to analyze and compare SSI surveillance data in orthopedic patients in the Military Medical Academy (MMA), Belgrade. **Methods.** A 4-year prospective cohort study was performed to identify the in-cidence rate and risk factors for SSI in orthopedic patients in the MMA, Belgrade. We collected data regarding pa-tients characteristics, health care and micro-organisms iso-lated in SSI. The National Nosocomial Infection Surveil-lance (NNIS) risk index was subsequently calculated for each patient. The Centers for Disease Control and Preven-tion criteria were used for the diagnosis of SSI. **Results.** Assessment of 3,867 patients after different orthopedic operations revealed SSI in 109 patients. The overall incidence rate of SSI was 2.8% with the decrease from 4.6% in 2007 to 1.6% in 2010. Using NNIS risk index for surgical procedures there were: 53.7% (2,077) patients with risk 0 – the incidence rate of 1.4%; 38.9% (1,506) patients with risk 1 – the incidence rate of 3.1%; 7.3% (281) patients with risk 2 – the incidence rate of 11.7%; 0.1% (3) patients with risk 3 – without infection within the risk. Multivariate logistic regression analysis identified 6 independent risk factors associated with SSI: contaminated or dirty wounds, smoking, preoperative infection, NNIS risk index, body mass index and the length of hospital stay. **Conclusion.** The results of our study are valuable confirmation of relations between risk factors and SSI in orthopedic patients. A decreasing incidence rate of SSI (from 4.6% to 1.6%) during a 4-year active surveillance approved its implementation as an important component of SSI reduction strategy.

Key words:
orthopedic procedures; surgical wound infection; risk factors; serbia.

Apstrakt

Uvod/Cilj. Aktivno praćenje važan je deo strategije sni-ženja učestalosti infekcije operativnog mesta (IOM). Cilj rada bio je analiza i poređenje podataka dobijenih praće-njem ortopedskih bolesnika u Vojnomedicinskoj akademiji (VMA), Beograd. **Metode.** U cilju utvrđivanja stope učestalosti IOM kod ortopedskih bolesnika u VMA, kao i faktora rizika od nastanka infekcije, sprovedeno je 4-godišnje prospektivno, kohortno istraživanje. Prikupljeni su podaci o bolesnicima, o pruženim medicinskim uslugama, kao i o uzročnicima IOM. Za svakog bolesnika određen je indeks rizika od nastanka IOM Nacionalnog sistema nadzora nad bolničkim infekcijama u SAD-a (NNIS). U cilju dijagnoze IOM upotrebljeni su kriteriju-mi Centra za prevenciju i kontrolu bolesti (CDC) u SAD-u. **Rezultati.** Istraživanjem je obuhvaćeno 3 867 bolesnika nakon različitih ortopedskih operacija, od kojih je 109 imalo IOM. Ukupna stopa učestalosti IOM iznosila je 2,8%, sa tendencijom sniženja od 4,6% u 2007. godini do 1,6% u 2010. Određivanjem indeksa rizika (NNIS) došlo se do sledećih rezultata: kod 53,7% (2 077) boles-nika sa rizikom 0 – stopa učestalosti bila je 1,4%; kod 38,9% (1 506) bolesnika sa rizikom 1 – stopa učestalosti bila je 3,1%; kod 7,3% (281) bolesnika sa rizikom 2 – stopa učestalosti bila je 11,7%; kod 0,1% (3) bolesnika sa rizikom 3 – nisu registrovane IOM. Multivarijantnom logističkom regresionom analizom identifikovano je šest nezavisnih faktora rizika povezanih sa nastankom IOM: kontaminirana ili prljava operativna mesta, pušenje duvana, preoperativna infekcija, NNIS indeks rizika, indeks telesne mase i dužina hospitalizacije. **Zaključak.** Podaci dobijeni našim istraživanjem značajni su za dokazivanje odnosa između faktora rizika i nastanka IOM kod ortopedskih bolesnika. Sniženje stope učestalosti IOM od 4,6% do 1,6% tokom 4 godine aktivnog praćenja dokazuje da njegova primena predstavlja važan deo strategije sniženja učestalosti IOM.

Ključne reči:
ortopedske procedure; rana, hirurška, infekcija; faktori rizika; srbija.

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Introduction

Surgical site infections (SSIs) continue to be a significant problem in surgical patients across the globe [1–3]. The impact of these infections can be devastating for patients as well as incurring additional hospital costs. Orthopedic SSIs have substantially greater physical limitations and significant reductions in their quality of life, prolong total hospital stays and increase healthcare costs by more than 300% [4].

Active surveillance is a cornerstone of orthopedic SSI detection and SSI rates accurate calculation within an institution. Orthopedic SSI surveillance is integral to hospital infection control and quality improvement programs, with feedback of SSI rates being an important component of SSI reduction strategies in different healthcare systems [5–7].

The aim of this study was to analyze and compare surveillance data from a large cohort of orthopedic patients of the University Clinic in Serbia during a 4-year period.

Methods

Setting

The Military Medical Academy (MMA), Belgrade, Serbia, a teaching hospital of University of Defense, is a 1,200-bed tertiary healthcare center. The Clinic for Orthopedic Surgery and Traumatology is a 72-bed department of MMA. The Department of Infection Control performs continuous surveillance on all surgical patients of MMA [3].

Study population

The personnel for infection control collected data related to patients [age, gender, tobacco use, body mass index (BMI), the presence of underlying diabetes mellitus], data related to health care [length of hospital stay (LHS), preoperative LHS, preoperative preparing, preoperative infection, immunosuppressive treatment, antibiotic prophylaxis, drainage, duration of drainage, central vascular catheter, urinary catheter].

The National Nosocomial Infections Surveillance System (NNIS) risk index was subsequently calculated on the basis of data relating to the operation: wound contamination class, duration of surgery, and the American Society of Anesthesiologists (ASA) score [8, 9]. The National Research Council operative site classification was used. It classifies surgical wounds as clean, clean/contaminated, contaminated, and dirty/infected [10]. The NNIS index ranges from 0 to 3. Each of the three risk indices is worth 1 point: contaminated or dirty surgical wound, ASA score greater than 2, and the duration of surgery greater than the 75th percentile for a specific group of surgical procedures [11].

For the diagnosis of SSIs, the Centers for Disease Control and Prevention (CDC) criteria were used [12]. SSIs were classified as superficial incisional, deep incisional or organ/space in consultation with orthopedic surgeon.

Only the first episode of SSIs was included for patients who had more than one SSI during the study period. The cumulative incidence of SSIs or the rate of SSIs (%) was based on SSIs detected during hospital stay combined with SSIs identified on readmission following the initial operation. No post-discharge surveillance was performed.

Study design

A prospective cohort study was performed to identify incidence rate and risk factors (RFs) for SSIs from January 1, 2007 to December 31, 2010.

Microbiological testing was performed at the Institute of Medical Microbiology at the MMA. Isolates were identified by routine methods [13].

The incidence rate was defined as the number of SSIs *per* 100 operative procedures.

Statistical analysis of data was done using the SPSS software package (SPSS, Chicago, IL, USA, version 11.00). The results are expressed as the mean ± SD or as the proportion of the total number of patients. In all studies, testing for significant differences was conducted by χ^2 test for categorical variables and Student's *t*-test for continuous variables. The factors were considered to be significant at a p value of ≤ 0.05. All p values were two-tailed. RFs independently associated with infections were identified by multivariate logistic regression analysis of variables selected by univariate analysis with a limit for entering and removing variables of 0.05.

Results

From January 1, 2007 to December 31, 2010, a total of 3,867 different orthopedic operative procedures were evaluated (Table 1). Among these, 109 were complicated by SSI. The overall cumulative incidence rate was 2.8%, with a decrease from 4.6% in 2007 to 1.6% in 2010 (Table 2). There were 30 (27.5%) superficial, 45 (41.3%) deep incisional, and 34 (31.2%) organ-spaces infections.

Table1

Surgical site infection (SSI) rates*, by operative procedures and risk index category

Operative procedure category	Risk index category			Cumulative rate (%)
	0	1	2,3	
	n, rate (%)	n, rate (%)	n, rate (%)	
Limb amputation	26 (11.5)	87 (5.7)	84 (15.5)	10.7
Open fracture	2 (0)	29 (0)	10 (0)	0
Reduction of long bone fracture	295 (2.7)	128 (3.9)	18 (22.2)	3.9
Repair of neck of femur	213 (0.9)	319 (1.6)	2 (50)	1.5
Hip prosthesis	1021 (0.6)	574 (1.9)	60 (6.7)	1.3
Knee prosthesis	173 (2.9)	145 (1.4)	46 (2.2)	2.2
Other musculoskeletal procedures	347 (1.4)	224 (8.5)	64 (15.6)	5.4

Table 2

Surgical site infection (SSI) rates* by operative procedures and annual distribution Per 100 operative procedures

Operative procedure category	2007 n, rate (%)	2008 n, rate (%)	2009 n, rate (%)	2010 n, rate (%)
Limb amputation	45 (15.6)	51 (11.8)	44 (9.1)	57 (7.0)
Open fracture	8 (0)	13 (0)	4 (0)	16 (0)
Reduction of long bone fracture	86 (4.7)	109 (8.3)	126 (2.4)	120 (0.8)
Repair of the neck of the femur	73 (2.7)	140 (1.4)	148 (0.7)	173 (1.7)
Hip prosthesis	306 (1.0)	504 (2.0)	347 (1.2)	498 (0.8)
Knee prosthesis	79 (2.5)	78 (2.6)	79 (1.3)	128 (2.3)
Other musculoskeletal procedures	141 (11.3)	125 (6.4)	206 (3.4)	163 (1.8)
Total	738 (4.6)	1020 (3.6)	954 (2.1)	1155 (1.6)

***Per* 100 operative procedures; n – numbers of operative procedures.**

Using the NNIS risk index, there were: 53.7% (2,077 surgical procedures) with risk 0 and the incidence rate of 1.4%; 38.9% (1,506) with risk 1 and the incidence rate of 3.1%; 7.3% (281) with risk 2 and the incidence rate 11.7%; 0.1% (3) with risk 3 and no infection within this risk.Table 2 shows the number of procedures and cumulative incidence rates by the NNIS risk index for the period February 2007 to 31 December 2010.

The mean age of patients was 64.10 years (range 10 to 97, median 69.00 years). There were 56.2% females and 43.8% males.

The median LHS was 6.00 (mean + SE = 7.39 + 0.13) days. The patients with SSI had 9.58 days of preoperative LHS, and without SSI 7.33 days of preoperative LHS. The median LHS was 14 days (mean + SE = 16.27 + 0.16), ranging from 3 to 116 days.

The characteristics of the patients and SSI related RF according to univariate analysis are shown in Table 3. Comparison of the patients with and without SSIs revealed significant differences. Univariate analysis showed that the occurrence of SSIs was significantly associated with the following categories: diabetes mellitus, smoking, BMI, ASA score, preoperative LHS, length of stay in hospital, preoperative showering, preoperative infection, immunosuppressive treatment, drainage of the surgical site, duration of drainage, contaminated and dirty/infected wound, central vascular catheter and NNIS risk index. Gender, Intensive Care Unit (ICU) stays, antibiotic prophylaxis, urinary catheter, preoperative shaving, and age were found not to be associated with SSI.

Multivariate logistic regression analysis identified six independant RFs associated with SSI occuring in these patients (Table 4).

Table 3

Potential risk factors for the development of surgical site infection (SSI) (univariate analysis)

Variable	Patients with SSI n = 109	Patients without SSI n = 3758	*p*-value	RR (95 CI %)
Patients characteristic				
diabetes mellitus, n (%)	28 (25.7)	517 (13.8)	0.001	2.167 (1.396–3.363)
smoking, n (%)	31 (28.4)	490 (13.0)	0.000	2.651 (1.730– 4.062)
BMI (kg^2/m^2), $\bar{x} \pm SD$	26.17 ± 4.0	27.18 ± 4.6	0.009	1.060 (1.014–1.108)
ASA > 2, n (%)	47 (43.1)	1214 (32.3)	0.019	1.589 (1.081–2.335)
Related to health care				
preoperative length of stay (days), $\bar{x} \pm SD$	7.33 ± 8.1	9.58 ± 12.2	0.007	1.020 (1.005–1.034)
length of stay in hospital (days), $\bar{x} \pm SD$	15.95 ± .000	27.4 ± 16.1	0.000	1.057 (1.045–1.069)
preoperative showering, n (%)	92 (84.4)	3487 (92.8)	0.001	.421 (.247– .761)
preoperative infection, n (%)	10 (9.2)	85 (2.3)	0.000	4.365 (2.200–8.659)
immunosuppressive treatment, n (%)	9 (8.8)	155 (4.1)	0.039	2.092 (1.038–4.216)
drainage, n (%)	29 (26.6)	368 (9.8)	0.000	3.339 (2.154–5.176)
duration drainage (days), $\bar{x} \pm SD$	0.30 ± 1.1	1.05 ± 2.2	0.000	1.279 (1.173–1.395)
contaminated and dirty/infected wound, n (%)	54 (49.5)	407 (10.8)	0.000	8.084 (5.477–11.931)
central vascular catheter, n (%)	6 (5.5)	43 (1.1)	0.000	5.033 (2.095–12.089)
NNIS risk index, n (%)			0.000	6.067 (3.954–9.309)
0	29 (26.61)	2048 (54.5)		
1	47 (43.12)	1459 (38.82)		
2	33 (30.27)	248 (6.6)		
3	0 (0)	3 (0.08)		

BMI – body mass index; ASA – American Society of Anesthesiologists; NNIS – National Nosocomial Infections System; RR – relative risk; CI – confidence interval; n (%) – numbers of patients (percentage); $\bar{x} \pm SD$ – mean ± standard deviation.

Table 4

Independent predictors of surgical site infection (SSI) by stepwise multivariate logistic regression

Variable	RR	95% CI	S.E.	*p*-value
Contaminated and dirty/infected wound	3.753	2.170–6.492	0.280	0.000
Smoking	2.576	1.593–4.164	0.245	0.000
Preoperative infection	2.512	1.129–5.588	0.408	0.024
NNIS risk index	2.141	1.069–4.289	0.354	0.032
Body mass index	1.066	1.017–1.118	0.024	0.008
Length of hospital stay	1.056	1.041–1.070	0.007	0.000

RR – relative risk; CI – confidence intervals; S.E. – standard error; NNIS – National Nosoconial Infecting System.

Microorganisms were isolated in 70 (64.2%) SSIs of the 109 recorded SSIs. Of these, one species was isolated from 58 SSIs, 2 from 10 SSIs, and 3 from 2 SSIs. *Staphylococcus aureus* (*S. aureus*) was most frequently isolated microorganisam 33/109 (45.7%) of laboratory confirmed SSIs (32 SSIs) of which 43.7% (18/32) were methicillin-resistant (MRSA). Next was *Acinetobacter* spp. (9 SSIs or 12.9% laboratory confirmed SSIs) of which 22.2% isolates were resistanat to carbapenems. This species followed by *Enterococcus* spp. (8 or 11.4% of laboratory confirmed SSIs) without registred resistance to vancomycin (VRE), *Klebsiella* spp. (7 SSIs or 10.0% of laboratory confirmed SSIs, of which 85.7% were the 3rd generation cephalosporin-resistant), and *Pseudomonas aeruginosa* (7 SSIs or 10.0% of laboratory confirmed SSIs of which 81.5% were resistant to fluoroquinolones, and 28.6% to carbapenems).

Discussion

The reduction in SSI incidence to a minimal level can produce great benefits for the patients and would economize resources.

In 2006 SSI surveillance has become an integral part of current hospital infection control programs in the MMA. This study provides important information about the incidence rate of orthopedic operative procedures, the orthopedic patient characteristics, RFs related to health care and microorganisms isolated from SSIs in a large group of patients admitted to the Clinic for Orthopedic Surgery and Traumatology during the study period.

The incidence rate of SSI (2.8%) found in the present study was similar to figures reported by the authors from developing countries [14]. The effectiveness of SSI surveillance in orthopedics has been demonstrated in different studies [5, 15, 16]. In our study the rate was 4.6% in the first year, which decreased to 1.6% in the fourth surveillance year.

Comparing our data with data from the systems of other countries, we found diferences in the infection rate for some procedures. Operative procedures for open fracture were not complicated with SSIs and data from this category should be interpreted with caution due to a small number of procedures (Table 1).

Also, we found that the incidence rate was much higher than that reported in the US and England for limb amputation [7, 17]. Limb amputation surgery showed decreases in SSI incidence from 15.6% in 2007 to 7.0% in 2010. That is similar to English analysis which showed consistent decreases in the inpatient SSI incidence [7].

Reduction in long bone fracture and repair of the neck of the femur were operative procedures which showed a decreasing SSI rate. Data from hospitals in England in 2011/12, showed year-on-year decreases in the incidence of SSIs for same operations, too [7].

SSIs were more common in our hospital than in hospitals in Scotland, England, and US after hip and knee protheses [7, 17, 18].

In our patients SSIs were more common in the category of other musculosceletal procedures than in the US and Brazil [14, 17], but we registered the trend of decreases in the SSI incidence from 11.3% in 2007 to 1.8% in 2010.

A number of factors may explain this difference, including different helthcare systems, type of hospital, practices, patient mix, length of stay etc.

Of 109 SSIs, 30 (27.5%) were superficial infections, 45 (41.3%) deep infections and 34 (31.2%) organ-spaces infections. In the US, some hospitals with a high volume of surgery perform surveillance only for deep incisional and organ/space infections, which termed the complex SSIs [19]. In our study complex infections were 72.5% of SSIs. Such infections require rehospitalization, revision surgery and intravenous antibiotic therapy.

An increased risk for infection in the presence of predisposing factors is of particular concern for orthopedic surgeons. The multivariate method used in our analysis allowed factors to be identified which had both a significant and an independent association with SSIs after orthopedic surgery: contaminated and dirty/infected wound, smoking, preoperative infection, NNIS risk index, body mass index, length of hospital stay (Table 4). The results of our multivariate analysis showed that the risk of SSIs increased consistently across all surgical categories where the wound class was contaminated/dirty. Although contaminated and dirty/infected wound is non-modifiable RF for SSIs, it is important to implement perioperative measures to optimize SSIs outcome. Serbian and MMA guidelines for SSIs prevention identify evidence-based measures in the perioperative period such as glucose control, skin antisepsis and antimicrobial prophylaxis to minimize the risk of wound infection [20]. Negative effects of smoking on conditions of the musculoskeletal system and treatment of these conditions, are well-documented [21]. The present study confirms that smoking is a strong predictor for SSIs in our orthopedic patients, too.

Microorganisms from a distant source of infection, principally through hematogenous spread, can cause SSIs in orthopedic patients [22]. The presence of preoperative infections significantly differed between our patients with and without SSIs. So,

the practice to prevent SSIs aimed to minimize the number of microorganisms introduced into the operative site [20].

The NNIS system provides risk index for stratification of SSIs. It is widely used, operation-specific and prospectively applied and validated method for accounting for differences in case mix. The important step in our study was SSI stratification rates according to NNIS risk index.

In a report on surveillance of SSIs in Europe the cumulative incidence by NNIS risk index varied from 0.7% for hip prothesis operation with risk index of 0, to 2.7% with risk index of 2 or 3 [2]. In our study NNIS risk index for the same operations varied from 0.6% to 6.7%. For knee prosthesis operation, surveillance in Europe showed a variation of incidence by NNIS from 0.6% with a risk index of 0, to 1.9% with the risk index of 2 or 3, while our resultes showed a variation of incidence by NNIS from 2.9% with risk index of 0, to 2.2% with risk index of 2 or 3.

Yuan and Chen [23] meta-analysis showed that obesity had about two fold increased risk of SSIs in orthopedics. Our study confirms that obesity increases SSIs in orthopedics patients because of BMI determined as independent RF for SSIs (Table 4).

The LHS could be a cause and/or consequence of SSIs. Whitehouse et al. [4] reported that orthopedic SSIs prolong total hospital stays by a median of 2 weeks *per* patient. Our study showed very similar results. The patients with SSI stayed in hospital 24.72 days *vs* patients without SSI who stayed in hospital 15.95 days.

S. aureus was the most frequently isolated microorganism – 45.7% of which 43.7% were methicillin-resistant *Staphylococcus aureus* (MRSA). In France and England this pathogen was also a predominant isolate in the orthopedic categories, with decreasing occurrence of MRSA, which could be explained by the impact of various national policies directed at controlling MRSA [5, 7, 24].

Acinetobacter spp. was second most frequent germ of SSIs in our patients. There is a study which provides important information about the RFs of nosocomial *Acinetobacter* spp. infections in a large cohort of surgical patients in MMA [25].

There is a limitation of our study due to no postdischarge surveillance performed to detect SSIs. The Finish authors showed that although postdischarge surveillance had a large impact on the rate of SSIs after orthopedic surgery, it detected only a minority of deep incisional and organ/space SSIs [26].

Conclusion

The results of our study are valuable in documenting the relations between risk factors and surgical site infections in patients undergoing orthopedic surgery. Comparison of our results with the results of healthcare systems from other countries suggests active surveillance as an important component of surgical site infections reduction strategy.

The results of this study were communicated with the orthopedic surgical team to initiate greater attention to the national recommendation in prevention and control of surgical site infections.

R E F E R E N C E S

1. *Edwards JR, Peterson KD, Andrus ML, Dudeck MA, Pollock DA, Horan TC.* National Healthcare Safety Network (NHSN) Report, data summary for 2006 through 2007, issued November 2008. Am J Infect Control 2008; 36(9): 609−26.
2. Surveillance of surgical site infections in Europe, 2008-2009. Stockholm: ECDC; 2012.
3. *Suljagić V, Jevtic M, Djordjevic B, Jovelic A.* Surgical site infections in a tertiary health care center: prospective cohort study. Surg Today 2010; 40(8): 763−71.
4. *Whitehouse JD, Friedman N, Kirkland KB, Richardson WJ, Sexton DJ.* The Impact of Surgical-Site Infections Following Orthopedic Surgery at a Community Hospital and a University Hospital: Adverse Quality of Life, Excess Length of Stay, and Extra Cost. Infect Control Hosp Epidemiol 2002; 23(4): 183−9.
5. *Mabit C, Marcheix PS, Mounier M, Dijoux P, Pestourie N, Bonnevialle P,* et al. Impact of a surgical site infection (SSI) surveillance program in orthopedics and traumatology. Orthop Traumatol Surg Res 2012; 98(6): 690−5.
6. *Health Protection Agency.* Sixth report of the mandatory surveillance of surgical site infection in orthopedic surgery, April 2004 to March 2010. London: Health Protection Agency; 2010. Available from: www.hpa.org.uk
7. *Health Protection Agency.* Surveillance of surgical site infections in NHS hospitals in England, 2011/2012. London: Health Protection Agency; 2012. Available from: www.hpa.org.uk
8. *Haley RW, Culver DH, Morgan WM, White JW, Emori TG, Hooton TM.* Identifying patients at high risk of surgical wound infection. A simple multivariate index of patient susceptibility and wound contamination. Am J Epidemiol 1985; 121(2): 206−15.
9. *Culver DH, Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG,* et al. Surgical wound infection rates by wound class, operative procedure and patient risk index: National Nosocomial Infections Surveillance System. Am J Med 1991; 91(Suppl 38): S152−7.
10. *National Academy of Sciences/National Research Council.* Postoperative wound infections the influence of ultraviolet irradiation of the operating room and of various other factors. Ann Surg 1964; 160(Suppl 2): 1−132.
11. *Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM.* CDC definitions for nosocomial infections, 1988. Am J Infect Control 1988; 16(3): 128−40.
12. *Horan TC, Gayner RP, Martone WJ, Jarvis WR, Emori TC.* CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical infections. Infect Control Hosp Epidemiol 1992; 13: 606−8.
13. *Koneman WE, Allen DS, Janda WM, Schreckenberger PC, Winn W.* Color Atlas and Textbook of Diagnostic Microbiology. Philadelphia: Lippincott-Raven Publishers; 1997.
14. *Ercole FF, Franco LM, Macieira TG, Wenceslau LC, de Resende HI, Chianca TC.* Risk of surgical site infection in patients undergoing orthopedic surgery. Rev Lat Am Enfermagem 2011; 19(6): 1362−8.
15. *Schneeberger PM, Smits MH, Zick RE, Wille JC.* Surveillance as a starting point to reduce surgical-site infection rates in elective orthopaedic surgery. J Hosp Infect 2002; 51(3): 179−84.

16. *Brandt C, Sohr D, Behnke M, Daschner F, Rüden H, Gastmeier P.* Reduction of surgical site infection rates associated with active surveillance. Infect Control Hosp Epidemiol 2006; 27(12): 1347−51.
17. *National Nosocomial Infections Surveillance System.* National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control. 2004; 32(8): 470−85.
18. *Health Protection Scotland.* Surveillance of Surgical Site Infection Independent Hospital Report, For procedures carried out from the 1 February 2004 to 30 June 2011. Glasgow: Health Protection Scotland; 2012.
19. *Yi M, Edwards JR, Horan TC, Berrios-Torres IS, Fridkin SK.* Improving Risk-Adjusted Measures of Surgical Site Infection for the National Healthcare Safety Network. Infect Control Hosp Epidemiol 2008; 29(10): 941−6.
20. *Marković-Denić, Šuljagić V, Bilanović D, Mandarić D, Milićević M.* Prevention of the surgical-site infections. Beograd: Institut za zaštitu zdravlja Srbije "Dr Milan Jovanović Batut", Ministrarstvo zdravlja Republike Srbije. 2005. (Serbian).
21. *Durand F, Berthelot P, Cazorla C, Farizon F, Lucht F.* Smoking is a risk factor of organ/space surgical site infection in orthopaedic surgery with implant materials. Int Orthop 2013; 37(4): 723−7.
22. *Cordero-Ampuero J, de Dios M.* What are the risk factors for infection in hemiarthroplasties and total hip arthroplasties. Clin Orthop Relat Res 2010; 468(12): 3268−77.
23. *Yuan K, Chen H.* Obesity and surgical site infections risk in orthopedics: a meta-analysis. Int J Surg 2013; 11(5): 383−8.
24. *Department of Health.* Screening for Meticillin-resistant Staphylococcus aureus (MRSA) colonisation - a strategy for NHS Trusts: A summary of best practice. London: Department of Health; 2007.
25. *Suljagić V, Jevtić M, Djordjević B, Romić P, Ilić* R, *Stanković N,* et al. Epidemiology of nosocomial colonization/infection caused by *Acinetobacter* spp. in patients of six surgical clinics in war and peacetime. Vojnosanit Pregl 2011; 68(8): 661−8.
26. *Huotari K, Lyytikäinen O.* Impact of postdischarge surveillance on the rate of surgical site infection after orthopedic surgery. Infect Control Hosp Epidemiol 2006; 27(12): 1324−9.

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ORIGINAL ARTICLE

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Association between risk factors, basal viral load, virus genotype and the degree of liver fibrosis with the response to the therapy in patients with chronic hepatitis C virus infection

Povezanost faktora rizika, bazalnog nivoa virusa, genotipa virusa i stepena fibroze jetre sa odgovorom na terapiju kod bolesnika sa hroničnom hepatitis C virusnom infekcijom

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Abstract

Background/Aim. Hepatitis C is an important sociomedical problem worldwide due to frequent progression to chronic disease, occurrence of liver cirrhosis and hepatocellular carcinoma. Standard pegylated interferon alfa 2a plus ribavirin therapy results in resolution of infection only in 50% of patients. The aim of this study was to determine the association of various factors with response to the therapy in patients with chronic heptitis C virus (HCV) infection. Age and sex of patients, inoculation risk factors, histopathological changes in the liver, viral load and HCV genotype were analyzed. **Methods.** The study included a group of 121 patients with chronic HCV infection. The treatment was carried out 24 weeks for virus genotype 2 and 3, and 48 weeks for genotype 1 and 4. The degree of histopathological changes in the liver was determined by hematoxylin and eosin staining, whereas polimerase chain reaction was used for HCV genotyping. **Results.** In the group of non-responding patients genotype 1 was represented with 100%, while in the other groups, although predominantly present, its percentage was lower. Unresponsiveness to therapy and relapse of disease were associated with higher viral load and advanced fibrosis. Intravenous use of psychoactive substances, as a risk factor, was present in a high percentage in the group of patients with sustained response, while blood transfusion and dialysis were leading risk factors in the group of relapse responders and non-responders. **Conclusion.** The results of our study showed that the treatment outcome of chronic HCV infection was associated with baseline HCV ribonucleic acid, HCV genotype, route of infection and the degree of histopathological changes in the liver.

Key words:
hepatitis c; hepatitis, chronic; treatment outcome; risk factors; genotype; histological techniques; disease transmission, infections.

Apstrakt

Uvod/Cilj. Hepatitis C virusna (HCV) infekcija predstavlja veliki medicinski, ekonomski i socijalni problem u svetu. Standardna terapija pegilovanim interferonom alfa 2a i ribavirinom dovodi do rezolucije bolesti kod samo oko 50% bolesnika. Cilj ovog rada bio je da se utvrdi povezanost faktora rizika od nastanka infekcije, genotipske zastupljenosti virusa i stepena patohistoloških promena jetre sa odgovorom na terapiju kod bolesnika sa hroničnom HCV infekcijom. **Metode.** Ispitivanjem je obuhvaćena grupa od 121 bolesnika sa hroničnom HCV infekcijom. Lečenje je sprovedeno tokom 24 nedelje za genotip virusa 2 i 3, i tokom 48 nedelja za genotip 1 i 4. Za određivanje genotipa virusa korišćena je metodologija lančane reakcije polimeraze. Stepen patohistoloških promena jetre određivan je standardnom hematoksilin-eozin metodom. **Rezultati.** U ispitivanoj grupi bolesnika najzastupljeniji HCV genotip bio je genotip 1. U grupi bolesnika bez odgovora na terapiju genotip 1 bio je zastupljen sa 100%, dok je u ostalim grupama, iako dominantno prisutan, njegov procenat bio znatno niži. Najveći broj virusnih čestica registrovan je u grupi bolesnika sa nepovoljnim odgovorom na terapiju. Najviši procenat bolesnika bez fibroze (F0) ili sa niskim stepenom fibroze (F1) nalazio se u grupi bolesnika sa povoljnim odgovorom na terapiju, dok se najveći broj bolesnika sa izraženom fibrozom (F3 i F4) nalazio među bolesnicima sa nepovoljnim odgovorom na terapiju. Intravenska upotreba psihoaktivnih supstanci kao faktor rizika bila je prisutna u visokom procentu

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kod bolesnika sa povoljnim odgovorom, dok su transfuzija krvi i dijaliza bili vodeći faktor rizika za bolesnike kod kojih je došlo do relapsa HCV i kod onih bolesnika koji nisu odgovorili na terapiju. **Zaključak.** Rezultati ove studije pokazuju da je ishod lečenja hronične HCV infekcije povezan sa bazalnim nivoom HCV ribonukleinske kiseline u momentu postavljanja dijagnoze, genotipom HCV virusa, načinom infekcije i stepenom oštećenja parenhima jetre.

Ključne reči:
hepatitis c; hepatitis, hronični; lečenje, ishod; faktori rizika; genotip; histološke tehnike; bolest, prenošenje.

Introduction

Hepatitis C virus (HCV) infection is a major medical, social and economic problem in the world. It is assumed that about 180 million people worldwide have chronic HCV infection [1, 2]. The discovery of HCV in 1989 clarified the etiology of a large number of posttransfusion hepatitis with unknown cause [3]. Until 1990, the most important route of infection was transfusion of blood and blood products, and today that is the intravenous use of psychoactive substances. Most patients with acute HCV infection have no distinct symptoms and the diagnosis is usually made accidentally, finding elevated activities of serum aminotransferases on routine biochemical testing. The outgo of acute HCV infection depends on many factors, such as virus genotype and the strength of host immune response. Nearly 75% of patients with acute hepatitis C develop chronic disease [4]. The progression of disease is associated with alcohol abuse [5], the presence of diabetes [6], age of the patient [7], co-infection with HIV and/or other primary hepatotropic viruses [8]. About 10–20% of patients with chronic hepatitis C will develop liver cirrhosis [4] and hepatocellular carcinoma occurs in about 1–5% of cases [9]. Standard treatment of hepatitis C using pegylated interferon alfa 2a (PEG-IFNα-2a) and ribavirin (RBV) is successful in only half of patients. Moreover, therapy is costly and has diverse side-effects (flu-like symptoms, depression, anemia, leucopenia, nausea, cough, rash, etc.). Thus, identifying factors that influence the outcome of therapy is of great importance.

The aim of this study was to determine the association of various factors with response to PEG-IFNα-2a plus RBV combined therapy in patients with chronic HCV infection.

Methods

This prospective study was carried out in the Department of Infectious Diseases, Clinical Center of Kragujevac, between 2005 and 2009. This study group consisted of 121 patients with chronic hepatitis C. Written informed consent was obtained from all patients according to the Declaration of Helsinki, and the local Ethics Committee approved the study. Anamnesis, biochemical analysis, liver biopsy, quantification of viral load and genotyping were acquired for each patient before the beginning of the treatment. Histopathological data were obtained by standard hematoxylin-eosin (HE) staining of biopsy specimens and liver damage was scored according to Knodell et al. [10]. The patients were treated with PEG-IFNα-2a (180 μg/week) and RBV (800–1200 mg/day body weight-adjusted) in a period of 24 weeks for genotype 2 and 3 and 48 weeks for genotype 1 and 4. Sustained virological response (SVR) was defined as an undetectable HCV ribonucleic acid (RNA) six months after completing the therapy. Non-responsiveness (NR) to the therapy was defined as detectable HCV RNA during and at end of the therapy. Reappearance of viral RNA after completing the therapy in patients whose serum HCV RNA was undetectable during or at the end of the treatment was categorized as relapse (relapse responders – RR).

All the results were statistically examined with the commercial SPSS program (version 19.0, SPSS Inc., Chicago, IL). Central tendency, variability and frequency were analyzed, according to the type of data collected, stratified by the subgroups of the patients of interest. Mann-Whitney *U*-test and Kruskal-Wallis-test were used for comparative analysis between the groups of nonparametric data. Contingency tables were used to analyze the relationship between two or more variables.

Results

Characteristics of the patients

The study group of 121 patients comprised of 80 (66%) males and 41 (34%) females with the average age 41.9 ± 14 years. The number of males was significantly higher than the number of females ($p = 0.004$). The route of infection was intravenous use of psychoactive substances (IVU PAS) in 41 (33.84%) patients, blood transfusion in 23 (19%), dialysis in 15 (12.4%), sexual contact in 3 (2.48%), professional exposure in 2 (1.65%), perinatal transmission in 1 (0.83%) patient, and for 36 (29.7%) patients the route of virus transmission was unknown (Figure 1A). The median viral load (HCV RNA titer) in the whole group was 3,839.500 IU/mL. HCV genotype 1 was dominant (83 of 121 patients, 68.6%), represented in statistically higher number than other genotypes ($p < 0.001$). Genotype 3 was registered in 33 (27.2%) patients, genotype 4 in 3 (2.6%) and genotype 2 in 2 (1.65%) patients (Figure 1B). Liver biopsy specimens were obtained from 104 patients and scored according to Knodell et al. [10]. The degree of fibrosis was evaluated as: no fibrosis (F0), recorded in 11 (10.6%) patients, F1 found in 48 (46.1%), F2 in 25 (24.0%), F3 in 14 (13.47%) and F4 in 6 (5.77%) patients (Figure 1C).

Regarding the response to the therapy, data were obtained for 95 subjects. SVR was achieved in 69 (72.63%) patients, 9 (9,47%) patients were NR to the therapy and 17 (17.89%) patients were RR (Figure 1D). According to treatment response all data were organized in three groups: SVR, RR and NR.

Viral factors influencing response to the therapy

The lowest median HCV RNA levels were registered in the patients with favorable response to the therapy (SVR – 2,378.250 IU/mL), higher in RRs (4,968,000 IU/mL) and the highest in NRs (6,021.000 IU/mL) (Figure 2).

Although dominant in all four groups, genotype 1 was the most frequent in NRs (9 of 9 patients; $p < 0.001$). The second most frequent was genotype 3, present in high percent in the group of SVRs (25 of 69 patients – 36.23%; $p < 0.001$), while lesser in RR (4 of 17 – 23.53% patients). Genotype 4 was found only in the SVR group in 3 (4.35%) of 69 patients. One patient with genotype 2 the group of RRs was registered in (5.88%) (Table 1).

Host factors influencing response to the therapy

Except for the SVR group ($p = 0.026$), there was no statistically significant difference in the age of the patients, as well as in the percent of the males and the females among the study groups (Table 1).

IVU PAS as the route of viral inoculation was present in statistically higher percentage in the group of the patients with SVR (43.48%; $p = 0.026$), while blood transfusion and

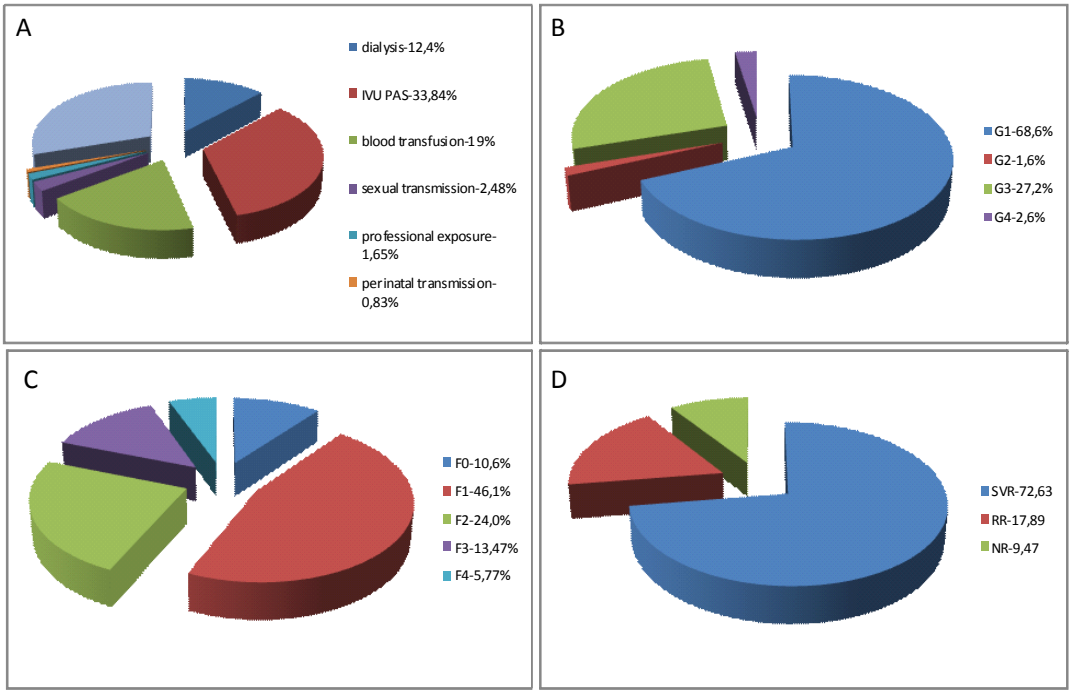

Fig. 1 – Characteristics of the patients: (A) Rate of hepatitis C virus (HCV) transmission route; (B) HCV genotypes; (C) Stage of fibrosis, and (D) Responsiveness to the therapy.
IVU PAS – intravenous use of psychoactive substances; G – genotype; F – fibrosis; SVR – sustained virological response; RR – relapse responder; NR – non-responsiveness.

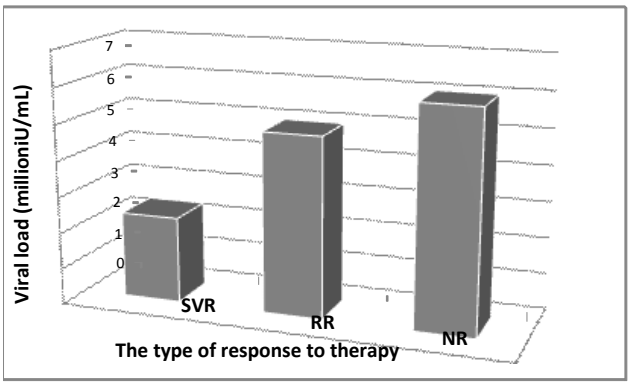

Fig. 2 – Median viral load related to the response to the therapy.
SVR – sustained virological response; RR relapse responder; NR – non-responsiveness;
SVR – sustained virological response; RR – relapse responder; NR – non-responsiveness; IVU PAS – intravenous use of psychoactive substances; *$p < 0.05$; **$p < 0.01$.

dialysis were the leading risk factors in the group of RRs (transfusion – 23.53%, dialysis – 17.65%) and NEs (33.3% both) (Table 1). Dialysis as the route of infection was most frequent in the group of NRs ($p = 0.023$).

Analysis of the relation between the degree of liver fibrosis and responsiveness to the therapy showed that the majority of the patients with F0 were in the group of SVRs (7 patients), and only one patient in the RR group. F1 and F2 were prevailing in the SVR (49.21% and 26.98%, respectively), in the RR group F1, F2 and F3 were represented in the similar percent (35.71%; 28.57%; 28.57%, respectively) and in the group of NRs the most frequent were F3 and F4 (33.3% each) (Table 1). F3 stage was significantly more immanent in the RR and NR patients ($p < 0.05$) and F4 in non-responders ($p < 0.05$).

whereas the lowest viral load was found in the SVRs. This data is in agreement with previous findings that baseline viral load correlates with treatment outcome, regardless the virus genotype [11].

In our study, genotype 1 was dominant, represented in all the groups, and the genotype 3 was second most frequent. All NR patients were infected with HCV-1. Indeed, this genotype is a more aggressive strain and most difficult to treat [12]. Clinical studies have shown that standard PEG-IFNα-2a plus RBV therapy is quite successful in case of genotype 3 [13]. Similarly, we found genotype 3 significantly more immanent in the group of SVRs. Genotype 4 is considered to be associated with progression to cirrhosis and worse response to the therapy [14, 15], although recent clinical trials have demonstrated the opposite results [16]. The results of our

Table 1

Response to pegylated interferon alfa-2a plus ribavirin therapy depending on the patients characteristics, route of hepatitis C virus (HCV) infection, HCV genotypes, and the stage of liver fibrosis

Characteristics of the patients	The patients grouped according to the treatment response		
	SVR (n = 69)	RR (n = 17)	NR (n = 9)
Age (years)	37*	46	48
Gender, n (%)			
male	46 (66.7)	10 (55.6)	6 (66.7%)
female	23 (33.33)	7 (44.4)	3 (33.33%)
Route of infection, n (%)			
IVU PAS	30 (43.48)*	2 (11.76)	1 (11.11)
transfusion	14 (20.29)	4 (23.53)	3 (33.33)
dialysis	4 (5.80)	3 (17.65)	3 (33.33)*
sexual transmission	0 (0)	1 (5.88)	0 (0)
professional exposure	1 (1.45)	0 (0)	0 (0)
perinatal transmission	1 (1.45)	0 (0)	0 (0)
unknown	19 (27.54)	7 (41.18)	2 (22.22)
HCV genotypes (G), n (%)			
G1	41 (59.42)	12 (70.59)	9 (100.0)**
G2	0 (0.0)	1 (5.88)	0 (0.0)
G3	25 (36.23)**	4 (23.53)	0 (0.0)
G4	3 (4.35)	0 (0.0)	0 (0.0)
Rate of fibrosis (F) stage, n (%)	SVR (n = 63)	RR (n = 14)	NR (n = 6)
F0	7 (11.11)	1 (7.14)	0 (0)
F1	31 (49.21)	5 (35.71)	1 (16.7)
F2	17 (26.98)	4 (28.57%)	1 (16.7)
F3	5 (7.94)	4 (28.57)*	2 (33.3%)*
F4	3 (4.76)	0 (0%)	2 (33.3)*

SVR – sustained virological response; RR – relapse responder; NR – non-responsiveness; IVU PAS – intravenous use of psychoactive substances; *$p < 0.05$; **$p < 0.01$.

Discussion

The ultimate goal of PEG-IFNα-2a plus RBV therapy is the resolution of HCV infection. Considering that only half of treated patients achieve SVR, it is of great importance to reveal factors that may influence the outcome of the therapy. In the present study in the group of 121 patients host and viral factors that can affect the response to PEG-IFNα-2a plus RBV therapy were analyzed.

The results of the study showed that the largest number of virus particles was registered in the group of NRs, study are in accordance with the latest, given that all patients infected with HCV-4 responded to the therapy.

The majority of studies point to age as predictive factor in reaching SVR [17]. In our study we also found this correlation. There was no statistically significant difference in the percent of males and females among the study groups. Similarly, previous studies have shown that gender have no influence in achieving SVR [18].

Up to 1990s the leading risk factor for HCV infection was blood transfusion. Ever since 1992, blood from donators has been screened for the presence of HCV. Nowadays the

most common route of infection is VU PAS, followed by transfusion of blood and blood derivates, long-term hemodialysis, organ transplantation, sexual contact, perinatal transmission, nosocomial transmission, tattoos or body piercings and professional exposure. The results of our study are in accordance with this data since the most numerous were the patients infected through intravenous use of drugs, the second most frequent risk factor were transfusion and dialysis, and in a small percent of patients sexual contact, professional exposure and perinatal transmission. Analyzing data we found that in the group of SVRs the most frequent routes of HCV transmission were IVV PAS and blood transfusion, while in the group of RRs and NRs dialysis and transfusion were the leading risk factors.

Chronic HCV infection gives rise to liver injury that can lead to formation of scar tissue, *ie* fibrosis. As inflammation continues, liver lesions are more massive and more liver tissue is replaced with nonfunctional connective tissue. About 10–20% of chronically infected patients can develop cirrhosis and liver cancer. Fibrosis is not an irreversible process. In patients who achieve SVR to the therapy, fibrosis stabilization and retraction occur [19]. However, the presence of progressive fibrosis predicts a lower response rate [20]. In the present study the majority of patients with stage F0 were in the group of SVRs and with increase of the stage of fibrosis the response rate was decreasing. The patients with advanced fibrosis (F2 and F3) were the most prevailing in the group of RRs, and the patients with high stage fibrosis (F4) in the group of NRs.

Conclusion

The results of this study showed that the majority of patients on pegylated interferon alfa-2a plus ribavarin responded to the therapy (71.88%). Hepatitis C virus genotype, viral load, age of the patients and the stage of fibrosis were related to the response to the therapy. Our study did not confirm the association between the gender of the patients and the treatment outcome. Intravenous use of psychoactive substances as the route of infection was the most frequent in the group of responders with sustained virological response, and transfusion and dialysis in the group of the patients with poor response to the therapy (relapse responders and non-responders).

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R E F E R E N C E S

1. *Lavanchy D.* The global burden of hepatitis C. Liver Int 2009; 29(Suppl 1): 74−81.
2. *Shepard CW, Finelli L, Alter MJ.* Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005; 5(9): 558−67.
3. *Esteban JI, Sauleda S, Quer J.* The changing epidemiology of hepatitis C virus infection in Europe. J Hepatol 2008; 48(1): 148−62.
4. *Afdhal NH.* The Natural History of Hepatitis C. Semin Liver Dis 2004; 24 Suppl 2: 3−8.
5. *Poynard T, Bedossa P, Opolon P.* Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997; 349(9055): 825−32.
6. *Zein CO, Levy C, Basu A, Zein NN.* Chronic hepatitis C and type II diabetes mellitus: a prospective cross-sectional study. Am J Gastroenterol 2005; 100(1): 48−55.
7. *Fried MW, Shiffman ML, Reddy RK, Smith C, Marinos G, Gonçales FL,* et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347(13): 975−82.
8. *Operskalski EA, Kovacs A.* HIV/HCV co-infection: pathogenesis, clinical complications, treatment, and new therapeutic technologies. Curr HIV/AIDS Rep 2011; 8(1): 12−22.
9. *Thompson C, Rogers G, Hewson P, Wright D, Anderson R, Cramp M,* et al. Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. Health Technol Assess 2007; 11(34): 1−206.
10. *Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N,* et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981; 1(5): 431−5.
11. *Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R,* et al. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. Hepatology 2003; 37(3): 600−9.
12. *Pozzato G, Moretti M, Crocé LS, Sasso F, Kaneko S, Unoura M,* et al. Interferon therapy in chronic hepatitis C virus: evidence of different outcome with respect to different viral strains. J Med Virol 1995; 45(4): 445−50.
13. *Sarin SK, Kumar CK.* Treatment of patients with genotype 3 chronic hepatitis C- current and future therapies. Liver Int 2012; 32(1 Suppl): 141−5.
14. *Zein NN, Rakela J, Krawitt EL, Reddy KR, Tominaga T, Persing DH.* Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Collaborative Study Group. Ann Intern Med 1996; 125(8): 634−9.
15. *Attaullah S, Khan S, Ali I.* Hepatitis C virus genotypes in Pakistan: a systemic review. Virol J 2011; 8: 433.
16. *Kamal SM, Nasser IA.* Hepatitis C genotype 4: What we know and what we don't yet know. Hepatology 2008; 47(4): 1371−83.
17. *Antonucci G, Angeletti C, Vairo F, Longo MA, Girardi E.* Age and prediction of sustained virological response to hepatitis C virus (HCV) infection treatment based on 28-day decrease in HCV RNA levels. J Infect Dis 2009; 200(9): 1484−5.
18. *Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R,* et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358(9286): 958−65.
19. *Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O,* et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 2000; 132(7): 517−24.
20. *Karino Y, Toyota J, Sugawara M, Miyazaki K, Kuwata Y, Yamazaki K,* et al. Hepatitis C virus genotypes and hepatic fibrosis regulate 24-h decline of serum hepatitis C virus RNA during interferon therapy in patients with chronic hepatitis C. J Gastroenterol Hepatol 2003; 18(4): 404−10.

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The Fracture Risk Assessment Tool (FRAX® score) in subclinical hyperthyroidism

Indeks za određivanje rizika od preloma kostiju (FRAX® skor) u supkliničkom hipertireoidizmu

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Abstract

Background/Aim. The Fracture Risk Assessment Tool (FRAX® score) is the 10-year estimated risk calculation tool for bone fracture that includes clinical data and hip bone mineral density measured by dual-energy x-ray absorptiometry (DXA). The aim of this cross-sectional study was to elucidate the ability of the FRAX® score in discriminating between bone fracture positive and negative pre- and postmenopausal women with subclinical hyperthyroidism. **Methods.** The bone mineral density (by DXA), thyroid stimulating hormone (TSH) level, free thyroxine (fT4) level, thyroid peroxidase antibodies (TPOAb) titre, osteocalcin and beta-cross-laps were measured in 27 pre- and postmenopausal women with newly discovered subclinical hyperthyroidism [age 58.85 ± 7.83 years, body mass index (BMI) 27.89 ± 3.46 kg/m^2, menopause onset in 46.88 ± 10.21 years] and 51 matched euthyroid controls (age 59.69 ± 5.72 years, BMI 27.68 ± 4.66 kg/m^2, menopause onset in 48.53 ± 4.58 years). The etiology of subclinical hyperthyroisims was autoimmune thyroid disease or toxic goiter. FRAX® score calculation was performed in both groups. **Results.** In the group with subclinical hyperthyroidism the main FRAX® score was significantly higher than in the controls (6.50 ± 1.58 *vs* 4.35 ± 1.56 respectively; $p = 0.015$). The FRAX® score for hip was also higher in the evaluated group than in the controls (1.33 ± 3.92 *vs* 0.50 ± 0.46 respectively; $p = 0.022$). There was no correlations between low TSH and fracture risk ($p > 0.05$). The ability of the FRAX® score in discriminating between bone fracture positive and negative pre- and postmenopausal female subjects ($p < 0.001$) is presented by the area under the curve (AUC) plotted *via* ROC analysis. The determined FRAX score cut-off value by this analysis was 6%, with estimated sensitivity and specificity of 95% and 75.9%, respectively. **Conclusion.** Pre- and postmenopausal women with subclinical hyperthyroidism have higher FRAX® scores and thus greater risk for low-trauma hip fracture than euthyroid premenopausal women. Our results point to the use of FRAX® calculator in monitoring pre- and postmenopausal women with subclinical hyperthyroidism to detect subjects with high fracture risk in order to prevent further fractures.

Key words:
hip fractures; risk assessment; questionnaires; postmenopause; hyperthyroidism.

Apstrakt

Uvod/Cilj. Alat za određivanje rizika od preloma kostiju (FRAX® skor) je matematički model za izračunavanje pretpostavljenog desetogodišnjeg rizika od preloma kostiju koji uključuje kliničke podatke i gustinu koštane mase u predelu vrata i butne kosti izmerene osteodenzitometrijom (*dual-energy x-ray absorptiometry* – DXA). Cilj ove studije preseka bio je da se proceni primenljivost skora FRAX® u prepoznavanju osoba sa povećanim rizikom od preloma kostiju i utvrdi da li supklinički hipertireoidizam nosi veći rizik od preloma kosti u odnosu na eutireoidno stanje. **Metode.** Mineralna koštana gustina merena DXA metodom, tireostimulišući hormon (TSH), slobodni tiroksin (fT4), antitela na tireoperoksidazu (TPOAt), osteokalcin i beta *cross-laps* mereni su kod 27 žena sa nedavno dijagnostikovanim supkliničkim hipertireoidizmom (58,85 ± 7.83 godina, indeks telesne mase (ITM) 27,89 ± 3,46 kg/m^2, nastanak menopauze u 46,88 ± 10,21 godini) i 51 žene uporedivih osobina (59,69 ± 5,72 god, ITM 27,68 ± 4,66 kg/m^2, nastanak menopauze u 48,53 ± 4,58 godini). FRAX® skor je upotrebljen za procenu rizika od preloma kostiju u obe grupe. **Rezultati.** Ukupni skor FRAX® (6,50 ± 1,58 *vs* 4,35 ± 1,56, $p = 0,015$) i skor FRAX® za prelom kuka (1,33 ± 3,92 *vs*

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0,50 ± 0,46, p= 0,022) bio je značajno veći u grupi sa supkliničkim hipertireoidizmom u odnosu na kontrolnu grupu. Nije bilo korelacije između nivoa TSH i rizika od frakture ($p > 0,05$). Kompetentnost skora FRAX® u razlikovanju pre-i postmenopauzalnih žena sa rizikom od frakture i bez rizika ($p < 0.001$) je prikazana površinom ispod krive (AUC) pomoću ROC analize. *Cut-off* vrednost skora FRAX® bila je u ovoj analizi 6%, sa pretpostavljenom senzitivnošću i specifičnošću od 95% i 75,9%. **Zaključak.** Pre- i postmenopauzalne žene sa supkliničkim hipertireoidizmom imaju veći skor FRAX® i time veći rizik od preloma kuka na malu traumu nego eutireoidne žene. Naši rezultati ukazuju na to da primena FRAX® kalkulatora u grupi pre- i postmenopauzalnih žena sa supkliničkim hipertireoidizmom doprinosi prepoznavanju osoba sa povećanim rizikom od preloma kostiju.

Ključne reči:
kuk, prelomi; rizik, procena; upitnici; postmenopauza; hipertireoidizam.

Introduction

Thyroid hormones are essential for bone development in children and acquisition of peak bone mass and bone turnover in adults [1–3]. In adults, thyroid hormones play important role as homeostatic regulators that maintain bone mass. Thyroid stimulating hormone (TSH) affects bone metabolism in direct pathway *via* specific receptors on the bone, although thyroid hormones exert catabolic effect on bone tissue by stimulating osteoclast activity [4, 5]. It is well known that overt hyperthyroidism and hypothyroidism increased the risk for bone fractures. Hyperthyroidism affects bone turnover by increasing bone resorption. Hypothyroidism suppresses bone formation and bone turnover, but underlying mechanism between hypothyroidism and fracture risk is not clear [6]. Some studies suggest that even mild or moderate thyroid disease is a respective risk factor for osteoporotic fractures, especially in postmenopausal women [7, 8]. Bone mineral density (BMD) is traditionally a predictive factor for osteoporotic fractures. The Fracture Risk Assessment Tool (FRAX® score) was recommended by the World Health Organization (WHO) [9]. This tool enabling a 10-year prediction for possible fractures, incorporates BMD measured by dual-energy-X-ray-absorptiometry (DXA) on the femoral neck, and a few of independent risk factors for fractures on low trauma like: age, previous fractures, parental hip fracture, body mass index (BMI), current smoking, usage of drugs which could affect bone density, alcohol abuse and poor health [10–15].

The aim of this cross-sectional study was to elucidate the ability of the FRAX® score in discrimination between bone fracture positive and negative pre- and postmenopausal women with subclinical hyperthyroidism in order to identify individuals at high risk for future osteoporotic fractures.

Methods

FRAX® score calculation (10-year estimated risk for bone fracture) and measurement of thyroid peroxydase antibodies (TPOAb), bone markers, osteocalcin and beta-cross-laps (β-cross-laps) were performed in the group of 27 peri- and postmenopausal women with newly discovered subclinical hyperthyroidism [age 58.85 ± 7.83 years, body mass index (BMI) 27.89 ± 3.46 kg/m^2, menopause onset in 46.88 ± 10.21 years] and 51 matched euthyroid controls (age 59.69 ± 5.72 years, BMI 27.68 ± 4.66 kg/m^2, menopause onset in 48.53 ± 4.58 years). The etiology of subclinical hyperthyroidism was autoimmune thyroid disease or toxic goiter.

The inclusion criteria for studied group were: women, 40–70 years of age, with the TSH level lower than 0.3 mIU/L and free thyroxin (fT4) level within the normal range. Additional including criteria were: no previous history of thyroid disease, no bowel disease with malabsorption and no steroid therapy longer than 6 months during the life. The studied and the control group were assessed via a questionnaire about independent risk factors for osteoporosis, such as previous fractures, current cigarette smoking, alcohol consumption, parental fractures and onset of menopause.

TSH and fT4 leveles measured by chemiluminescent microparticle immunoassay (CMIA) (Abbott, ARCHITECT ci8200). Reference ranges for TSH were 0.35–4.94 mIU/mL with analytical sensitivity of ≤ 0.1 μIU/mL and for fT4 9.0–19.1 pmol/L with analytical sensitivity of ≤ 0.4 ng/dL. The TPOAb was measured by CMIA for the quantitative determination of the IgG class of TPOAb in human serum and plasma (Abbott, ARCHITECT *i* system,). Reference values were < 5.61 IU/mL. Osteocalcin and β-cross-laps were determined by electrochemiluminescence immunoassay (ECLIA) (Roche, Cobas e601). The reference range for osteocalcin was 15–46 ng/mL, and for β-cross-laps 104–1008 pg/mL. The bone mineral density was measured by dual energy X-ray bone densitometer Lunar DPX. Measuring was performed on the lumbar spine and left femoral neck. BMD was expressed as standard deviation (SD) in T-score. The fracture risk was calculated by the FRAX® score assessment for Turkey [16, 17].

Statistical analysis

Statistical analysis was performed using SPSS statistical software (SPSS for Windows, release 20.0, SPSS, Chicago, IL). Data were expressed as mean values with SD or as absolute numbers with percentages. Numeric variables were analysed using Student's *t*-test or the Mann-Whitney *U*-test (for skewed data), while categorical data were analyzed using a χ^2 test and Fisher's exact test, as appropriate. Odds ratios (ORs) for vertebral and hip fracture in relation to thyroid function were determined using unadjusted and adjusted logistic regression (adjusted for age, BMI and BMD, expressed as T-score). Stepwise adjusted regression analysis of relationships between thyroid status and BMD, bone turnover and FRAX® scores was performed after adjustment for age, BMI and smoking. The ability of the FRAX® score and TSH in discriminating between bone fracture positive and negative perimenopausal women with subclinical hyperthyroidism was described by

the Receiver Operating Characteristic (ROC) curve method. The curves were drawn by plotting the sensitivity against the false positive rate (1-specificity), for varying the cut-off of the FRAX® score and TSH levels. The area under the curve (AUC) represents a quantitative measure of predictive value of TSH and FRAX® score for bone fracture. In all tests, *p* value < 0.05 was considered to be statistically significant.

The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the institutional Ethics Board of the Faculty of Medicine, University of Belgrade, Serbia.

Results

The anthropometric, biochemical, BMD and personal history data of both examined groups are presented in Table 1.

There were no significant differences in mean values of the evaluated descriptive parameters including parents' fractures, current smoking, type 2 diabetes mellitus, as well as treatment with corticosteroids. Also, there were no significant differences between groups concerning age, BMI and fat mass.

The TSH level was significantly lower in subjects with subclinical hyperthyroidism in comparison with the control group (0.0730 ± 0.05 *vs* 2.23 ± 0.94 mIU/mL, respectively; *p* < 0.001). The fT4 level significantly higher in the examined group than in the healthy subjects (15.88 ± 2.21 *vs* 13.82 ± 1.31 pmol/L, respectively; *p* < 0.001). TPOabs were more prevalent in subclinical hyperthyroid women than in the healthy women (44.4% *vs* 3.9%, respectively; *p* < 0.001).

BMD in the lumbar spine was not significantly lower in the studied than in the control (-1.24 ± 1.10 *vs* -1.13 ± 1.59, respectively; *p* = 0.73). The hip T-score was lower in the examined group than in the controls (-1.34 ± 0.73 *vs* -0.68 ± 0.90, respectively; *p* = 0.002). There was no significant difference in bone markers – osteocalcin (25.99 ± 12.74 *vs* 21.79 ± 5.34 ng/mL, respectively; *p* = 0.22) and β-cross-laps (374.97 ± 180.68 *vs* 306.88 ± 110.73, pg/mL respectively; *p* = 0.18) between the two groups.

The main fracture risk, main FRAX® score, was higher in the examined than in the control group (6.50 ± 1.58 *vs* 4.35 ± 1.56 respectively; *p* = 0.015) and fracture risk for hip was also higher in the group with subclinical hyperthyroidism (1.33 ± 3.92 *vs* 0.50 ± 0.46 respectively; *p* = 0.022) (Table 1).

Unadjusted and adjusted (for age, BMI and lumbar spine T-score) logistic regression analysis indicated that the parameter of thyroid function and bone markers in subclinical hyperthyroid women were not related to fracture (*p* > 0.05) (Table 2).

Table 1

Characteristics of the subclinical hyperthyroid and healthy women

Parameter	Subclinical hyperthyroidism	Healthy women	*p*
Subjects, n	27	51	
Age (years), x̄ ± SD	58.85 ± 7.83	59.69 ± 5.72	0.593
BMI (kg/m^2), x̄ ± SD	27.91 ± 4.57	27.68 ± 4.66	0.830
Fat mass, (%) x̄ ± SD	42.15 ± 10.21	43.41 ± 5.79	0.530
Menopause (years), x̄ ± SD	46.88 ± 10.21	48.53 ± 4.58	0.343
Current smoking, n (%)	6 (22.2%)	10 (19.6%)	0.786
Diabetes mellitus, n (%)	1 (3.7%)	2 (3.9%)	0.960
Parental fractures, n (%)	3 (11.1%)	5 (9.8%)	0.856
TSH (mU/L), x̄ ± SD	0.0730 ± 0.05	2.23 ± 0,94	< 0.001
FT4 (mU/L), x̄ ± SD	15.88 ± 2.21	13.82 ± 1.31	< 0.001
TPOAb, n (%)	12 (44.4%)	2 (3.9%)	< 0.001
T score (L1-L4), x̄ ± SD	-1.24 ± 1.10	-1.13 ± 1.59	0.738
T score hyp, x̄ ± SD	-1.34 ± 0 .73	-0.68 ± 0 .90	0.002
Osteocalcin, x̄ ± SD	25.99 ± 12.74	21.79 ± 5.34	0.225
β-Cross-laps, x̄ ± SD	374.97 ± 180.68	306.88 ± 110.73	0.188
FRAX® (main), x̄ ± SD	6.50 ± 1,58	4.35 ± 1.56	0.015
FRAX® (femoral neck), x̄ ± SD	1.33 ± 3.92	0.50 ± 0.46	0.022
Previous fractures, n (%)	3 (11.1%)	1 (2.0%)	0.081

BMI – body mass index; TSH – thyroid stimulating hormone; FT4 – free thyroxine; TPOAb – thyroid peroxydase antibodies; FRAX – the Fracture Risk Assessment Tool score.

Table 2

Relationship between thyroid function tests and fracture

Parameter	Unadjusted logistic regression model			Adjusted* logistic regression model		
	OR *per* unit			OR *per* unit		
	p	change	95% CI for OR	*p*	change	95% CI for OR
TSH	0.320	0.618	1.240–1.594	0.343	0.628	0.240–1.643
FT4	0.251	1.334	0.816–2.180	0.402	1.266	0.729–2.198
TPOAb	0.999	0.000	0.000	0.998	0.000	0.000
OC	0.445	0.946	0.822–1.090	0.895	1.009	0.880–1.158
BCL	0.483	0.997	0.989–1.005	0.871	1.001	0.991–1.011
BMI	0.579	1.061	0.860–1.310			

***Adjusted for age, BMI and T score for L1-L4; OR – odds ratio; CI – confidence level; TSH – thyroid stimulating hormone; fT4 – free thyroxine level; TPOAb – thyroid peroxydase antibodies; OC – osteocalcin; BCL – beta-cross-laps; BMI – body mass index.**

The ability of the TSH and FRAX® score in discriminating between bone fracture positive and negative pre- and postmenopausal female subjects is presented by AUC plotted in ROC analysis (Table 3, Figure 1).

Table 3
ROC analysis of the association between TSH and FRAX® score

Parameters	Area	SE	*p*	95% CI for SE
TSH	0.644	0.162	0.336	0.326–0.961
Main FRAX® score	0.998	0.003	0.001	0.992–1.005
Hip FRAX® score	0.750	0.192	0.094	0.373–1.127

TSH – thyroid stimulating hormone; FRAX® – Fracture Risk Assessment Tool; ROC – receiver operating characteristics; SE – standard error; CI – confindence interval.

The determined FRAX® score cut-off value by this analysis is 6%, with estimated sensitivity and specificity of 95% and 75.9%, respectively (Figure 1).

The association was found between TSH and the main FRAX® score (p = 0.001). The relationship between thyroid function tests, T-score, markers of bone turnover and FRAX® scores after adjustment for age or age, BMI and smoking is presented in Table 4.

A significant association was found between serum TSH ($p < 0.001$), fT4 ($p = 0.02$) and femoral neck BMD (Table 4).

TSH was in association with hip FRAX® score and that was statistically significant ($p = 0.046$) (Figure 2).

The association ($p = 0.008$) between fT4 and main FRAX® score was also found as well as between fT4 and hip FRAX® score ($p = 0.014$) (Figures 3 and 4)

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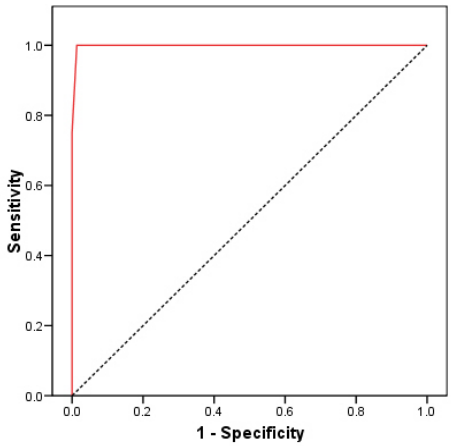

Fig. 1 – The ability of TSH in discriminating between bone fracture positive and negative pre- and postmenopausal female subjects.
TSH – thyroid stimulating hormone.

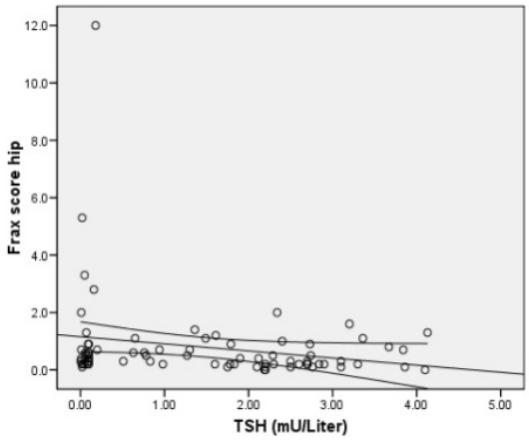

Fig. 2 – The hip FRAX® and TSH.
TSH – thyroid stimulating hormone; FRAX® – Fracture Risk Assessment Tool.

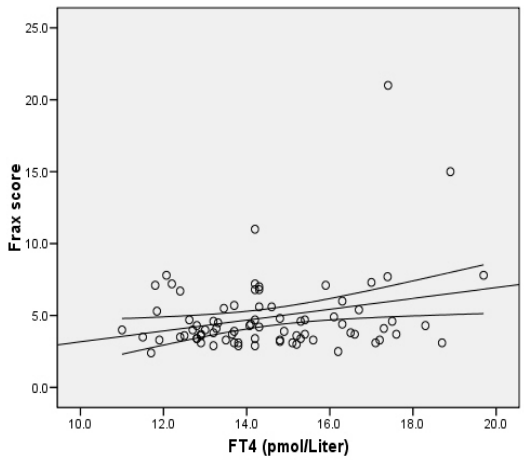

Fig. 3 – The main FRAX® score and FT4.
FRAX® – Fracture Risk Assessment Tool; fT4 – free thyroxine.

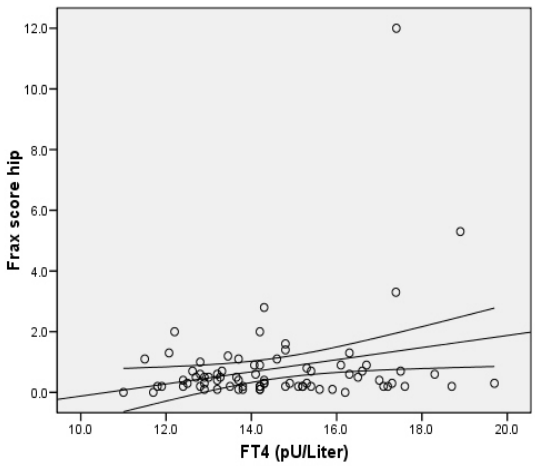

Fig. 4 – The hip FRAX® score and FT4.
FRAX® – Fracture Risk Assessment Tool; fT4 – free thyroxine.

Table 4

Relationship between thyroid function tests, T-score, markers of bone turnover and FRAX® scores

Parameters	TSH (mU/L) Adjusted linear regressive model			FT4 (mU/liter) Adjusted linear regressive model			TPOAb Adjusted linear regressive model	
	Model R^2	β coefficient (95% CI)	*p*	Model R^2	β coefficient (95% CI)	*p*	OR (95% CI for OR)	*p*
T skor								
L1–L4	0.040	0.157 (-0.059–0.374)	0.151	0.013	-0.094 (-0.426–0.238)	0.573	1.093 (0.707–1.690)	0.653
Hip	0.181	0.621 (0.304–0.939)	< 0.001	0.077	-0.602 (-1.113–0.092)	0.021	0.763 (0.366–1.590)	0.470
Bone marker								
OC	0.105	-0.025 (-0.071–0.020)	0.258	0.069	-0.036 (-0.110–0.038)	0.327	1.105 (1.010–1.209)	0.029
BCL	0.102	-0.002 (-0.005–0.001)	0.282	0.046	0.001 (-0.004–0.006)	0.655	1.009 (1.002–1.016)	0.012
FRAX® score								
Main	0.063	-0.110 (-0.220–0.000)	0.050	0.100	0.225 (0.062–0.389)	0.008	0.905 (0.651–1.258)	0.553
Hip	0.066	-0.208 (-0.413–0.004)	0.046	0.087	0.384 (0.081–0.687)	0.014	0.862 (0.426–1.743)	0.679

TSH – thyroid stimulating hormone; FT4 – free thyroxine; TPOAb – tyroid peroxydase antibodies; OC – osteocalcin; BCL – beta cross laps; BMI – body mass index; FRAX® – Fracture Risk Assessment Tool; CI – confidence interval; OR – odds ratio.

Discussion

The 10-year fracture risk in pre- and postmenopausal women with subclinical hyperthyroidism was compared with fracture risk in euthyroid women matched by age, BMI, age of menopause onset and percentage of fat mass. Some studies show that even a small variation in thyroid hormones level may affect bone quality [18]. First fracture appeared earlier in women with hyperthyroidism or thyroid cancer than in women without thyroid disease [19]. A meta analyse demonstrate that alkaline phosphatase activity may be decreased in femoral bone marrow cell cultures but not in vertebral bone marrow cells due to the excess of T3 [20]. Similar results were found in a study on animal model. That study shows that gene expression markers for osteoblast and osteoclast in levothyroxine (L-T4) treated rats are increased in the femoral bone but not in the lumbar spine [21]. A recent study indicates that the combination of L-T4 and levothyronine (L-T3) in the treatment of hypothyroidism causes a higher rate of bone resorption [22]. The level fT4 in the upper normal reference range but not low TSH level was independently related to decreased BMD in the lumbar spine in perimenopausal women [23]. Increased risk for fractures around the time of diagnosis has been reported in older men with subclinical hyper-or hypothyroidism [24]. The effect of thyroid hormones on bone metabolism is site specific. That was shown in a study on subclinical thyroid dysfunctional group (subclinical hyperthyroidism and hypothyroidism), compared with euthyroid controls [18]. Our results demonstrate that BMD in the lumbar spine was not lower in the group with subclinical hyperthyroidism than in the controls (-1.24 ± 1.10 *vs* -1.13 ± 1.59 respectively; $p = 0.73$) while the hip T-score was significantly lower in the examined group than in the controls (-1.34 ± 0 .73 *vs* -0.68 ± 0 .90 respectively; $p = 0.002$).

Excess of thyroid hormone, endogenous or due to overdose in thyroid replacement therapy even in asymptomatic persons, may be associated with elevated biochemical bone markers and poor bone mass [25–29]. In our study a significant association between serum TSH ($p < 0.001$), fT4 ($p = 0.02$) and femoral neck BMD was established. There was no association between TSH and fT4 and lumbar or hip BMD regarding bone markers. BMD is valuable but still not enough sensitive predictive fracture risk factor in population of postmenopausal women [30]. Some studies suggest that more than 50% of women with vertebral fractures have normal BMD and they do not meet criteria for osteoporosis; on the contrary, some premenopausal women with low BMI have relatively low fracture rates [31]. After surgical treatment of overt hyperthyroidism BMD and fracture risk decreased [32].

Considering BMD limitation to predict fractures, new fracture assessment tools were established in order to improve the prediction of osteoporotic fractures. One of them is the FRAX® score, as a computer-based algorithm for calculation 10-year hip or other bone fracture probability, obtained clinical risk factors, habits and hip BMD [15, 30, 33–36]. In our study, ROC analysis showed association between FRAX® score and TSH. The ability of TSH and FRAX® score in discriminating between bone fracture positive and negative pre- and postmenopausal female subjects is presented by the AUC plotted in ROC analysis. TSH was in association with hip FRAX® score ($p = 0.046$). The association between fT4 and the main FRAX® score was found ($p = 0.008$) as well as between fT4 and hip FRAX® score ($p = 0.014$).

The results regarding the contribution of bone markers in fracture risk were inconsistent [37–39]. Some studies did not find any difference among bone markers between patients with subclinical hyper- or hypothyroidism and healthy controls. Other studies reported the increase in bone markers in

perimenopausal women with hypothyroidism, whereas other investigators did not find any changes in bone markers in a similar study [40, 41]. We, also, did not find a difference in the levels of osteocalcin and β-cross-laps between the two groups ($p > 0.05$) as well as the association between bone markers and TSH or fT4 and FRAX® score.

The group with subclinical hypothyroidism had more prevalence of TPOAb than the control group ($p < 0.001$). The association was found between TPOAb and osteocalcin ($p = 0.029$) and cross-laps-levels ($p = 0.012$) but there was no association between TPOAt and fracture risk. Previously, was suggested that autoimmune thyroid disease in subclinical hypothyroid women increased hip fracture risk [42]. The influence of autoimmune disease on bone is complex and it is manifested as immunoregulatory imbalance. Alterations in homeostatic mechanisms might explain an imbalance of osteoblastic activity [43]. Osteopenia could be a consequence of chronic inflammatory autoimmune disorders with alteration osteclastic activity in new bone formation. The major regulators of bone destructions in autoimmune disorders are divided into two groups: proosteoclastogenic inflammatory cytokines (RANKL L) and antiosteclastogenic ones (OPG, IFN γ and IL 4). The influence of inflammation on bone is determinated with the duration of autoimmune disorders [44]. The association between TPOAb and osteocalcin in our study indicates that TPOAb (or inflammatory cytokines included in inflammatory response) may stimulate bone resorption. Short duration of autoimmune thyroid disease may be the explanation for missing association between TPOAb and FRAX® score.

The limitation of our study was that we did not know the duration of subclinical hyperthyroidism before the diagnosis was established.

Conclusion

Pre- and postmenopausal women with subclinical hyperthyroidism have higher FRAX® scores and, thus, greater risk for low-trauma hip fracture than euthyroid pre- and postmenopausal women. The results obtained in this study point out the use of FRAX® calculator in monitoring pre-and postmenopausal women with subclinical hyperthyroidism to detect subjects with high fracture risk and prevent future osteoporotic fractures.

REFERENCES

1. *Williams GR.* Extrathyroidal expression of TSH receptor. Ann Endocrinol (Paris) 2011; 72(2): 68−73.
2. *Wojcicka A, Bassett DJ, Williams GR.* Mechanisms of action of thyroid hormones in the skeleton. Biochim. Biophys Acta 2013; 1830(7): 3979−86.
3. *Gogakos AI, Duncan BJ, Williams GR.* Thyroid and bone. Arch Biochem Biophys 2010; 503(1): 129−36.
4. *Mazziotti G, Porcelli T, Patelli I, Vescovi PP, Giustina A.* Serum TSH values and risk of vertebral fractures in euthyroid post-menopausal women with low bone mineral density. Bone 2010; 46(3): 747−51.
5. *Bassett DJ, Williams GR.* Critical role of the hypothalamic-pituitary-thyroid axis in bone. Bone 2008; 43(3): 418−26.
6. *Murphy E, Williams GR.* The thyroid and the skeleton. Clin Endocrinol (Oxf) 2004; 61(3): 285−98.
7. *Duntas LH.* Subclinical thyroid disorders: The menace of the Trojan horse. J Endocrinol Invest 2003; 26(5): 472−80.
8. *Lakatos P.* Thyroid hormones: beneficial or deleterious for bone. Calcif. Tissue Int 2003; 73(3): 205−9.
9. *Kanis JA, Johnell O, Oden A, Johansson H, Mccloskey E.* FRAX™ and the assessment of fracture probability in men and women from the UK. Osteoporosis Int 2008; 19(4): 385−97.
10. *Unnanuntana A, Gladnick BP, Donnelly E, Lane JM.* The assessment of fracture risk. J Bone Joint Surg Am 2010; 92(3): 743−53.
11. *Pearce EN.* Thyroid dysfunction in perimenopausal and postmenopausal women. Menopause Int 2007; 13(1): 8−13.
12. *Shidara K, Inaba M.* Bone metabolic marker for osteoporosis. Nippon Rinsho 2009; 67(5): 927−31.
13. *Kamel HK, Hussain MS, Tariq S, Perry HM, Morley JE.* Failure to diagnose and treat osteoporosis in elderly patients hospitalized with hip fracture. Am J Med 2000; 109(4): 326−8.
14. *Follin SL, Black JN, McDermott MT.* Lack of diagnosis and treatment of osteoporosis in men and women after hip fracture. Pharmacotherapy 2003; 23(2): 190−8.
15. *Baddoura R, Hoteit M, El-Hajj FG.* Osteoporotic fractures, DXA, and fracture risk assessment: Meeting future challenges in the Eastern Mediterranean Region. J Clin Densitom 2011; 14(4): 384−94.
16. *Cauley JA, El-Hajj FG, Arabi A, Fujiwara S, Ragi-Eis S, Calderon A,* et al. Official Positions for FRAX® clinical regarding international differences from Joint Official Positions Development Conference of the International Society for Clinical Densitometry and International Osteoporosis Foundation on FRAX®. J Clin Densitom 2011; 14(3): 240−2.
17. *Ilias I, Spanoudi F, Koukkou E, Nikopoulou SC.* Use of the FRAX calculator with and without bone mineral density in Greek women. Hormones (Athens) 2012; 11(2): 222−3.
18. *Solomon BL, Wartofsky L, Burman KD.* Prevalence of fractures in postmenopausal women with thyroid disease. Thyroid 1993; 3(1): 17−23.
19. *Milne M, Kang MI, Quail JM, Baran DT.* Thyroid hormone excess increases insulin-like growth factor I transcripts in bone marrow cell cultures: Divergent effects on vertebral and femoral cell cultures. Endocrinology 1998; 139(5): 2527−34.
20. *Suwanwalaikorn S, Ongphiphadhanakul B, Braverman LE, Baran DT.* Differential responses of femoral and vertebral bones to long-term excessive L-thyroxine administration in adult rats. Eur J Endocrinol 1996; 134(5): 655−9.
21. *Fadeyev VV, Morgunova TB, Melnichenko GA, Dedov II.* Combined therapy with L-thyroxine and L-triiodothyronine compared to L-thyroxine alone in the treatment of primary hypothyroidism. Hormones (Athens) 2010; 9(3): 245−52.
22. *van Rijn LE, Pop VJ, Williams GR.* Low bone mineral density is related to high physiological levels of free thyroxine in perimenopausal women. Eur J Endocrinol 2014; 170(3): 461−8.
23. *Lee JS, Buzková P, Fink HA, Vu J, Carbone L, Chen Z,* et al. Subclinical thyroid dysfunction and incident hip fracture in older adults. Arch Intern Med 2010; 170(21): 1876−83.
24. *Lee WY, Oh KW, Rhee EJ, Jung CH, Kim SW, Yun EJ,* et al. Relationship between subclinical thyroid dysfunction and femoral neck bone mineral density in women. Arch Med Res 2006; 37(4): 511−6.
25. *Chapurlat RD, Garnero P, Bréart G, Meunier PJ, Delmas PD.* Serum type I collagen breakdown product (serum CTX) predicts hip fracture risk in elderly women: The EPIDOS study. Bone 2000; 27(2): 283−6.

26. *Grimnes G, Emaus N, Joakimsen RM, Figenschau Y, Jorde R.* The relationship between serum TSH and bone mineral density in men and postmenopausal women: The Tromsø study. Thyroid 2008; 18(11): 1147−55.
27. *Mosekilde L, Eriksen EF, Charles P.* Effects of thyroid hormones on bone and mineral metabolism. Endocrinol. Metab. Clin North Am 1990; 19(1): 35−63.
28. *Krakauer JC, Kleerekoper M.* Borderline-low serum thyrotropin level is correlated with increased fasting urinary hydroxyproline excretion. Arch Intern Med 1992; 152(2): 360−4.
29. *Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y*, et al. TSH is a negative regulator of skeletal remodeling. Cell 2003; 115(2): 151−62.
30. *Lau B, Cole SR, Gange SJ.* Competing risk regression models for epidemiologic data. Am J Epidemiol 2009; 170(2): 244−56.
31. *Tromp AM, Ooms ME, Popp-Snijders C, Roos JC, Lips P.* Predictors of fractures in elderly women. Osteoporos Int 2000; 11(2): 134−40.
32. *Vestergaard P, Mosekilde L.* Fractures in patients with hyperthyroidism and hypothyroidism: A nationwide follow-up study in 16,249 patients. Thyroid 2002; 12(5): 411−9.
33. *Dawson-Hughes B, Tosteson AN, Melton LJ, Baim S, Favus MJ, Khosla S,* et al. Implications of absolute fracture risk assessment for osteoporosis practice guidelines in the USA. Osteoporos Int 2008; 19(4): 449−58.
34. *Watts NB.* Fracture Risk Assessment Tool (FRAX®): Applications in clinical practice. Womens Health 2011; 20(4): 525−31.
35. *Leslie WD, Majumdar SR, Lix LM, Johansson H, Oden A, McCloskey E,* et al. High fracture probability with FRAX usually indicates densitometric osteoporosis: implications for clinical practice. Osteoporos Int 2012; 23(1): 391−7.
36. *Leslie WD, Lix LM, Langsetmo L, Berger C, Goltzman D, Hanley DA,* et al. Construction of a FRAX® model for the assessment of fracture probability in Canada and implications for treatment. Osteoporos Int 2011; 22(3): 817−27.
37. *Bauer DC, Garnero P, Harrison SL, Cauley JA, Eastell R, Ensrud KE,* et al. Biochemical markers of bone turnover, hip bone loss, and fracture in older men: the MrOS study. J Bone Miner Res 2009; 24(12): 2032−8.
38. *Ross PD, Kress BC, Parson RE, Wasnich RD, Armour KA, Mizrahi IA.* Serum bone alkaline phosphatase and calcaneus bone density predict fractures: A prospective study. Osteoporos Int 2000; 11(1): 76−82.
39. *Lee J, Vasikaran S.* Current recommendations for laboratory testing and use of bone turnover markers in management of osteoporosis. Ann Lab Med 2012; 32(2): 105−12.
40. *Martini G, Gennari L, De PV, Pilli T, Salvadori S, Merlotti D,* et al. The effects of recombinant TSH on bone turnover markers and serum osteoprotegerin and RANKL levels. Thyroid 2008; 18(4): 455−60.
41. *Johnell O, Kanis JA, Oden A, Johansson H, De LC, Delmas P*, et al. Predictive value of BMD for hip and other fractures. J Bone Miner Res 2005; 20(7): 1185−94.
42. *Polovina S, Popovic V, Duntas L, Milic N, Micic D.* Frax score calculations in postmenopausal women with subclinical hypothyroidism. Hormones (Athens) 2013; 12(3): 439−48.
43. *Singh A, Mehdi AA, Srivastava RN, Verma NS.* Immunoregulation of bone remodelling. Int J Crit Illn Inj Sci 2012; 2(2): 75−81.
44. *Schett G.* The multiple faces of autoimmune mediated bon loss. Nat Rev Endocrinol 2010; 6(12): 698−706.

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Analysis of risk factors for occlusions of a synthetic femoropopliteal bypass graft

Analiza faktora rizika od okluzije sintetskog grafta kod femoropoplitealnog bajpasa

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Abstract

Background/Aim. Femoropopliteal bypass is a revascularization technique of lower extremities with excellent outcome. The great saphenous vein is the best graft material, but if it is not adequate or has been removed, synthetic grafts are an useful alternative. Graft occlusion is the most significant complication with the most serious consequences. The aim of this study was to analyse predictive factors for the synthetic femoropopliteal bypass occlusions. **Methods.** This retrospective case-control study included all patients who underwent synthetic femoropopliteal bypass due to peripheral arterial occlusive disease at the Vascular Surgery Center, Clinical Center of Kragujevac, Serbia, from 2007 to 2013. The cases group were the patients with femoropopliteal graft occlusion (n = 44), with the control group consisted of the patients without such an outcome (n = 88). **Results.** Significant effects to occlusion were: concomitant cardiovascular disease ($_{adjusted}$OR 27.05; 95% CI 4.74; 154.35), a type of femoropopliteal bypass ($_{adjusted}$OR 16.50; 95% CI 4.05; 67.24), previous vascular intervention ($_{adjusted}$OR 4.67; 95% CI 1.20; 18.14), clinical stage of the disease ($_{adjusted}$OR 3.73; 95% CI 1.94; 7.18), administration of postoperative oral anticoagulant therapy ($_{adjusted}$OR 0.05; 95% CI 0.01; 0.23) and the use of angiotensin converting enzyme inhibitors ($_{adjusted}$OR 0.14; 95% CI 0.03; 0.70). A significant synergism was shown for the following combinations of the observed risk factors: type of femoropopliteal bypass and cardiovascular disease, type of femoropopliteal bypass and previous vascular intervention, previous vascular intervention and cardiovascular disease, previous vascular intervention and beta blockers, cardiovascular disease and diabetes, type of femoropopliteal bypass and antiaggregant therapy, clinical stage of disease and cardiovascular disease, previous vascular intervention and antiaggregant therapy. **Conclusion.** Concomitant cardiovascular disease, below-knee femoropopliteal bypass, advanced stage of vascular disease and non-use of anticoagulant therapy and angiotensin-converting enzyme inhibitors are the significant predictors of graft occlusion after synthetic femoropopliteal bypass. Their synergistic effect determines the importance of diabetes, use of beta blockers and platelet antiaggregant therapy.

Key words:
graft occlusion, vascular; biocompatible materials; femoral artery; saphenous vein; risk factors.

Apstrakt

Uvod/Cilj. Femoropoplitealni (FP) bajpas je revaskularizaciona tehnika donjih ekstremiteta sa odličnim ishodom. Velika vena safena najbolji je materijal za graft, ali ako nije adekvatna ili je uklonjena, sintetički graftovi su korisna alternativa. Najznačajnija komplikacija sa ozbiljnijim posledicama je okluzija grafta. Cilj rada bio je da se analiziraju faktori za okluziju sintetičkog grafta kod FP bajpasa. **Metode.** Ova retrospektivna studija tipa slučaj-kontrola obuhvatila je sve bolesnike kod kojih je urađen FP bajpas sintetskim graftom zbog arterijske bolesti donjih ekstremiteta u Centru za vaskularnu hirurgiju Kliničkog centra u Kragujevcu od 2007. do 2013. godine. Grupu slučajeva činili su bolesnici sa okluzijom femoropoplitealnog bajpasa (n = 44), a kontrolnu grupu činili su bolesnici bez takvog ishoda (n = 88). **Rezultati.** Značajan uticaj na okluziju imali su prateća kardiovaskularna oboljenja ($_{adjusted}$OR 27,05; 95% CI 4,74; 154,35), vrsta femoropoplitealnog bajpasa ($_{adjusted}$OR 16,50; 95% CI 4,05; 67,24), prethodna vaskularna intervencija ($_{adjusted}$OR 4,67; 95% CI 1,20; 18,14), klinički stadijum bolesti ($_{adjusted}$OR 3,73; 95% CI 1,94; 7,18), davanje postoperativne antikoagulantne terapije ($_{adjusted}$OR 0,05; 95% CI 0,01; 0,23) i korišćenje inhibitora angiotenzin konvertujućeg enzima ($_{adjusted}$OR 0,14; 95% CI 0,03; 0,70). Značajan zajednički uticaj imale su sledeće kombinacije posmatranih faktora rizika: tip femoropoplitealnog bajpasa i kardiovaskularnih oboljenja, tip femoropoplitealnog bajpasa i prethodnih vaskularnih postupaka, prethodnih vaskularnih postupaka i kardiovaskularnih oboljenja, prethodnih vaskularnih postupaka i upotrebe beta blokatora, kardiovaskularnih oboljenja i dijabetesa, tip femo-

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ropoplitealnog bajpasa i antiagregacione terapije, kliničkog stadijuma bolesti i kardiovaskularnih oboljenja, prethodnih vaskularnih postupaka i antiagregacione terapije. **Zaključak.** Prateća kardiovaskularna oboljenja, potkoleni femoropoplitealni bajpas, uznapredovali stadijum vaskularne bolesti i nekorišćenje antikoagulantne terapije i inhibitora angiotenzin-konvertujućeg enzima značajni su prediktori okluzije grafta nakon sintetskog femororpoplitealnog bajpasa. Zajednički uticaj ukazuje i na značaj dijabetesa, upotrebe beta blokatora i trombocitne antiagregacione terapije.

Ključne reči:
vaskularni graft, okluzija; biokompatibilni materijali; a. femoralis; v. saphena; faktori rizika.

Introduction

Femoropopliteal (FP) bypass is a revascularization technique of lower extremities with the excellent outcome. The great saphenous vein is the best graft material for FP bypass in patients with peripheral arterial occlusive disease [1] and compared to synthetic graft has better patency and limb salvage [2]. If the great saphenous vein is not adequate or has been removed, synthetic grafts appear to be the useful alternative. The initial outcome after FP bypass is good graft patency and blood supply [1]. In approximately 40% of patients with chronic critical limb ischemia within 6 months a major limb amputation is necessary if revascularization of the extremity has been not done [3].

Graft occlusion is the most significant complication with the most serious consequences after FP bypass revascularization. According to the time of onset, occlusion is classified into early (< 30 days), intermediary (30 day–2 years) and late (> 2 years) [4, 5]. Graft occlusion occurs in 25–35% of patients within 2 years after synthetic FP bypass reconstruction [4, 6, 7].

Literature review shows that FP bypass graft occlusions are related to patient age [8], gender [9, 10], race [8], the clinical stage of vascular disease [1, 4, 9, 11, 12], type of FP bypass [4, 11], type of graft [9, 13] and previous vascular interventions [2, 12, 14–16].

The literature shows contradictory and rare results of the effects of concomitant chronic disease, medications [8, 11, 15, 17, 18], and smoking [8, 12] on graft occlusions after synthetic FP bypass.

The aim of this research was to analyse the significance of insufficiently known and contradictory predictive factors for synthetic FP bypass graft occlusions according to previous studies, as well as their mutual interaction (possible additive effect of potential risk factors).

Methods

In this retrospective case-control study we examined potential risk factors for the development of graft occlusion after no heparinized synthetic [Dacron collagen coated (polyethylene terephthalate); expanded PTFE (polytetrafluoroethylene)] FP bypass. Data were collected from the medical documentation of the patients.

This study included all patients with FP bypass due to peripheral arterial occlusive disease at the Vascular Surgery Center, Clinical Center of Kragujevac, Serbia, during the 7-year period, from 2007 to 2013. Criteria for femoropopliteal bypass were type C and D lesions by TransAtlantic Intersociety Consensus (TASC) classification. Indications for operative treatment of femoropopliteal bypass were significant intermitent claudication and critical limb ischemia. The average follow-up period after FP bypass was 42 months. After FP bypass the therapy with statins was not given on routine basis. The cases group were the patients with FP early, intermediary and late graft occlusion, and the control group consisted of patients without such an outcome. The graft occlussion after FP bypass determination was based on the clinical, ultrasound and angiografic examinations with patients who were coming on regural checkups. The study was approved by the Ethics Committee of the Clinical Center Kragujevac (01-728/2014).

The case group included the patients with detected graft occlusion after synthetic FP bypass. A control group consisted of patients without graft occlusions after synthetic FP bypass. Patients with incomplete data were not included in the study. We found out 4 patients with incomplete medical documentation (one patient had occlussion of the graft).

We used a case-control design matched by age and gender. Two controls were randomly selected for each case-group patient with more controls, utilizing the Excel RANDBETWEEN function. The degree of exposure to risk factors that we studied (independent variables) was determined by statistical analysis of medical records of the cases group and the controls.

With such parameters, there was a total of 132 subjects in both groups: 44 in the group of cases and 88 controls, with the proviso that the patients be distributed between the groups in the ratio of 1 : 2 (for each patient from the group of the cases there were two control patients).

Inclusion criterion was: already underwent synthetic FP revascularization of lower extremities due to chronic peripheral arterial occlusive disease.

Exclusion criterion was: patients with incomplete medical records.

The variables measured in the study were: independent variables (causes) – clinical stage of disease according to Rutherford criteria, previous vascular intervention (aorto-bifemoral bypass, contralateral amputation, ipsilateral and contralateral FP bypass, graft revision and endovascular procedures), the type of supporting medicaments (antiaggregation, anticoagulant, antihypertensive, and other) and tobacco use; dependent variables (outcome) was: graft occlusion after synthetic FP bypass; confounding variables were: gender, age, concomitant chronic disease (cardiovascular disease, diabetes, and other).

Continuous variables were reported as mean ± standard deviation (SD) in the text and tables, and categorical variables were presented as proportions. Student's *t*-test was

used for independent (small) samples when comparing the mean values of continuous variables and alternative non-parametric test was used if the outcome did not follow a normal distribution (the Kolmogorov-Smirnov test χ^2 test was used to examine differences between categorical variables, and Fisher's test was used if the frequency of certain samples was low. The effect of independent and confounding variables on the dichotomous outcome (graft occlusion after synthetic FP bypass) and mutual interaction of predictor variables were analyzed using binary logistic regression, and the results were presented as adjusted odds ratios (OR). Statistically significant results were all the results where the probability of the hypothesis is less than 5% ($p < 0.05$). The results were presented in the tabelar form. The software package SPSS, the version 18.0, was used for statistical analysis.

Results

Baseline characteristics of participants and the differences between them are presented in Table 1.

The results of logistic regression analysis (Coh & Snell R square 0.439, Nagelkerke R square 0.610, Hosmer-Lemeshow χ^2 2.414, df = 8, $p = 0.966$) with the adjustment for potential confounders are given in Table 2.

Confounding factors that increase the risk for the occurrence of occlusions after synthetic FP bypass were found for: the concomitant cardiovascular disease ($_{adjusted}$OR 27.05; 95%

Table 1

Baseline characteristics of the cases and the controls

Variable	Cases (n = 44)	Controls (n = 88)	Test value and significance	Crude odds ratios with confidence intervals (1.96 SE)
Sex (M/F)	13/31	24/64	$\chi^2 = 0.75$ $p = 0.784$	0.89 (0.40; 1.99)
Age (years), mean ± SD	62.66 ± 8 .88	63.52 ± 9.17	$t = -0.515$ $p = 0.607$	0.99 (0 .95; 1.03)
Clinical stage of the disease according to Rutherford, n				
I	0	0		
II	0	16		
III	4	21	$\chi^2 = 16.24$ $p = 0.006$	1.88 (1.31; 2.70)
IV	15	21		
V	20	23		
VI	5	7		
Previous vascular intervention (no/yes), n	28/16	66/22	$\chi^2 = 1.85$ $p = 0.174$	1.71 (0.78; 3.74)
Cardiovascular diseases (no/yes), n	7/37	35/53	$\chi^2 = 7.70$ $p = 0.006$	3.49 (1.40; 8.70)
Diabetes (no/yes), n	30/14	61/27	$\chi^2 = 0.02$ $p = 0.89$	1.05 (0.48; 2.30)
Type of femoropopliteal bypass (above-knee, below-knee), n	12/32	57/31	$\chi^2 = 16.53$ $p = 0.001$	4.90 (2.21; 10.85)
OAC (yes/no), n	34/10	50/38	$\chi^2 = 5.30$ $p = 0.021$	0.39 (0.17; 0.88)
Platelet AA (acetylsalicylic acid and clopidogrel/ acetylsalicylic acid/ clopidogrel/without acetylsalicylic acid and clopidogrel), n	8/20/2/14	30/33/0/25	$\chi^2 = 7.16$ $p = 0.067$	1.22 (0.89; 1.65)
ACE inhibitors (no/yes), n	14/30	27/61	$\chi^2 = 0.51$ $p = 0.774$	0.94 (0.43; 2.03)
Beta blockers (no/yes), n	26/18	51/37	$\chi^2 = 0.02$ $p = 0.901$	0.95 (0.46; 1.99)
Duration of smoking (years), mean ± SD	29.45 ± 15 .05	26.24 ± 16 .55	$U = 1764.0$ $p = 0.40$	1.01 (0.99; 1.04)

SE* – significant difference; SD – standard deviation; OAC – postoperative vitamin K antagonist oral anticoagulant therapy (warfarin or acenokumarol) to INR (2–3); AA – postoperative antiaggregant therapy (acetylsalicylic acid or clopidogrel); ACE – angiotensin-converting enzyme.

Table 2

Crude and adjusted odds ratios of the risk factors

Risk factors	Crude OR (95%CI)	Adjusted OR (95%, CI)
Cardiovascular disease	3.49 (1.40–8.70)	27.05 (4.74, 154.35)
Type of femoropopliteal bypass (above-knee, below-knee)	4.90 (2.21–10.85)	16.50 (4.05, 67.24)
Previous vascular intervention	1.71 (0.78–3.74)	4.67 (1.20, 18.14)
Clinical stage of disease according to Rutherford	1.88 (1.31–2.70)	3.73 (1.94, 7.18)
ACE inhibitors	0.94 (0.43–2.034)	0.14 (0.03, 0.70)
OAC	0.39 (0.17–0.88)	0.05 (0.01, 0.23)

CI – confidence intervals; OR – odds ratio (for the sake of clarity only significant associations are shown in the table (95% CI of adjusted OR does not include value of 1); ACE – angiotensin-converting enzyme; OAC – postoperative vitamin K antagonist oral anticoagulant therapy (warfarin or acenokumarol) to INR (2–3).

CI 4.74; 154.35), type of FP bypass ($_{adjusted}$OR 16.50; 95% CI 4.05; 67.24), previous vascular intervention ($_{adjusted}$OR 4.67; 95% CI 1.20; 18.14), clinical stage of disease according to Rutherford ($_{adjusted}$OR 3.73; 95% CI 1.94; 7.18), the administration of postoperative oral anticoagulant (OAC) therapy ($_{adjusted}$OR 0.05; 95% CI 0.01; 0.23). Angiotensin-converting enzyme inhibitors (ACEI) plaied the protective role in the occurrence of graft occlusion after synthetic FP bypass ($_{adjusted}$OR 0.14; 95% CI 0.03; 0.70).

A significant synergistic effect was found for the combination of type of FP bypass and cardiovascular disease ($_{adjusted}$OR 32.76; 95% CI 7.31; 146.81), type of FP bypass and previous vascular intervention ($_{adjusted}$OR 7.56; 95% CI 1.58; 36.26), previous vascular intervention and cardiovascular disease ($_{adjusted}$OR 7.31; 95% CI 1.77; 30.20), previous vascular intervention and beta blockers ($_{adjusted}$OR 5.79; 95% CI 1.13; 29.56), cardiovascular disease and diabetes ($_{adjusted}$OR 4.37; 95% CI 1.17; 16.23), type of FP bypass and platelet antiaggregant therapy ($_{adjusted}$OR 2.60; 95% CI 1.42; 4.74), clinical stage of disease according to Rutherford and cardiovascular disease ($_{adjusted}$OR 2.47; 95% CI 1.68; 3.63), previous vascular intervention and platelet antiaggregant (AA) therapy ($_{adjusted}$OR 2.13; 95% CI 1.01; 4.52), postoperative OAC therapy and ACEI ($_{adjusted}$OR 0.09; 95% CI 0.02; 0.39) (Table 3).

By examining more risk factors, there was a synergistic effect found between the previous vascular intervention, cardiovascular disease and diabetes ($_{adjusted}$OR 9.98; 95% CI 1.42; 70.16); previous vascular intervention, cardiovascular disease and type of FP bypass ($_{adjusted}$OR 6.97; 95% CI 1.55; 31.41); cardiovascular disease, type of FP bypass and platelet AA therapy ($_{adjusted}$OR 3.00; 95% CI 1.55; 5.78); clinical stage of disease according to Rutherford, cardiovascular disease and type of FP bypass ($_{adjusted}$OR 2.15; 95% CI 1.59; 2.91).

We have determined that the angiographic patency of three, two or one crural recipient artery in the group of cases was observed in 11, 14 and 19 patients (25.0%, 31.8%, and 43.2%, respectively), and in the group of controls it was observed in 29, 36 and 23 patients (33.0%, 40.9%, and 26.1%, respectively). In the group of cases there were 11 patients with Dacron and 33 patients with PTFE graft (25% and 75%, respectively). In the group of controls there were 58 patients with Dacron and 30 patients with PTFE graft (65.9% and 34.1%, respectively) The femoropopliteal patency before the occlusion appeared was on the average 317.95 ± 123.49 days. From the 44 patients that were in the group of cases, the amputation of the limb after the occlusion of the graft was necessary for 23 (52.27%) patients. Perioperative surgical site infections after femoropopliteal bypass were found in 6.82% (9/132) of the patients.

Table 3

Synergistic effects of the risk factors

Risk factors	Crude OR (95%, CI)	AdjustedOR (95%, CI)
Type of femoropopliteal bypass and Cardiovascular disease	5.58 (2.54, 12.25)	32.76 (7.31, 146.81)
Type of femoropopliteal bypass and Previous vascular intervention	4.56 (1.56, 13.33)	7.56 (1.58, 36.26)
Previous vascular intervention and Cardiovascular disease	2.47 (1.05, 5.79)	7.31 (1.77, 30.20)
Previous vascular intervention and Beta blockers	1.56 (0.58, 4.20)	5.79 (1.13, 29.56)
Cardiovascular disease and Diabetes	1.75 (0.76, 4.04)	4.37 (1.17, 16.23)
Type of femoropopliteal bypass and platelet AA	1.69 (1.18, 2.40)	2.60 (1.42, 4.74)
Clinical stage of disease according to Rutherford and Cardiovascular disease	1.47 (1.21, 1.80)	2.47(1.68, 3.63)
Previous vascular intervention and platelet AA	1.36 (0.90, 2.07)	2.13(1.01, 4.52)
Clinical stage of disease according to Rutherford and Type of femoropopliteal bypass	1.48 (1.24, 1.76)	1.97(1.47, 2.64)
Clinical stage of the disease according to Rutherford and Diabetes	1.08 (0.92, 1.28)	1.30(1.02, 1.66)
Clinical stage of the disease according to Rutherford and platelet AA	1.12 (1.04, 1.21)	1.26(1.10, 1.44)
OAC and ACE hibitors	0.45 (0.18, 1.14)	0.09(0.02, 0.39)
Previous vascular intervention and Cardiovascular disease and Diabetes	2.69 (0.68, 10.58)	9.98(1.42, 70,16)
Cardiovascular disease and Type of femoropopliteal bypass and ACE inhibitors	4.21 (1.94, 9.15)	9.55(3.12, 29.25)
Previous vascular intervention and Cardiovascular disease and Type of femoropopliteal bypass	4.02 (1.35, 11.93)	6.97(1.55, 31,41)
Cardiovascular disease and Type of femoropopliteal bypass and platelet AA therapy	2.04 (1.31, 3.16)	3.00(1.55, 5.78)
Clinical stage of the disease according to Rutherford and Cardiovascular disease and Type of femoropopliteal bypass	1.52 (1.27, 1.81)	2.15(1.59, 2.91)

CI – confidence intervals; OR – odds ratio; AA – postoperative antiaggregant therapy (acetylsalicylic acid and clopidogrel/acetylsalicylic acid/clopidogrel/without acetylsalicylic acid and clopidogrel); OAC – postoperative vitamin K antagonist oral anticoagulant therapy (warfarin or acenokumarol) to INR (2–3); ACE – angiotensin converting enzyme; AA – postoperative antiaggregant therapy (acetylsalicylic acid and clopidogrel/acetylsalicylic acid/clopidogrel/without acetylsalicylic acid and clopidogrel).

Discussion

We matched the patients in the study by sex and age, given the proven impact of predictive factors on graft occlusion [2, 9–12, 18] after synthetic FP bypasses to determine the significance of other variables.

If the great saphenous vein is not available Dacron has a small patency benefit if compared to PTFE in above-knee FP reconstruction [19, 20] and PTFE graft is an alternative to a below-knee FP bypass [4, 21]. We used a Dacron graft for above-knee and PTFE graft for below-knee FP bypass.

We found that cardiovascular disease is important risk factor for the development of graft occlusion after synthetic FP bypass. According to the literature, accompanying cardiovascular diseases indicate poor graft patency [2] and limb salvage (HR 3.68, 95% CI 1.51–8.94) [22] and in such patients the endovascular approach is recommended [23]. These results suggest that in patients with peripheral arterial occlusive disease and concomitant coronary artery disease is necessary to conduct adequate cardiac and arterial blood pressure monitoring for long-term patency of synthetic FP bypass.

In this study we determined the statistical significance of the type of FP bypass for graft occlusion after synthetic FP bypass and these results also correspond to the previous research [9]. We conclude that in patients with synthetic FP below-knee bypass there is an increased risk of graft occlusion [6, 11].

We noted that previous vascular intervention (aortobifemoral bypass, contralateral amputation, ipsilateral and contralateral FP bypass, graft revision and endovascular procedures) are important risk factors for the development of graft occlusions after synthetic FP bypass. These results indicate that in patients with vascular and endovascular interventions there is the increased risk of graft occlusion due to advanced vascular disease. Similar results have been shown in other studies [2, 15, 24]. One-year graft occlusion after bypass procedure is significantly higher in patients with prior vascular intervention (31% *vs* 20%; $p = 0.046$ and 28% *vs* 18%; $p = 0.009$) [15].

In our study clinical stage of disease according to Rutherford criteria is an important risk factor for the development of graft occlusions after synthetic FP bypass. Our conclusion is that there is an increased risk of graft occlusion in patients with advanced atherosclerosis and clinical stage of peripheral arterial occlusive disease of the lower extremity, especially in those with chronic critical limb ischemia. These results are consistent with other studies that show the importance of clinical stage of disease for graft occlusion [2, 6, 8, 12, 25, 26]. The difference in patency and limb salvage rate among patients surgically treated for intermittent claudication, pain at rest or tissue loss was statistically significant [4].

We determined a statistical significance of ACEI for graft occlusions. The use of ACEI can play important role in prevention of atherosclerosis, delay of its progression and reduction of vascular events. ACEI play the protective role in the occurrence of graft occlusion after synthetic FP bypass because they reduce the likelihood of occlusion.

We found a significant difference in the prevention of graft occlusion after synthetic FP bypass associated with postoperative vitamin K antagonist, OAC therapy. The use of adequate OAC therapy reduces the likelihood of synthetic FP graft occlusion. Increase in the risk of graft occlusion and ischemia without the use of OAC indicated that OAC therapy was useful especially in PTFE graft [27]. OAC may be specially useful for FP bypass procedure with a low flow (midgraft velocity ≤ 45 cm/s) [1].

A synergism was observed in several risk factors (Table 3). Some of these interactions were also shown in previous studies [2, 15, 17, 28–31], and some were shown here for the first time. Synergistic effect determined the importance of diabetes, arterial hipertension and the use of platelet AA therapy. This result indicates that in diabetic patients with advanced chronic critical limb ischemia, and the accompanying cardiovascular disease, there is an increased risk of graft occlusion after synthetic FP bypass [2, 15, 30]. Also, arterial hypertension increased risk of graft occlusion after synthetic FP bypass. .In patients with advanced vascular disease, concomitant cardiovascular disease and below-knee type of bypass, use of platelet AA after synthetic FP bypass decreased risk of graft occlusion.

A major hindrance in the study was its limitation to one center and its retrospective design. Despite the useful strategies for minimization of possible subjectivity in sampling and measuring the outcomes, such as case-control matching, a lot of patients were not included due to incomplete patient files, and true randomization of matched controls was not possible. These circumstances hampered our ability to analyse the impact of some potentially important factors on the occurrence of graft occlusion after synthetic FP bypass.

The modern trend in vascular surgery is the use of a hybrid method, where in the same patient during the same operation both vascular and endovascular procedures are performed. The hybrid method provides the possibility of simultaneous improvement of the outflow by performing angioplasty of tibial artery during the femoropopliteal reconstruction, thereby reducing the risk of graft occlusion [32].

Conclusion

Concomitant cardiovascular disease, below-knee femoropopliteal bypass, advanced stage of vascular disease and chronic limb ischemia were significant risk factors for graft occlusion after synthetic femoropopliteal bypass. Angiotensin converting enzyme inhibitors and postoperative oral anticoagulant therapy play the protective role against the occurrence of graft occlusion after synthetic femoropopliteal bypass. Synergistic effect determines the importance of diabetes, use of beta blockers and platelet antiaggregant therapy.

Identifying risk factors enables more effective preventive actions and modification by reducing incidence of graft occlusions, major amputation or disability of the patient. Further studies will identify additional risk factors, and achieve better graft patency rates after synthetic femoropopliteal bypass.

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REFERENCES

1. *Takagi H, Goto S, Matsui M, Manabe H, Umemoto T.* A contemporary meta-analysis of Dacron versus polytetrafluoroethylene grafts for femoropopliteal bypass grafting. J Vasc Surg 2010; 52(1): 232−6.
2. *Debus ES, Larena-Avellaneda A, Heimlich F, Goertz J, Fein M.* Alloplastic bypass material below the knee: actual rationale. J Cardiovasc Surg (Torino) 2013; 54(Suppl 1): 159−66.
3. *Dohmen A, Eder S, Euringer W, Zeller T, Beyersdorf F.* Chronic critical limb ischemia. Dtsch Arztebl Int 2012; 109(6): 95−101.
4. *Baldwin ZK, Pearce BJ, Curi MA, Desai TR, McKinsey JF, Bassiouny HS*, et al. Limb salvage after infrainguinal bypass graft failure. J Vasc Surg 2004; 39(5): 951−7.
5. *Goodney PP, Nolan BW, Schanzer A, Eldrup-Jorgensen J, Bertges DJ, Stanley AC,* et al. Factors associated with amputation or graft occlusion one year after lower extremity bypass in northern New England. Ann Vasc Surg 2010; 24(1): 57−68.
6. *Pulli R, Dorigo W, Castelli P, Dorrucci V, Ferilli F, De BG,* et al. Midterm results from a multicenter registry on the treatment of infrainguinal critical limb ischemia using a heparin-bonded ePTFE graft. J Vasc Surg 2010; 51(5): 1167−77.
7. *Hertzer NR, Bena JF, Karafa MT.* A personal experience with the influence of diabetes and other factors on the outcome of infrainguinal bypass grafts for occlusive disease. J Vasc Surg 2007; 46(2): 271−9.
8. *Singh N, Sidawy AN, de Zee KJ, Neville RF, Akbari C, Henderson W.* Factors associated with early failure of infrainguinal lower extremity arterial bypass. J Vasc Surg 2008; 47(3): 556−61.
9. *Tangelder MJ, Algra A, Lawson JA, Eikelboom BC.* Risk factors for occlusion of infrainguinal bypass grafts. Eur J Vasc Endovasc Surg 2000; 20(2): 118−24.
10. *Alpagut U, Ugurlucan M, Banach M, Mikhailidis DP, Dayioglu E.* Does gender influence the patency of infrainguinal bypass grafts. Angiology 2008; 59(3): 278−82.
11. *Brumberg RS, Back MR, Armstrong PA, Cuthbertson D, Shames ML, Johnson BL,* et al. The relative importance of graft surveillance and warfarin therapy in infrainguinal prosthetic bypass failure. J Vasc Surg 2007; 46(6): 1160−6.
12. *Lancaster RT, Conrad MF, Patel VI, Cambria RP, LaMuraglia GM.* Predictors of early graft failure after infrainguinal bypass surgery: a risk-adjusted analysis from the NSQIP. Eur J Vasc Endovasc Surg 2012; 43(5): 549−55.
13. *Schanzer A, Hevelone N, Owens CD, Belkin M, Bandyk DF, Clowes AW,* et al. Technical factors affecting autogenous vein graft failure: observations from a large multicenter trial. J Vasc Surg 2007; 46(6): 1180−90.
14. *Bosiers M, Deloose K, Verbist J, Schroë H, Lauwers G, Lansink W,* et al. Heparin-bonded expanded polytetrafluoroethylene vascular graft for femoropopliteal and femorocrural bypass grafting: 1-year results. J Vasc Surg 2006; 43(2): 313−8.
15. *Nolan BW, de Martino RR, Stone DH, Schanzer A, Goodney PP, Walsh DW,* et al. Prior failed ipsilateral percutaneous endovascular intervention in patients with critical limb ischemia predicts poor outcome after lower extremity bypass. J Vasc Surg 2011; 54(3): 730−5.
16. *Baril DT, Goodney PP, Robinson WP, Nolan BW, Stone DH, Li Y,* et al. Prior contralateral amputation predicts worse outcomes for lower extremity bypasses performed in the intact limb. J Vasc Surg 2012; 56(2): 353−60.
17. *Brown J, Lethaby A, Maxwell H, Wawrzyniak AJ, Prins MH.* Antiplatelet agents for preventing thrombosis after peripheral arterial bypass surgery. Cochrane Database Syst Rev 2008; 8(4): CD000535.
18. *Henke PK, Blackburn S, Proctor MC, Stevens J, Mukherjee D, Rajagopalin S,* et al. Patients undergoing infrainguinal bypass to treat atherosclerotic vascular disease are underprescribed cardioprotective medications: effect on graft patency, limb salvage, and mortality. J Vasc Surg 2004; 39(2): 357−65.
19. *Twine CP, Mclain AD.* Graft type for femoro-popliteal bypass surgery. Cochrane Database Syst Rev 2010; 12(5): CD001487.
20. *Jensen LP, Lepäntalo M, Fossdal JE, Røder OC, Jensen BS, Madsen MS*, et al. Dacron or PTFE for above-knee FP bypass. a multicenter randomised study. Eur J Vasc Endovasc Surg 2007; 34(1): 44−9.
21. *Van Det RJ, Vriens BH, van der Palen J, Geelkerken RH.* Dacron or ePTFE for femoro-popliteal above-knee bypass grafting: short- and long-term results of a multicentre randomised trial. Eur J Vasc Endovasc Surg 2009; 37(4): 457−63.
22. *Engelhardt M, Boos J, Bruijnen H, Wohlgemuth W, Willy C, Tannheimer M,* et al. Critical limb ischaemia: initial treatment and predictors of amputation-free survival. Eur J Vasc Endovasc Surg 2012; 43(1): 55−61.
23. *Antoniou GA, Chalmers N, Georgiadis GS, Lazarides MK, Antoniou SA, Serracino-Inglott F,* et al. A meta-analysis of endovascular versus surgical reconstruction of femoropopliteal arterial disease. J Vasc Surg 2013; 57(1): 242−53.
24. *Taylor SM, York JW, Cull DL, Kalbaugh CA, Cass AL, Langan EM.* Clinical success using patient-oriented outcome measures after lower extremity bypass and endovascular intervention for ischemic tissue loss. J Vasc Surg 2009; 50(3): 534−41.
25. *Söderström M, Arvela E, Aho PS, Lepäntalo M, Albäck A.* High leg salvage rate after infrainguinal bypass surgery for ischemic tissue loss (Fontaine IV) is compromised by the short life expectancy. Scand J Surg 2010; 99(4): 230−4.
26. *Smeets L, Ho GH, Tangelder MJ, Algra A, Lawson JA, Eikelboom BC,* et al. Outcome after occlusion of infrainguinal bypasses in the Dutch BOA Study: comparison of amputation rate in venous and prosthetic grafts. Eur J Vasc Endovasc Surg 2005; 30(6): 604−9.
27. *Jackson MR, Johnson WC, Williford WO, Valentine JR, Clagett PG.* The effect of anticoagulation therapy and graft selection on the ischemic consequences of femoropopliteal bypass graft occlusion: results from a multicenter randomized clinical trial. J Vasc Surg 2002; 35(2): 292−8.
28. *Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL.* ACC/AHA 2005 guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): executive summary a collaborative report. J Am Coll Cardiol 2006; 21(47): 1239−312.
29. *Mohler E 3rd, Giri J; ACC; AHA.* Management of peripheral arterial disease patients: comparing the ACC/AHA and TASC-II guidelines. Curr Med Res Opin 2008; 24(9): 2509−22.
30. *Moxey PW, Hofman D, Hinchliffe RJ, Jones K, Thompson MM, Holt PJ.* Trends and outcomes after surgical lower limb revascularization in England. Br J Surg 2011; 98(10): 1373−82.
31. *Inan B, Aydin U, Ugurlucan M, Aydin C, Teker ME.* Surgical treatment of lower limb ischemia in diabetic patients - long-term results. Arch Med Sci 2013; 9(6): 1078−82.
32. *Pulli R, Dorigo W, Guidotti A, Fargion A, Alessi IA, Pratesi C.* The Role of Infrainguinal Bypass Surgery in the Endovascular Era. Ann Vasc Dis 2014; 7(1): 7−10.

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Value of bacterial culture of vaginal swabs in diagnosis of vaginal infections

Vrednost bakterijske kulture vaginalnog brisa u dijagnozi vaginalne infekcije

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Abstract

Bacground/Aim. Vaginal and cervical swab culture is still very common procedure in our country's everyday practice whereas simple and rapid diagnostic methods have been very rarely used. The aim of this study was to show that the employment of simple and rapid diagnostic tools [vaginal fluid wet mount microscopy (VFWMM), vaginal pH and potassium hydroxide (KOH) test] offers better assessment of vaginal environment than standard microbiologic culture commonly used in Serbia. **Methods.** This prospective study included 505 asymptomatic pregnant women undergoing VFWMM, test with 10% KOH, determination of vaginal pH and standard culture of cervicovaginal swabs. Combining findings from the procedures was used to make diagnoses of bacterial vaginosis (BV) and vaginitis. In addition, the number of polymorphonuclear leukocytes (PMN) was determined in each sample and analyzed along with other findings. Infections with *Candida albicans* and *Trichomonas vaginalis* were confirmed or excluded by microscopic examination. **Results.** In 36 (6%) patients cervicovaginal swab cultures retrieved several aerobes and facultative anaerobes, whereas in 52 (11%) women *Candida albicans* was isolated. Based on VFWMM findings and clinical criteria 96 (19%) women had BV, 19 (4%) vaginitis, and 72 (14%) candidiasis. Of 115 women with BV and vaginitis, pH 4.5 was found in 5, and of 390 with normal findings 83 (21%) had vaginal pH 4.5. Elevated numbers of PMN were found in 154 (30%) women – in 83 (54%) of them VFWMM was normal. Specificity and sensitivity of KOH test and vaginal pH determination in defining pathological vaginal flora were 95% and 81%, and 79% and 91%, respectively. **Conclusion.** Cervicovaginal swab culture is expensive but almost non-informative test in clinical practice. The use of simpler and rapid methods as vaginal fluid wet mount microscopy, KOH test and vaginal pH offers better results in diagnosis, and probably in the treatment and prevention of sequels of vaginal infections.

Key words:
vaginal diseases; infection; gravidity; diagnostic techniques, obstetrical and gynecological; sensitivity and specificity; serbia.

Apstrakt

Uvod/Cilj. U postojećim uslovima rada u kliničkoj praksi zastupljeno je korišćenje skupog testa kulture cervikovaginalnog brisa i pored postojanja bržih i jednostavnih metoda u otkrivanju vaginalnih infekcija. Cilj ove studije bio je da se pokaže da uvođenje jednostavnih i brzih dijagnostičkih postupaka: mikroskopski pregled nativnog preparata vaginalnog sekreta (MPNPVS), pH vagine i test sa kalijumom hidoksidom (KOH) test omogućava bolju procenu stanja vaginalne flore od standardne mikrobiološke kulture koja se često koristi u Srbiji. **Metode.** Ovim prospektivnim istraživanjem obuhvaćeno je 505 asimptomatskih trudnica kod kojih je rađen MPNPVS, test sa 10% KOH, određivan pH vagine i standardna kultura cervikovaginalnog brisa. Kombinujući rezultate ovih procedura postavljana je dijagnoza bakterijske vaginoze (BV) i vaginitisa. Istovremeno određivan je broj polimorfonuklearnih leukocita (PMN) u svakom uzorku i analiziran zajedno sa ostalim nalazima. Na osnovu mikroskopskog nalaza potvrđivana je ili isključivana infekcija *Candidom albicans* i *Trichomonas vaginalisom*. **Rezultati.** Kod 36 (6%) trudnica kulturom cervikovaginalnog brisa dobijeno je nekoliko vrsta aeroba i fakultativnih anaeroba, dok je kod 52 (11%) žena izolovana *Candidia albicans*. Na osnovu MPNPVS i kliničkih kriterijuma 96 (19%) žena imalo je BV, 19 (4%) vaginitis, i 72% (14%) kandidijazu. Od 115 trudnica sa BV i vaginitisom, pH 4,5 nađen je kod 5, a od 390 trudnica sa normalnim nalazom njih 83 (21%) imalo je pH 4,5. Povećan broj PMN nađen je kod 154 (30%) žena, a kod njih 83 (54%) MPNPVS bio je normalan. Specifičnost i senzitivnost KOH testa i pH vagine u proceni stanja vaginalne flore iznosili su 95% i 81%, odnosno 79% i 91%. **Zaključak.** Kultura cervikovaginalnog brisa je skup i najčešće nekoristan test u kliničkoj praksi. Upotreba jednostavnih i bržih metoda kao što su mikroskopski pregled nativnog preparata vaginalnog sekreta, test sa KOH i određivanje pH vagine daju bolje rezultate u dijagnozi, kao i prevenciji i lečenju posledica do kojih vaginalne infekcije mogu da dovedu.

Ključne reči:
vagina, bolesti; infekcija; trudnoća; dijagnostičke tehnike, akušerstvo i ginekologija; senzitivnost i specifičnost; srbija.

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Introduction

Some 15 years ago Wiesenfeld and Macio [1] drew attention to a surprisingly rare use of simple tests like vaginal fluid wet mount microscopy (VFWMM), 10% potassium hydroxide (KOH) test, and vaginal pH as well as a relatively high proportion of patients (17%) sampled for vaginal swab culture despite its unproven value in the diagnosis of common vaginal infections. Vaginal and cervical swab culture is still very common procedure in our country's everyday practice whereas simple and rapid diagnostic methods have been very rarely used. Newer culture-independent techniques have shown that vagina hosts more than 300 different microorganisms of which over 95% cannot be cultured [2–5]. It is not surprising if we take into account the fact that a microorganism has been displaced from its natural habitat where it has optimal amounts of nutrients and oxygen, optimal temperature and pH, and the presence of other microorganisms, into an artificial environment. Hence, the standard microbiological cultivation (with no use of special transport and growth media, anaerobic conditions, cocultivation etc.) results in the selection of rare microorganisms capable of surviving new harsh growth conditions. Consequently, aerobic and facultative anaerobic organisms are commonest growths of cervical and vaginal swab cultures although it is well-known that vagina is colonized mostly by anaerobes in the ratio 10 : 1 [6–9].

The aim of the study was to show that cervical and vaginal swab culture has no value in everyday clinical practice, and that the routine use of straightforward procedures (VFWMM, vaginal pH and KOH test) may result in the better diagnosis of vaginal infections and prevention of serious complications that may follow [10–14].

Methods

This prospective study included 505 pregnant and asymptomatic women (24–28 week of pregnancy) seen during a regularly planned appointments. A swab was taken from both the cervical canal and vaginal side wall, put into a sterile dry tube, and transported to the laboratory within 3 hours. Chocolate agar was used as growth medium. The second swab taken from the vaginal side wall was smeared onto a slide, covered by a drop of saline, and the mount viewed under a phase-contrast microscope (magnification 400×). After completion of VFWMM, a drop of 10% KOH was dripped onto one end of the mount in order to check for the presence of yeasts. All the patients underwent 10% KOH test and in all vaginal pH was determined by a test strip (Merck pH, range 4.0 to 7.0). The predominance of lactobacilli over small bacterial forms (SBF), negative KOH test, and vaginal pH < 4.5 were considered normal findings, irrespective of the polymorphonuclear (PMN) numbers (Figure 1a). The diagnosis of BV was made in patients whose VFWM had more SBF than lactobacilli (irrespective of the presence of clue cells), normal PMN numbers, and in those with pH > 4.5 and/or positive KOH test (Figures 1b and 1c). In contrast to Amsel's criteria, the presence of clue cells and abnormal homogenous off-white vaginal discharge were not considered obligatory criteria for the diagnosis of BV [15]. In patients with the predominance of SBF, elevated PMN numbers and pH > 4.5, in whom infections with *Candidia albicans* (10% KOH) and *Trichamonas vaginalis* had been excluded, the diagnosis of vaginitis was made (Figures 1d, 2a-d). The term aerobic vaginitis is not used as the diagnosis was not made on the basis of the original Donder's criteria [16]. The PMN number was assessed semi-quantitatively on at least 10 fields of view (FV) at 400× as follows (Figure 3): group 0 – PMN absent or much less numerous than epithelial cells (EC); group 1 – PMN seen on more than 50% of FV but their numbers still less than that of EC; group 3 – PMN seen on most FV and their numbers equal or higher than the numbers of EC; group 4 – PMN seen on most FV and their numbers much higher than the numbers of EC. The groups 0 and 1 were considered normal according to the PMN number, and the other two groups were considered pathological.

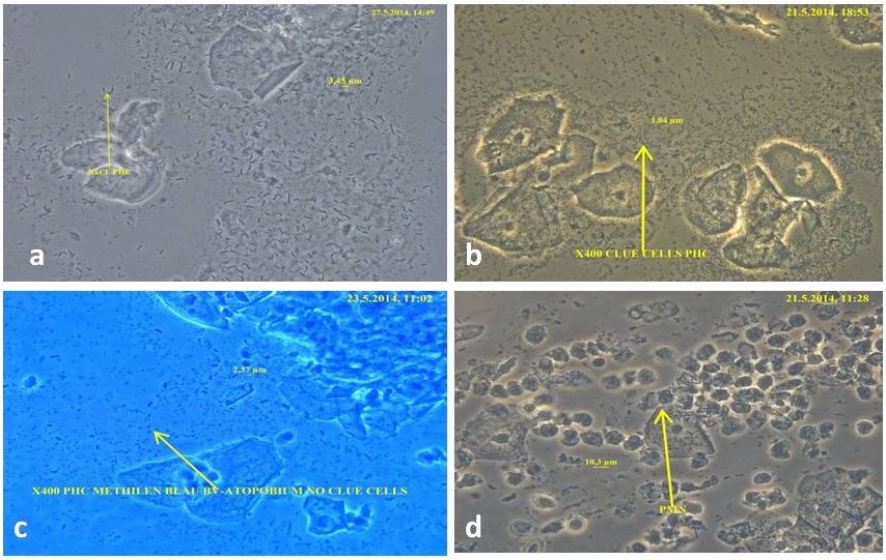

Fig. 1 – Vaginal fluid wet mount microscopy.

a) Normal flora: predominance of *lactobacilli* over small bacterial forms (SBF); b) Bacterial *vaginosis* (BV): "clue cells"; c) BV, "clue cells" absent, predominance of SBF, polymorphonuclear (PMN) numbers normal, pH > 4.5; d) vaginitis: predominance of SBF, elevated PMN numbers, pH > 4.5.

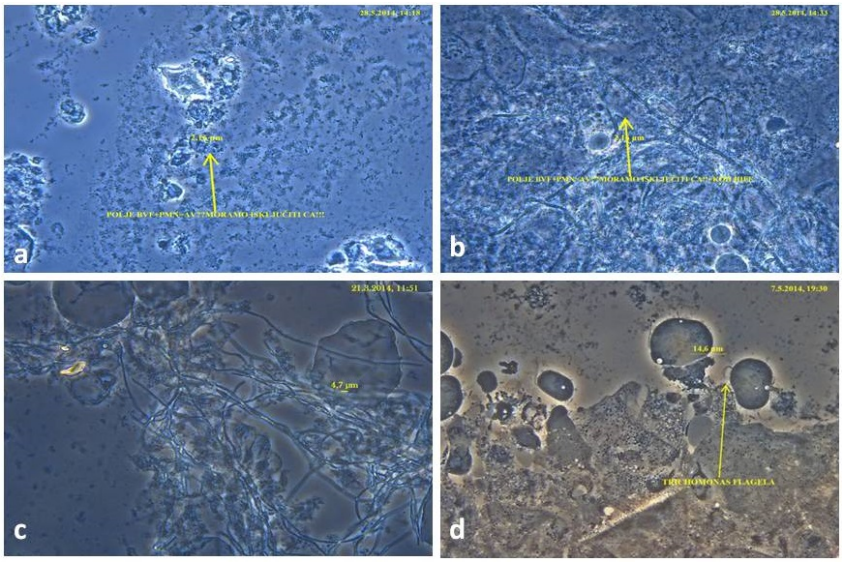

Fig. 2 – Vaginal fluid wet mount microscopy.

a)Vaginitis: predominance of SBF, elevated polymorphonuclear (PMN) numbers, pH > 4.5; b) *Candida albicans* with bacterial vaginosis; c) *Candida albicans* with normal flora; d) *Trichomonas vaginalis* with bacterial vaginosis.

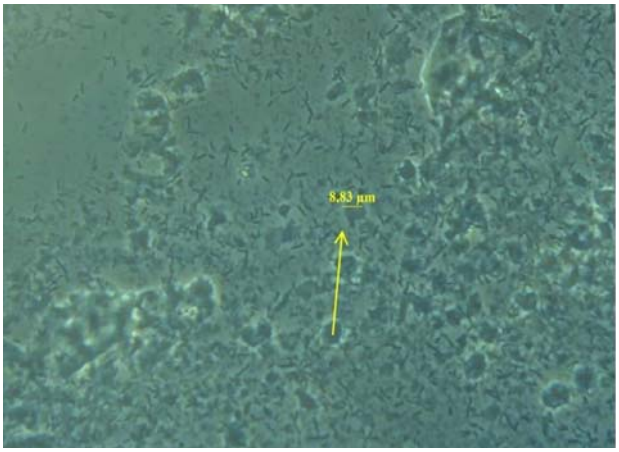

Fig. 3 – Vaginal fluid wet mount microscopy: large numbers of polymorphonuclear leukocytes (PMN) accompanied by the normal number of *Lactobacilli*.

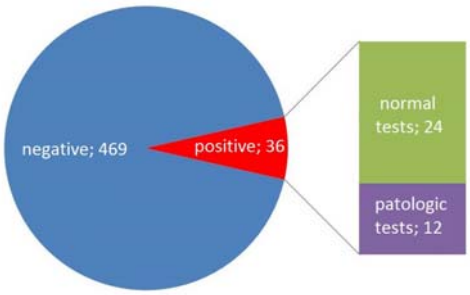

Fig. 4 – Patients with sterile and culture-positive cervicovaginal swab cultures; the latter subdivided into those with normal vaginal fluid wet mount microscopy (VFWMM), potassium hydroxide (KOH) test and vaginal pH, and those with at least one pathological of those tests.

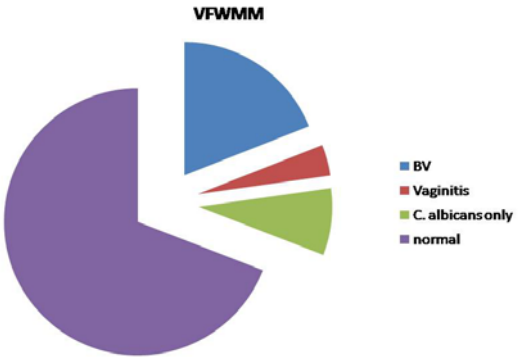

Fig. 5 – Proportion of women with positive vaginal fluid wet mount microscopy findings and diagnosis of bacterial vaginosis (BV), vaginitis, and isolated *Candida albicans*.

Results

Cervicovaginal culture and vaginal fluid wet mount microscopy

In 36 (6%) patients cervicovaginal swab cultures retrieved the following bacteria: *Coagulase negative staphylococcus* (n = 11), *Enterococcus* (n = 8), *Escherichia coli* (n = 5), *Staphylococcus aureus* (n = 5), *Streptococcus beta haemolyticus* (n = 5), *Streptococcus viridans* (n = 1), and *Proteus mirabilis* (n = 1). In 24 (66%) of those 36 patients VFWMM was normal, vaginal pH was < 4.5 and KOH test was negative (Figure 4). BV was diagnosed in 96 (19%) of the patients [concomitant *C. albicans* in 28 (30%)]. Vaginitis was confirmed in 19 (4%) of the patients of whom 3 also had *C. albicans* (Figure 5). Solely one of 19 patients with vaginitis had normal vaginal pH, and KOH test was positive in 12/19 patients. Of 72 patients in whom we microscopically observed *C. albicans*, 41 had normal background flora, i.e. the majority of bacterial forms were *Lactobacilli*.

Vaginal pH and KOH test

Of 115 patients with BV and vaginitis, 5 had pH < 4.5 (false negative finding), whereas among 390 patients with normal findings, 83 had pH > 4.5. False negative KOH test was found in 22 (of 115) patients, whereas 34 patients within the healthy group had a positive KOH test. Original authors' criteria were used as gold standards and specificity, sensitiv-

ity, positive and negative predictive values, and total precision for each of the two tests in relation to the two groups of patients were calculated. Similar values were obtained for both tests even when in both groups patients with *C. albicans* had been either retained or removed (Table 1).

Table 1

Characteristics of two simple and rapid bedside tests [vaginal pH and potassium hydoxide (KOH) test] in the diagnosis of abnormal vaginal conditions as compared against vaginal fluid wet mount microscopy (VFWMM)

Test	True positive	False positive	Sensitivity	Specificity	PPV	NPV	TP
pH 4.5	110	83	95%	79%	56%	98%	83%
KOH	93	34	81%	91%	73%	94%	89%

PPV – positive predictive value; NPV – negative predictive value; TP – total precision.

Polymorphonuclear leukocytes

Pathological numbers of PMN were found in 154 (30%) patients, of whom 83 (54%) had normal and 71 (46%) pathological VFWMM (Figure 6). Among the latter there were: BV (n = 10), BV + *C. albicans* (n = 14), vaginitis (n = 19) and *C. albicans* (n = 28). In 72 patients with the microscopic evidence of yeast infection, 45 (63%) had a pathological number of PMN, whereas within the group of patients with *C. albicans* and predominantly lactobacillar flora (41 patients), 28 (68%) had the elevated number of PMN.

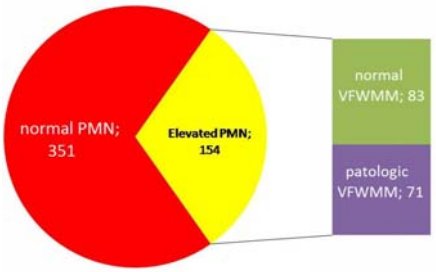

Fig. 6 – Vaginal fluid wet mount microscopy (VFWMM). Proportion of patients with elevated polymorphonuclear (PMN) numbers and their subdivision into those with positive and negative findings on the vaginal fluid wet mount microscopy.

Discussion

More than 30 years ago Hammerschlag et al. [6, 7] published results of vaginal swab microbiologic cultures in healthy, 2-month- to 15-year-old girls showing that vagina is colonized even at such an early age by a wide range of bacteria and yeasts: *Diphtheroids* (78%), *Staphylococcus epidermidis* (73%), *α-hemolytic streptococci* (39%), *Escherichia coli* (34%), *Candida species* (28%), *Ureoplasma urealyticum* (27%), *Klebsiella* (15%), *Enterococcus species* 10%, *Group D streptococci* (8.5%), *Staphylococcus aureus* (7%), *Mycoplasma hominis* (6%), *Haemophilus influenzae* (5%), *Pseudomonas aeruginosa* (5%), *Proteus* (5%). Methodologically similar studies on women in reproductive age, apart from the above bacteria, isolated, more or less frequently, other aerobes and facultative anaerobes (*Peptostreptococcus, Gardnerella, Bacteroides, Veillonella, Bifidobacterium,* etc.) [9]. We cultured similar bacteria. Isolation of any of the bacteria cannot be automatically regarded a cause of any signs or symptoms of the patients. It is clear from our study (Figures 4 and 5) that if relied on cervicovaginal swab cultures only, most women with pathological vaginal findings would have been undiagnosed and untreated. On the other hand, some patients with normal vaginal flora would be probably, due to isolation of a bacterium, unnecessarily treated. Thus, in practice we would have better results if we based our diagnosis on the examination under a speculum (precision 30%) than if culture results guided our treatment decisions. Let us discuss the value of these methods in specific vaginal conditions.

Bacterial vaginosis. In contrast to most infectious diseases that we diagnose by isolation or identification of specific microorganism, commonly without its quantification, in this polymicrobial condition we cannot pinpoint a single organism so that culture findings (including isolation of *Gardinerella vaginalis*) are useless in the diagnosis of the syndrome. The simplest but very important step during the workup would be to perform in every woman, along with the routine gynecologic exam, the test with 10% KOH and to determine the vaginal pH [17]. As seen from our results, this simple addition would enable us to select, in the majority of cases, women requiring further diagnostic workup. The workup generally means VFWMM or microscopy of Gram-stained samples, but not the cervicovaginal swab culture. In very few women the microscopic findings do not correlate with signs and symptoms and then we have to consider further microbiologic or molecular biologic tests in order to identify specific organisms like *C. albicans, T. vaginalis*, *Neisseria gonorrhoeae*. Considering a high prevalence of BV (12–50%), the fact that it is in half of cases completely asymptomatic but anyway may lead to serious complications, it is a high time to institute educational programs for our gynecologists to embrace VFWMM. In this way they may timely diagnose and treat BV, thus preventing its serious complications [8].

C. albicans. In this study, with blood agar cultivation, *C. albicans* was isolated in 56 patients (not shown), whereas fungal elements were seen on VFWMM (10% KOH) in 72 (14%) women. Obviously, special media are better suited for *C. albicans* isolation. It is known that some 15–30% patients have mixed infections. In line with this, 38% of our patients with BV had concomitant *C. albicans*. Moreover, as 63% women with *C. albicans* had elevated PMN numbers despite the fact that the majority (68%) had a predominant lactobacillar flora, it is obvious that elevated PMN numbers necessitate a search for *C. albicans*.

Vaginitis. Many forms of vaginitis have been described in the literature so far, from aerobic, cytolytic, atrophic, desquamative, and inflammatory to ulcerative but here we de-

cided to use simply the term vaginitis [18]. The crucial single feature by which we may microscopically differentiate vaginitis from BV is, in our opinion, elevated and normal PMN number, respectively. Of course, only after a prior exclusion of *C. albicans* and *T. vaginalis* infections. In both BV and vaginitis the bacterial background is very similar: more small bacterial forms than *Lactobacilli*. Microscopically during VFWMM it is impossible to make a distinction between the flora of BV (anaerobes, e.g. *Atopobium vagine*) and aerobic vaginitis (aerobes, e.g. *Coccae*). Moreover, *Lactobacillus iners* and *Gardnerella vaginalis*, the two most common bacterial species detected by molecular biological studies, are so similar phenotypically that cannot be differentiated on VFWMM under the 400× magnification. We also think that the presence of clue cells should not be a prerequisite for the diagnosis of BV during VFWMM as, by our experience, many women fulfilling other three Amsel's criteria have no clue cells. This is probably the reason why the diagnosis of BV is made more frequently according to the Nugent's criteria – most of the women are actually classified into the intermediary group [19]. Moreover, in the study, 18 of 19 women diagnosed with vaginitis had a pathological vaginal pH strengthening the view that the microscopic examination is the only way to differentiate between BV and vaginitis.

Polymorphonuclear leukocytes. Although it is a widespread belief that PMNs seen under the microscope represent a form of inflammation, a disease, it is common to encounter many women with elevated PMN and normal numbers of *Lactobacilli*. In our cohort of patients, 54% with elevated PMN had predominant lactobacillar flora. Verhelst et al. [20] have delineated a separate group of women in their new classification of Gram-stained sample analysis (so-called Claeys' criteria). Their microscopic findings are characterized by a large number of PMN accompanied by a normal number of *Lactobacilli* (Figure 3). In our study 16% of the patients had such findings without detectable *C. albicans* confirming that this group of women should be paid a special attention in future studies in order to find a cause of such unusual constellation of microscopic findings. Finally, analysis of PMN should become a part of routine VFWMM.

Until recently most of our knowledge about vaginal microbiology has come from studies based upon the culture. However, as about 95% of microorganisms within the vaginal flora cannot be cultivated, other, mostly molecular biology-based techniques are increasingly used to define vaginal microflora [21–23]. These techniques will undoubtedly help us to understand dynamics and intricate interactions among the microorganisms in maintenance of healthy vaginal environment or development of pathological conditions. This will make us to redefine terms of normal and pathologic within the vaginal ecosystem [4–8]. Bacterial communities should be analyzed not only qualitatively and quantitatively but also functionally so that we are able to define healthy vaginal microbiome of an individual woman (personalized medicine). Till that time, for everyday practice, VFWMM and/or Gram-stained vaginal samples along with vaginal pH and KOH test should be routine methods to assess the vaginal flora and discriminate between healthy and diseased.

Conclusion

Despite abundant evidence dispelling a reasonable value of culturing vaginal and/or cervical swabs in clinical practice, it is still by large the most frequent diagnostic procedure in this country, and, not uncommonly, a major tool to distinguish between normal and pathological vaginal flora. Yet, the deployment of only two simple tests (vaginal pH and KOH test) when there is no resources for microscopic examination offer much better results than standard swab cultures. Moreover, the techniques are simple, rapid and inexpensive, and may be easily performed even in the setting of primary healthcare level. The wider use of the techniques would result in better management of vaginal infections and their consequences.

REFERENCES

1. *Wiesenfeld HC, Macio I.* The infrequent use of office-based diagnostic tests for vaginitis. Am J Obstet Gynecol 1999; 181(1): 39−44.
2. *Zhou X, Bent SJ, Schneider MG, Davis CC, Islam MR, Forney LJ.* Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. Microbiology 2004; 150(Pt 8): 2565−73.
3. *Zozaya-Hinchliffe M, Lillis R, Martin DH, Ferris MJ.* Quantitative PCR assessments of bacterial species in women with and without bacterial vaginosis. J Clin Microbiol 2010; 48(5): 1812−9.
4. *Fredricks DN, Fiedler TL, Marrazzo JM.* Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med 2005; 353(18): 1899−911.
5. *Srinivasan S, Morgan MT, Liu C, Matsen FA, Hoffman NG, Fiedler TL,* et al. More than meets the eye: associations of vaginal bacteria with gram stain morphotypes using molecular phylogenetic analysis. PLoS One 2013; 8(10): e78633.
6. *Hammerschlag MR, Alpert S, Rosner I, Thurston P, Semine D, McComb D*, et al. Microbiology of the vagina in children: normal and potentially pathogenic organisms. Pediatrics 1978; 62(1): 57−62.
7. *Hammerschlag MR, Alpert S, Onderdonk AB, Thurston P, Drude E, McCormack WM,* et al. Anaerobic microflora of the vagina in children. Am J Obstet Gynecol 1978; 131(8): 853−6.
8. *Klebanoff MA, Schwebke JR, Zhang J, Nansel TR, Yu K, Andrews WW.* Vulvovaginal symptoms in women with bacterial vaginosis. Obstet Gynecol 2004; 104(2): 267−72.
9. *Jaquiery A, Stylianopoulos A, Hogg G, Grover S.* Vulvovaginitis: clinical features, aetiology, and microbiology of the genital tract. Arch Dis Child 1999; 81(1): 64−7.
10. *Larsen B, Monif GR.* Understanding the bacterial flora of the female genital tract. Clin Infect Dis 2001; 32(4): e69−77.
11. *Donders GG, Vereecken A, Dekeersmaecker A, Van Bulck B, Spitz B.* Wet mount microscopy reflects functional vaginal lactobacillary flora better than Gram stain. J Clin Pathol 2000;53(4):308-13.

12. *Hemalatha R, Ramalaxmi BA, Swetha E, Balakrishna N, Mastromarino P.* Evaluation of vaginal pH for detection of bacterial vaginosis. Indian J Med Res 2013; 138(3): 354−9.
13. *Thulkar J, Kriplani A, Agarwal N.* Utility of pH test & Whiff test in syndromic approach of abnormal vaginal discharge. Indian J Med Res 2010; 131:445-8.
14. *Pavletic AJ, Hawes SE, Geske JA, Bringe K, Polack SH.* Experience with routine vaginal pH testing in a family practice setting. Infect Dis Obstet Gynecol 2004; 12(2): 63−8.
15. *Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK.* Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983; 74(1): 14−22.
16. *Donders GG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B.* Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. BJOG 2002; 109(1): 34−43.
17. *Hemalatha R, Ramalaxmi BA, Swetha E, Balakrishna N, Mastromarino P.* Evaluation of vaginal pH for detection of bacterial vaginosis. Indian J Med Res 2013; 138(3): 354−9.
18. *Sobel JD.* Vaginitis. N Engl J Med 1997; 337(26): 1896−903.
19. *Donders GG.* Definition and classification of abnormal vaginal flora. Best Pract Res Clin Obstet Gynaecol 2007; 21(3): 355−73.
20. *Verhelst R, Verstraelen H, Claeys G, Verschraegen G, van Simaey L, de Ganck C,* et al. Comparison between Gram stain and culture for the characterization of vaginal microflora: definition of a distinct grade that resembles grade I microflora and revised categorization of grade I microflora. BMC Microbiology 2005; 5(1): 61.
21. *Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, Mcculle SL,* et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA 2011; 108(Suppl 1): 4680−7.
22. *Brotman RM, Ravel J, Cone RA, Zenilman JM.* Rapid fluctuation of the vaginal microbiota measured by Gram stain analysis. Sex Transm Infect 2010; 86(4): 297−302.
23. *Zhou X, Brown CJ, Abdo Z, Davis CC, Hansmann MA, Joyce P,* et al. Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. ISME J 2007; 1(2): 121−33.

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Angiogenesis: A special reference to corneal neovascularization

Angiogeneza: poseban osvrt na neovaskularizaciju rožnjače

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History

Angiogenesis, the term coined in 1935 [1] means the development of new blood vessels from the pre-existing capillaries and venulae. It is fundamental for reproduction, development and reparation, when strict regulation and short duration prevent the uncontrolled growth of neovascularization [2]. In pathological conditions, represented by solid tumors and a myriad of the neovascular diseases which involve retina, uvea and cornea, the disbalanced regulation leads to the lasting, life- or sight-threatening vascular proliferation.

Algire's [3] observation that the tumors actively attract blood vessels, and Michealson's [4] conclusion that a diffusible substance, factor X, present in the extravascular retina in various concentrations, is necessary for retinal neovascularization, were the beacons along the path which lead to the right direction: development of the field of angiogenesis research fifty years ago, started by the pioneering work of Folkman et al. [5]. Their hypothesis concerning the potential anticancer effects of angiogenesis inhibitors, based on the observation that the growth of solid tumors depends on their vascularization, [6] had been questioned until the isolation of a tumor factor responsible for angiogenesis [7].

Folkman's laboratory introduced new methods necessary for the new field of research: corneal pocket assay, polymers for the sustained release of macromolecules, chorioallantoic membrane, and capillary endothelial cell culture [8, 9]. Matrigel was added later [9]. These new tools helped in the discovery of the first angiostimulators: basic and acid fibroblast growth factor (bFGF, aFGF), and angiogenin [10]. Angiomodulators, most notably heparin, were added to the concept of angiogenesis [11]. Heparin antidote, protamine, was the first angioinhibitor with the known structure [12]. Then followed the discovery of a potent angioinhibitory effect of heparin in the presence of cortisone, [13–15] and of two endogenous angioihibitors, angiostatin [16] and endostatin [17].

Growth factors

A large number of molecules which stimulate or inhibit angiogenesis are known today. A key role among stimulators, especially in the eye, plays vascular endothelial growth factor (VEGF) [18, 19]. Its inhibition has been used for the therapy of various diseases of different organs, from colorectal cancer to age related macular degeneration, just because they share pathological angiogenesis in common.

VEGF is a potent hemoatracatant and endothlial cell mitogen. Its angiogenic action is regulated by hypoxia [20]. It is a dominant factor of ocular and general angiogenesis, being a perfect match for „Factor X" postulated by Michaelson [4] as early as 1948. A single gene is coding for binding VEGF A, B, C. D to the tyrosin-kinase receptors VEGFR [1–3]. Various isoforms of VEGF, created by alternate splicing, [21] enable this growth factor to act in more than one way: to stimulate angiogenesis and vascular permeability, participate in organ development and vasculogenesis, maintain small fenestrated blood vessels, and protect nerve cells in the retina and elsewhere [22, 23].

Basic fibroblast growth factor, although unable to promote neither retinal nor choroidal neovascularization alone, can act in synergism with VEGF [24]. Due to the lack of a signaling pathway for its release from cells and membranes, b FGF can act only upon their injury. Yet, its proangiogenic role is established by the findings of high levels of b FGF in the vitreous of the eyes with proliferative diabetic retinopathy, and large tumors, as well as the rodent corneal neovascularization after

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implantation of bFGF [25]. Finally, a simultaneous inhibition of both b FGF and VEGF activities in vitro is more efficient than inhibition of only VEGF [26].

Findings of the raised vitreous levels of erythropoietin in proliferative diabetic retinopathy, and inhibition of neovascularization in the ischemic murine retina indicate that this, otherwise blood-forming substance has a role in angiogenesis [27]. This complex process is also influenced by angiopoietins, cyclooxygenases [28], platelet derived growth factor (PDF), hepatocyte growth factor (HGF), placenta growth factor (PlGF), transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), and interleukines [29].

Switch to angiogenic phenotype

The sequence of events during angiogenesis is: burst of endothelial cells mitotic activity under the influence of an angiostimulator; creation of a break in the basement membrane; degradation of extracellular matrix; creation of a columnar structure through which blood starts to flow; termination of the process. For this to happen, a large number of receptors and ligands must be successively activated, while keeping a delicate balance of numerous stimulatory and inhibitory signals. Differently put, an angiogenic phenotype is achieved through a switch in the balance of angiogenesis stimulation and inhibition towards the former. As soon as the switch, usually a hypoxic stimulus, is turned on, the activity of angiogenic factors is up-regulated; [30] the shape of the cells is changed making cells succeptible to the action of angiogenic factors; [31–33] growth factors are released from their bound state; [34–36] pericytes, a barrier to angiogenesis, are lost and macrophages activated; [37–40] genes coding for angioinhibitors are inactivated; [41] proteolysis of large molecules to which angionhibitors are often bound is halted; soluble, decoy receptors, [42] like Fit-1, stop to bind angiostimulators, primarily VEGF; and notch signaling [43] starts navigating activated cells through a matrix prepared by proteinases [44] and bi-directional transmembrane receptors, integrins [45].

Corneal avascularity

One of the most useful ways towards understanding corneal angiogenesis is to study avascularity of the nomal cornea. The avascularity and easy accessability of cornea made it one of the most frequently used model of angiogenesis research [9, 46]. As Cogan [47] nicely put it: „Any theory which claims to explain corneal neovasculogenesis must account for the absence of blood vessels in the normal cornea. Unlike most other tissues, except probably cartilage, the cornea has no vessels and yet it is in immediate proximity of structures having blood vessels. No anatomic boundary separates the vascular limbus from the avascular cornea. An adequate explanation for this anatomic paradox would undoubtedly account for neovascularization of the cornea“. He believed that compactness of the normal cornea presented a barrier, while corneal edema was a condition for the invasion of blood vessels [48]. However, the shape of neovascularization induced by an isolated experimental corneal lesion led Campbell and Michaelson [49] to postulate the presence of a diffusible stimulator of blood vessel growth [49]. Ashton and Cook [50], in a lengthy critical review, added one more possible cause, hypoxia, which acted *per se* or by inducing the activity of a diffusible factor. These statements are nowadays incorporated into the growing body of recently accumulated data on angiogenesis.

All corneal layers participate in the maintenance of avascularity. The intact epithelium prevents both from corneal edema and activation of stromal proteinases, active players along the cascade of angiogenesis. This layer also contains a high expression of soluble VEGF receptors, [51] which bind and inactivate this potent mitogen of vascular endothelial cells [52]. Consequently, antagonization of one of these soluble receptors by a tripeptide modulates angiogenesis [53]. These decoy receptors are considered as the key players in maintenance of corneal avascularity. However, a recent observation of simultaneous suppression of corneal inflammation and neovascularization by netrin, [54] a member of the family of proteins similar to laminin, previously thought to be involved in neurogenesis only, adds to the complexity of the proposed mechanism. These substances seem to originate from the superficial limbus, [55, 56] possibly from its stem cells, which are likely to have a task more complex than epithelial regeneration [57]. The appearance of new blood vessels in cases with stem cell deficiency supports this line of thinking.

Both epithelial cells and keratocytes show the expression of a potent angioinhibitor, thrombospondin [58, 59]. Thrombospodins induce apoptosis of vascular endothelial cells and shield them from the bFGF activity [60]. Both epithelium and endothelium of the rat cornea show the expression of pigment epithelium derived factor (PEDF), one of the most potent angioinhibitors, which is able to block a VEGF receptor [61].

Matrix metaloproteinases (MMPs) are also expressed in various corneal cells. These zinc-dependent endopeptidases are able either to stimulate (MMP2, MMP14) or to inhibit (MMP3, MMP7) angiogenesis. The latter is achieved by degradation of collagen XIII and plasminogen, leaving active endostatin and angiostatin [62]. It has recently been shown that these enzymes can also have an anti-inflammatory effect by changing a gene expression [63].

In conclusion, corneal avascularity is maintained by homeostasis, which includes a well-known edema-preventing balance between corneal swelling pressure and dehydration, as well as an equilibrum of numerous pro- and anti-angiogenic activities.

Corneal neovascularization

Corneal neovacularization (CONV) is formed when blood vessels from the limbus penetrate the avascular corneal tissue (Figure 1).

Subepithelial neovascularization is characterized by direct arborization of blood vessels creating a pannus, which

splits the space between the epithelium and the Bowman's membrane. Interstitial new blood vessels follow the direction of collagen fibers and grow in a brush-like fashion. The deepest stromal neovascularization has an umbilical shape at first, and a membranous shape upon further growth [64]. CONV is essentially a reparatory attempt in response to hypoxia created by infection, trauma, immune reaction, tumor growth and stem cell loss. Accompanying processes and sequellae are: inflammation with cellular infiltration, edema, fibrous scarring, fatty deposit, and the loss of corneal immune privilege [60, 65]. The price of this reparatory process, which occasionally saves the ocular globe, is often high, and can be expressed in visual and aesthetic loss.

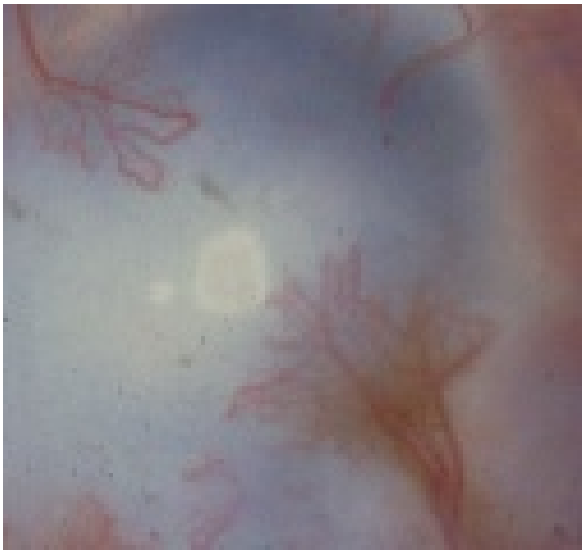

Fig. 1 – Corneal neovascularization.

Trachoma and onchocerciasis, both characterized by dramatic CONV, are among the world's most frequent causes of blindness. Their erradication needs measures that belong to economy rather that to angiogenesis research and therapy. But, about four percent of the population of the developed world also suffers from corneal neovascularization, mostly caused by herpes, with almost fifteen hundred thousand new cases every year [66]. The world statistics reports fourty thousand new cases of a drastic monocular visual loss or blindness per year [67]. Other infective agents, like pseudomonas, chlamidia and fungi are less frequent causes of visual loss. The non-infective causes of CONV are: contact lens wear, ocular surface diseases, corneal graft rejection, eye drops with preservative, and trauma, especially chemical burns. The socioeconomic significance of CONV is not negligeble, and the new treatment modalities can lessen the burden carried by many individuals and the society.

A key player in the ocular as well as corneal angiogenic cascade, like everywhere in the body, is VEGF. Its richest corneal resources are the epithelium, vascular endothelial cells, macrophages, and fibroblasts [60]. VEGF expression is significantly upregulated in inflammed and vascularized corneas [68]. On the other hand, CONV stops when VEGF or its receptors are inhibited, or when signals for VEGF release are blocked [69, 70].

Other minor factors involved in CONV are bFGF, released from basement membranes after injury; [34, 35, 71,] PDGF, which stimulates VEGF transcription and brings pericytes to block apoptosis of new vascular buds; [72] and angiopoietin [29]. Recent observations add epoxyeicosanoids to this list. These products of arachidonic acid metabolism control inflammatory and angiogenic response to injury, as a part of tissue and organ reparation and regeneration [73].

Treatment of CONV

Corticosteroids are still the mainstay of the therapy for CONV, sometimes aided by non-specific anti-inflammatory agents (NSAID) or cyclosporine. Physical methods include diathermy and photodynamic therapy, while transplantation of the limbus is beyond the scope of this review.

Corticosteroids

Corticosteroids act mainly against inflammation by prevention of neutrophyl and macrophage accumulation (a hallmark of the late sensitivity reaction), their adhesion to the capillary endothelial cells, and formation of plasminogen activator [74]. As Professor Claes Dohlman used to teach, this is why these medications helped the success of keratoplasty more than any surgical minutia.

Antiangiogenic effects of corticosteroids do not depend on their gluco- or mineralo-corticoid action. It seems to be achieved by capillary basement membrane degradation [75]. and is enhanced in the presence of heparin or its pentasacharide fragment [14, 15]. Unfortunately, these potent drugs have many side-effects: they are associated with masking of the signs of bacterial infection, progression of herpetic keratitis, and corneal melt if given later than a week after a chemical burn [76]. Prolonged topical corticosteroid therapy may cause cataract [77] and glaucoma [78].

Physical methods

Photodynamic therapy can occlude larger blood vessels. It includes an intravenous injection and the use of argon or diode laser beam. It is a costly procedure, and the injected substance may be potentially harmful [79].

Fine needle diathermy is quite easy to perform. Its best indication is occlusion of one or few larger blood vessel prior to keratoplasty. A long-lasting effect in a bunch of small vessels is hard to achieve [80, 81].

NSAID

Topical NSAID inhibits angiogenesis in rat cornea [82]. These medications inhibit cyclooxygenases, and the consequence is a low level of prostaglandines produced from arachidonic acid. Their use is limited to the early stages of angiogenesis, until accumulation of a large quantity of VEGF is created. Occasional corneal melts have been reported during the use of NSAID. Even one drop of a preservative-free NSAID can result in intense burning sensation.Therefore, caution and close observation are advised during their use [83–85].

Other medications

Well-known angioinhibitors, cyclosporine A, [86] methotrexate, [87] and tacrolimus [88] are mainly used as a substitution of corticosteroids, when a prolonged therapy after complicated keratoplasty is needed. Angioinhibitory action of thalidomide was unknown until it produced a tragic effect of inborn phocomelia [89, 90]. Thalidomide has recently been found useful in the treatment of some malignant tumors and uveitis, [91] but its use in CONV inhibition has been checked only experimentally. Amiloride, a competive inhibitor of urokinase type-plasminogen activator system, has been shown to inhibit CONV in various experimental models, [92, 93] but without a clinical use. It is interesting to speculate whether the concentrations of this drug, widely used as a diuretic, can produce inconspicuous angioinhibition.

Anti-VEGF therapy

According to a recent meta analysis, [94] a few studies have shown that a VEGF blocade may compete with corticosteroids as the therapy of choice in cases of CONV. One of the jewels in the crown of a half a century long angiogenesis research [95] was USA Food and Drug Administartion (FDA) aproval of bevacizumab, a humanized monoclonal antibody against VEGF A (Avastin, Genentech, Roche), for the adjuvant treatment of metastatic colorectal cancer first, and some other solid malignant tumors later [96, 97]. Intravitreal injections of this drug have also revolutionarized the therapy of the wet form of age-related macular degeneration, helped in the resolution of diffuse diabetic macular edema, and tried in various other ocular angiogenic diseases [98, 99]. However, the effect of topical bevacizumab in experimental CONV is only partial [100]. It is possible that a humanized antibody cannot exert a full effect in experimental animals, while the inhbition of CONV in humans is significant [101–105]. Subconjunctival and deep stromal injections of bevacizumab have been given in hope for a better effect on the deep CONV [106]. A better drug penetration into the cornea has been tried with a fragment of bevacizumab, ranibizumab, which has a small molecule and a higher affinity for VEGF. Its effect, more on the vessel diameter than on the involved surface area, has been only slighly better than the effect of bevacizumab [107]. The cost/effectiveness ratio of these two drugs calls for further investigations [69].

As previously stated, VEGF has more than one function. Possible late complications of huge doses of this drug given in oncology have not been excluded, but they are not within the scope of ophthalmology. However, corneal epithelial defects have been observed after topical application of bevacizumab [103, 108–110]. Pegabtanib, which binds only one VEGF isoform, offers less probability of complications which is unfortunately associated with a lesser effect [111]. A novel therapeutic approach, VEGF trap, is the use of a soluble receptor molecule, aflibercept, that includes sequences from VEGFR 1 and 2, and posseses a high binding affinity for VEGF-A and B, as well as for PlGF. Its prevents VEGF fom binding to its natural receptor and from promoting proliferation and migration of vascular endothelial cells. The effect of VEGF trap lasts twice as long as the effect of VEGF blockade by monoclonal antibodies [112, 113].

Another approach is silencing of a gene for VEGF production by one of ribonucleic acids, which inhibits the post-transcrptional processing [114] and signals from tyrosin-kinase receptors [115].

Targets other than VEGF

Among the substances which have also been tried for CONV inhibition are topical netrins; [54] infliximab, a monoclonal antibody against TNF-α; [116] and doxycycline. Doxycycline is a tetracycline and a potent MMP inhibitor [117]. It aseems to be one of the smart drugs, and has recently been used in oncology, [118] cardiology, [119] and neurology [120]. Its topical administration inhibits CONV in both experimental animals, [121] and in patients [122] by a MMP-independent mechanism [123]. Doxycycline acts in synergism with bevacizumab, and can additionally protect the corneal epithelium from untoward effects of the latter [124].

Conclusion

It appears that the ocular anti-angiogenic therapy in the years ahead will find more use of vascular endothelial growth factor trap and integrins, and less of corticosteroids and monoclonal antibodies against vascular endothelial growth factor. Inexpensive therapy with a well-known drug, doxycycline, might be used when corneal neovascularization is associated with disturbed epithelium. In the distant future treatment of corneal neovascularization will probably be based upon targeting a specific gene.

R E F E R E N C E S

1. *Hertig AT.* Angiogenesis in the early human chorion and in the placenta of the macaque monkey. Contrib Embryol Carnegie Inst 1935; 25(146): 37−81.
2. *Folkman J, Shing Y.* Angiogenesis. J Biol Chem 1992; 267(16): 10931−4.
3. *Algire GH.* Microscopic studies of the early growth of a transplantable melanoma of the mouse, using the transparent-chamber technique. J Natl Cancer Inst 1943; 4(1): 13−20.
4. *Michaelson IC.* The mode of development of the vascular system of the retina with some observations on its significance in certain retinal diseases. Trans Ophthalmol Soc UK 1948; 68: 137−80.
5. *Folkman J, Long D, Becker FF.* Growth and metastasis of tumor in organ culture. Cancer 1963; 16: 452−67.
6. *Folkman J.* Tumor angiogenesis: therapeutic implications. N Engl J Med 1971; 285(21): 1182−6.

7. *Folkman J, Merler E, Abernathy C, Williams G.* Isolation of a tumor factor responsible for angiogenesis. J Exp Med 1971; 133(2): 275−88.
8. *Nikolic Lj.* Angiogenesis. In: *Nikolic Lj, Danjo S*, editors. Diabetic retinopathy. Beograd: Zavod za udžbenike i nastavna sredstva; 1999. p. 75−98. (Serbian)
9. *Auerbach R, Lewis R, Shinners B, Kubai L, Akhtar N.* Angiogenesis assays: a critical overview. Clin Chem 2003; 49(1): 32−40.
10. *Folkman J, Klagsbrun M.* Vascular physiology. A family of angiogenic peptides. Nature 1987; 329(6141): 671−2.
11. *Folkman J, Klagsbrun M.* Angiogenic factors. Science 1987; 235(4787): 442−7.
12. *Taylor S, Folkman J.* Protamine is an inhibitor of angiogenesis. Nature 1982; 297(5864): 307−12.
13. *Folkman J, Langer R, Linhardt R, Haudenschild C, Taylor S.* Angiogenesis inhibition and tumor regression caused by heparin or a heparin fragment in the presence of cortisone. Science 1983; 221(4612): 719−25.
14. *Crum R, Szabo S, Folkman J.* A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. Science 1985; 230(4732): 1375−8.
15. *Nikolic L, Friend J, Taylor S, Thoft RA.* Inhibition of vascularization in rabbit corneas by heparin: cortisone pellets. Invest Ophthalmol Vis Sci 1986; 27(4): 449−56.
16. *O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M*, et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 1994; 79(2): 315−28.
17. *O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS,* et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 1997; 88(2): 277−85.
18. *Dvorak HF, Brown LF, Detmar M, Dvorak AM.* Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995; 146(5): 1029−39.
19. *Ferrara N, Gerber H, le Couter J.* The biology of VEGF and its receptors. Nat Med 2003; 9(6): 669−76.
20. *Shima DT, Adamis AP, Ferrara N, Yeo KT, Yeo TK, Allende R,* et al. Hypoxic induction of endothelial cell growth factors in retinal cells: identification and characterization of vascular endothelial growth factor (VEGF) as the mitogen. Mol Med 1995; 1(2): 182−93.
21. *Tischer E, Mitchell R, Hartman T, Silva M, Gospodarowicz D, Fiddes JC*, et al. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. J Biol Chem 1991; 266(18): 11947−54.
22. *Yamazaki Y, Morita T.* Molecular and functional diversity of vascular endothelial growth factors. Mol Divers 2006; 10(4): 515−27.
23. *D'Amore PA.* Vascular endothelial cell growth factor-a: not just for endothelial cells anymore. Am J Pathol 2007; 171(1): 14−8.
24. *Ljubimov AJ.* Growth factor synergy in angiogenesis. In: *Penn JS*, editor. Retinal and choroidal angiogenesis. Dordrecht, Nederlands: Springer; 2008. p. 289−310.
25. *Nugent MA, Iozzo RV.* Fibroblast growth factor-2. Int J Biochem Cell Biol 2000; 32(2): 115−20.
26. *Stahl A, Paschek L, Martin G, Feltgen N, Hansen LL, Agostini HT.* Combinatory inhibition of VEGF and FGF2 is superior to solitary VEGF inhibition in an in vitro model of RPE-induced angiogenesis. Graefes Arch Clin Exp Ophthalmol 2009; 247(6): 767−73.
27. *Watanabe D, Suzuma K, Matsui S, Kurimoto M, Kiryu J, Kita M,* et al. Erythropoietin as a retinal angiogenic factor in proliferative diabetic retinopathy. N Engl J Med 2005; 353(8): 782−92.
28. *Smith WL, DeWitt DL, Garavito RM.* Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000; 69: 145−82.
29. *Kwanta A.* Ocular angiogenesis: the role of growth factors. Acta Ophthalmol Scand 2006; 84(3): 282−8.
30. *Shweiki D, Itin A, Soffer D, Keshet E.* Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992; 359(6398): 843−5.
31. *Balsara RD, Merryman R, Virjee F, Northway C, Castellino FJ, Ploplis VA.* A deficiency of uPAR alters endothelial angiogenic function and cell morphology. Vascular Cell 2011; 3(1): 10.
32. *Singhvi R, Kumar A, Lopez GP, Stephanopoulos GN, Wang DI, Whitesides GM,* et al. Engineering cell shape and function. Science 1994; 264(5159): 696−8.
33. *Ingber DE.* Fibronectin controls capillary endothelial cell growth by modulating cell shape. Proc Natl Acad Sci U S A 1990; 87(9): 3579−83.
34. *Folkman J, Klagsbrun M, Sasse J, Wadzinski M, Ingber D, Vlodavsky I.* A heparin-binding angiogenic protein--basic fibroblast growth factor--is stored within basement membrane. Am J Pathol 1988; 130(2): 393−400.
35. *Nikolic LB.* Angiogenesis in rabbit cornea after disruption of descemet's membrane in the presence of heparin. Vestn Oftalmol 1998; 114(4): 35−8.
36. *Wang F, Sloss C, Zhang X, Lee SW, Cusack JC.* Membrane-bound heparin-binding epidermal growth factor like growth factor regulates E-cadherin expression in pancreatic carcinoma cells. Cancer Res 2007; 67(18): 8486−93.
37. *Speiser P, Gittelsohn AM, Patz A.* Studies on diabetic retinopathy. 3. Influence of diabetes on intramural pericytes. Arch Ophthalmol 1968; 80(3): 332−7.
38. *Orlidge A, D'Amore PA.* Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. J Cell Biol 1987; 105(3): 1455−62.
39. *Ramsauer M, D'Amore PA.* Getting Tie(2)d up in angiogenesis. J Clin Invest 2002; 110(11): 1615−7.
40. *Gerhardt H, Betsholtz C.* Endothelial-pericyte interactions in angiogenesis. Cell Tissue Res 2003; 314(1): 15−23.
41. *Dameron KM, Volpert OV, Tainsky MA, Bouck N.* Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science 1994; 265(5178): 1582−4.
42. *Kendall RL, Thomas KA.* Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci U S A 1993; 90(22): 10705−9.
43. *Phng LK, Gerhardt H.* Angiogenesis: a team effort coordinated by notch. Dev Cell 2009; 16(2): 196−208.
44. *Pepper MS.* Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. Arterioscler Thromb Vasc Biol 2001; 21(7): 1104−17.
45. *Barczyk M, Carracedo S, Gullberg D.* Integrins. Cell Tissue Res 2010; 339(1): 269−80.
46. *Nikolic LJ.* Experimental keratoplasty: a model for corneal vascularization research. Srp Arh Celok Lek 1986; 113: 135−44.
47. *Cogan D.* Corneal vascularization. Invest Ophthalmol 1962;1 :253−61.
48. *Cogan DG.* Vascularization of the cornea: its experimental induction by small lesions and a new theory of its pathogenesis. Arch Ophthalmol 1949; 41(4): 406−16.
49. *Campbell FW, Michaelson IC.* Blood-vessel formation in the cornea. Br J Ophthalmol 1949; 33(4): 248−55.
50. *Ashton N, Cook C.* Mechanism of corneal vascularization. Br J Ophthalmol 1953; 37(4): 193−209.
51. *Ambati BK, Nozaki M, Singh N, Takeda A, Jani PD, Suthar T,* et al. Corneal avascularity is due to soluble VEGF receptor-1. Nature 2006; 443(7114): 993−7.
52. *Cursiefen C, Chen L, Saint-Geniez M, Hamrah P, Jin Y, Rashid S,* et al. Nonvascular VEGF receptor 3 expression by corneal epithelium maintains avascularity and vision. Proc Natl Acad Sci U S A 2006; 103(30): 11405−10.
53. *Ponticelli S, Marasco D, Tarallo V, Albuquerque RJ, Mitola S, Takeda A,* et al. Modulation of angiogenesis by a tetrameric

tripeptide that antagonizes vascular endothelial growth factor receptor 1. J Biol Chem 2008; 283(49): 34250−9.
54. *Han Y, Shao Y, Lin Z, Qu Y, Wang H, Zhou Y,* et al. Netrin-1 simultaneously suppresses corneal inflammation and neovascularization. Invest Ophthalmol Vis Sci 2012; 53(3): 1285−95.
55. *Davanger M, Evensen A.* Role of the pericorneal papillary structure in renewal of corneal epithelium. Nature 1971; 229(5286): 560−1.
56. *Ebato B, Friend J, Thoft RA.* Comparison of central and peripheral human corneal epithelium in tissue culture. Invest Ophthalmol Vis Sci 1987; 28(9): 1450−6.
57. *Ti S, Grueterich M, Espana EM, Touhami A, Anderson DF, Tseng SC.* Correlation of long term phenotypic and clinical outcomes following limbal epithelial transplantation cultivated on amniotic membrane in rabbits. Br J Ophthalmol 2004; 88(3): 422−7.
58. *Armstrong DJ, Hiscott P, Batterbury M, Kaye S.* Corneal stromal cells (keratocytes) express thrombospondins 2 and 3 in wound repair phenotype. Int J Biochem Cell Biol 2002; 34(6): 588−93.
59. *Sekiyama E, Nakamura T, Cooper LJ, Kawasaki S, Hamuro J, Fullwood NJ,* et al. Unique distribution of thrombospondin-1 in human ocular surface epithelium. Invest Ophthalmol Vis Sci 2006; 47(4): 1352−8.
60. *Azar DT.* Corneal angiogenic privilege: angiogenic and antiangiogenic factors in corneal avascularity, vasculogenesis, and wound healing (an American Ophthalmological Society thesis). Trans Am Ophthalmol Soc 2006; 104: 264−302.
61. *Cai J, Jiang WG, Grant MB, Boulton M.* Pigment epithelium-derived factor inhibits angiogenesis via regulated intracellular proteolysis of vascular endothelial growth factor receptor 1. J Biol Chem 2006; 281(6): 3604−13.
62. *Pepper MS.* Extracellular proteolysis and angiogenesis. Thromb Haemost 2001; 86(1): 346−55.
63. *Khamis ZI, Zorio DA, Chung LW, Sang QA.* The Anti-inflammatory Role of Endometase/Matrilysin-2 in Human Prostate Cancer Cells. J Cancer 2013; 4(4): 296−303.
64. *Mann I, Pirie A, Pullinger BD.* An experimental and clinical study of the reaction of the anterior segment of the eye to chemical injury, with special reference to chemical warfare agents. Br J Ophthalmol 1948; 13(Suppl): 171.
65. *Chang JH, Gabison EE, Kato T, Azar DT.* Corneal neovascularization. Curr Opin Ophthamol 2001; 12(4): 242−9.
66. *Lee P, Wang CC, Adamis AP.* Ocular neovascularization: an epidemiologic review. Surv Ophthalmol 1998; 43(3): 245−69.
67. *Farooq AV, Shukla D.* Herpes simplex epithelial and stromal keratitis: an epidemiologic update. Surv Ophthalmol 2012; 57(5): 448−62.
68. *Philipp W, Speicher L, Humpel C.* Expression of vascular endothelial growth factor and its receptors in inflamed and vascularized human corneas. Invest Ophthalmol Vis Sci 2000; 41(9): 2514−22. 69.
69. *Stevenson W, Cheng S, Dastjerdi MH, Ferrari G, Dana R.* Corneal neovascularization and the utility of topical VEGF inhibition: ranibizumab (Lucentis) vs bevacizumab (Avastin). Ocul Surf 2012; 10(2): 67−83.
70. *Kim LA, D'Amore PA.* A brief history of anti-VEGF for the treatment of ocular angiogenesis. Am J Pathol 2012; 181(2): 376−9.
71. *Hajrasouliha AR, Sadrai Z, Chauhan SK, Dana R.* b-FGF induces corneal blood and lymphatic vessel growth in a spatially distinct pattern. Cornea 2012; 31(7): 804−9.
72. *Song S, Ewald AJ, Stallcup W, Werb Z, Bergers G.* PDGFRbeta+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. Nat Cell Biol 2005; 7(9): 870−9.
73. *Panigrahy D, Kalish BT, Huang S, Bielenberg DR, Le HD, Yang J,* et al. Epoxyeicosanoids promote organ and tissue regeneration. Proc Natl Acad Sci U S A 2013; 110(33): 13528−33.
74. *Haynes RC, Murad F.* Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of adrenocortical steroid biosynthesis. In: Gilman AG, *Goodman LS, Rall TW, Murad F*, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. New York: Macmillan Publishing Company; 1985. p. 1459−89.
75. *Ingber DE, Madri JA, Folkman J.* A possible mechanism for inhibition of angiogenesis by angiostatic steroids: induction of capillary basement membrane dissolution. Endocrinology 1986; 119(4): 1768−75.
76. *Renfro L, Snow JS.* Ocular effects of topical and systemic steroids. Dermatol Clin 1992; 10(3): 505−12.
77. *Thorne JE, Woreta FA, Dunn JP, Jabs DA.* Risk of cataract development among children with juvenile idiopathic arthritis-related uveitis treated with topical corticosteroids. Ophthalmology 2010; 117(7): 1436−41.
78. *Bremer F.* Origin of corticosteroid glaucoma. Bull Soc Belge Ophtalmol 2007; (304): 111−6. (French)
79. *Sheppard JD, Epstein RJ, Lattanzio FA, Marcantonio D, Williams PB.* Argon laser photodynamic therapy of human corneal neovascularization after intravenous administration of dihematoporphyrin ether. Am J Ophthalmol 2006; 141(3): 524−9.
80. *Pillai CT, Dua HS, Hossain P.* Fine needle diathermy occlusion of corneal vessels. Invest Ophthalmol Vis Sci 2000; 41(8): 2148−53.
81. *Maguire MG, Stark WJ, Gottsch JD, Stulting RD, Sugar A, Fink NE,* et al. Risk factors for corneal graft failure and rejection in the collaborative corneal transplantation studies. Collaborative Corneal Transplantation Studies Research Group. Ophthalmology 1994; 101(9): 1536−47.
82. *Takahashi K, Saishin Y, Saishin Y, Mori K, Ando A, Yamamoto S,* et al. Topical nepafenac inhibits ocular neovascularization. Invest Ophthalmol Vis Sci 2003; 44(1): 409−15.
83. *Zanini M, Savini G, Barboni P.* Corneal melting associated with topical diclofenac use after laser-assisted subepithelial keratectomy. J Cataract Refract Surg 2006; 32(9): 1570−2.
84. *Asai T, Nakagami T, Mochizuki M, Hata N, Tsuchiya T, Hotta Y.* Three cases of corneal melting after instillation of a new nonsteroidal anti-inflammatory drug. Cornea 2006; 25(2): 224−7.
85. *Moisseiev E, Varssano D.* Comparison of Ocular Tolerability Between Preserved and Preservative-Free Diclofenac Sodium Drops. J Ocul Pharmacol Ther 2011; 27(4): 333−7.
86. *Lipman RM, Epstein RJ, Hendricks RL.* Suppression of corneal neovascularization with cyclosporine. Arch Ophthalmol 1992; 110(3): 405−7.
87. *Joussen AM, Kruse FE, Völcker HE, Kirchhof B.* Topical application of methotrexate for inhibition of corneal angiogenesis. Graefes Arch Clin Exp Ophthalmol 1999; 237(11): 920−7.
88. *Sloper CM, Powell RJ, Dua HS.* Tacrolimus (FK506) in the management of high-risk corneal and limbal grafts. Ophthalmology 2001; 108(10): 1838−44.
89. *D'Amato RJ, Loughnan MS, Flynn E, Folkman J.* Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci U S A 1994; 91(6): 4082−5.
90. *McBride WG.* Thalidomide and congenital anomalies. Lancet 1961; 2: 1358.
91. *Parentin F, da Pozzo S, Lepore L, Perissutti P.* Thalidomide effectiveness for bilateral chronic idiopathic anterior uveitis in a three-year-old child. Ophthalmologica 2001; 215(1): 70−3.
92. *Avery RL, Connor TB, Farazdaghi M.* Systemic amiloride inhibits experimentally induced neovascularization. Arch Ophthalmol 1990; 108(10): 1474−6.
93. *Ignjatović Z, Nikolić L.* Inhibition of angiogenesis in the cornea with amiloride. Srp Arh Celok Lek 1996; 124(5-6): 120−3. (Serbian)
94. *Papathanassiou M, Theodoropoulou S, Analitis A, Tzonou A, Theodossiadis PG.* Vascular endothelial growth factor inhibitors for treatment of corneal neovascularization: a meta-analysis. Cornea 2013; 32(4): 435−44.
95. *Cao Y, Arbiser J, D'Amato RJ, D'Amore PA, Ingber DE, Kerbel R,* et al. . Forty-year journey of angiogenesis translational research. Sci Transl Med 2011; 114(3): 114rv3.

96. *Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W,* et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350(23): 2335−42.
97. *Shih T, Lindley C.* Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. Clin Ther 2006; 28(11): 1779−802.
98. *Gunther JB, Altaweel MM.* Bevacizumab (Avastin) for the treatment of ocular disease. Surv Ophthalmol 2009; 54(3): 372−400.
99. *Ristic D, Vukosavljevic M, Draganic B, Cerovic V, Petrovic N, Janicijevic-Petrovic M.* The effect of intravitreal administration of bevacizumab on macular edema and visual acuity in age-related macular degeneration with subfoveolar choroidal neovascularisation. Vojnosanit Pregl 2013; 70(7): 660−3.
100. *Manzano RP, Peyman GA, Khan P, Carvounis PE, Kivilcim M, Renet M,* et al. Inhibition of experimental corneal neovascularisation by bevacizumab (Avastin). Br J Ophthalmol 2007; 91(6): 804−7.
101. *de Stefano J, Kim T.* Topical bevacizumab therapy for corneal neovascularization. Arch Ophthalmol 2007; 125(6): 834−6.
102. *Bock F, Koenig Y, Kruse F, Baier M, Cursifen C.* Bevacizumab(Avastin) eye drops inhibit corneal neovacularization. Graefes Arch Clin Exp Ophthalmol 2008; 246(2): 281−4.
103. *Kim SW, Ha BJ, Kim EK, Tchah H, Kim TI.* The effect of topical bevacizumab on corneal neovascularization. Ophthalmology 2008; 115(6): e33−8.
104. *Dastherdi MH, Al-Arafaj KM, Nallasamy N, Pineda R, Pavan-Langston D, Dana R.* Topical bevacizumab in the treatment of corneal neovascularization; results of a prospective, open-label, non-comparative study. Arch Ophthalmol 2009; 127(4): 381−9.
105. *Cheng SF, Dastjerdi MH, Ferrari G, Okanobo A, Bower KS, Ryan DS,* et al. Short-term topical bevacizumab in the treatment of stable corneal neovascularization. Am J Ophthalmol 2012; 154(6): 940−8e1.
106. *Young SN, Lichtinger A, Kim P, Mohammadpour M.* Combined use of subconjunctival and intracorneal bevacizumab injection for corneal neovascularization. Cornea 2011; 30(10): 1110−4.
107. *Ferrari G, Dastjerdi MH, Okanobo A, Cheng S, Amparo F, Nallasamy N,* et al. Topical ranibizumab as a treatment of corneal neovascularization. Cornea 2013; 32(7): 992−7.
108. *Kim TI, Chung JL, Hong JP, Min K, Seo KY, Kim EK.* Bevacizumab application delays epithelial healing in rabbit cornea. Invest Ophthalmol Vis Sci 2009; 50(10): 4653−9.
109. *Kim EC, Ryu HW, Lee HJ, Kim MS.* Bevacizumab eye drops delay corneal epithelial wound healing and increase the stromal response to epithelial injury in rats. Clin Experiment Ophthalmol 2013; 41(7): 694−701.
110. *Koenig Y, Bock F, Horn F, Kruse F, Straub K, Cursiefen C.* Short- and long-term safety profile and efficacy of topical bevacizumab (Avastin) eye drops against corneal neovascularization. Graefes Arch Clin Exp Ophthalmol 2009; 247(10): 1375−82.
111. *Pieramici DJ, Rabena MD.* Anti-VEGF therapy: comparison of current and future agents. Eye 2008; 22(10): 1330−6.
112. *Oliveira HB, Sakimoto T, Javier JA, Azar DT, Wiegand SJ, Jain S,* et al. VEGF Trap (R1R2) suppresses experimental corneal angiogenesis. Eur J Ophthalmol 2010; 20(1): 48−54.
113. *Stewart MW.* Aflibercept (VEGF Trap-Eye) for the treatment of exudative age-related macular degeneration. Expert Rev Clin Pharmacol 2013; 6(2): 103−13.
114. *Dykxhoorn DM, Novina CD, Sharp PA.* Killing the messenger: short RNAs that silence gene expression. Nat Rev Mol Cell Biol 2003; 4(6): 457−67.
115. *Hos D, Bock F, Dietrich T, Onderka J, Kruse FE,* Thierauch KH, et al. Inflammatory corneal (lymph)angiogenesis is blocked by VEGFR-tyrosine kinase inhibitor ZK 261991, resulting in improved graft survival after corneal transplantation. Invest Ophthalmol Vis Sci 2008; 49(5): 1836−42.
116. *Kim JW, Chung SK.* The effect of topical infliximab on corneal neovascularization in rabbits. Cornea 2013; 32(2): 185−90.
117. *Moses MA, Harper J, Folkman J.* Doxycycline treatment for lymphangioleiomyomatosis with urinary monitoring for MMPs. N Engl J Med 2006; 354(24): 2621−2.
118. *Golub LM, Lee HM, Ryan ME, Giannobile WV, Payne J, Sorsa T.* Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. Adv Dent Res 1998; 12(2): 12−26.
119. *Cerisano G, Buonamici P, Valenti R, Sciagrà R, Raspanti S, Santini A,* et al. Early short-term doxycycline therapy in patients with acute myocardial infarction and left ventricular dysfunction to prevent the ominous progression to adverse remodelling: the TIPTOP trial. Eur Heart J 2014; 35(3): 184−91.
120. *Malik YS, Sheikh MA, Zhu X.* Doxycycline can stimulate cytoprotection in neural stem cells with oxygen-glucose deprivation-reoxygenation injury: a potential approach to enhance effectiveness of cell transplantation therapy. Biochem Biophys Res Commun 2013; 432(2): 355−8.
121. *Aydin E, Kivilcim M, Peyman GA, Esfahani MR, Kazi AA, Sanders DR.* Inhibition of experimental angiogenesis of cornea by various doses of doxycycline and combination of triamcinolone acetonide with low-molecular-weight heparin and doxycycline. Cornea 2008; 27(4): 446−53.
122. *Jovanovic V, Nikolic L.* The effect of topical doxycycline on corneal neovascularization. Curr Eye Res 2014; 39(2): 142−8.
123. *Gilbertson-Beadling S, Powers EA, Stamp-Cole M, Scott PS, Wallace TL, Copeland J,* et al. The tetracycline analogs minocycline and doxycycline inhibit angiogenesis in vitro by a non-metalloproteinase-dependent mechanism. Cancer Chemother Pharmacol 1995; 36(5): 418−24.
124. *Su W, Li Z, Li Y, Lin M, Yao L, Liu Y,* et al. Doxycycline enhances the inhibitory effects of bevacizumab on corneal neovascularization and prevents its side effects. Invest Ophthalmol Vis Sci 2011; 52(12): 9108−15.

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CASE REPORTS

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Does thrombolytic therapy harm or help in ST elevation myocardial infarction (STEMI) caused by the spontaneous coronary dissection?

Da li trombolitička terapija utiče negativno ili pozitivno na infarkt miokarda sa ST-elevacijom (STEMI) nastao spontanom disekcijom koronarne arterije?

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Abstract

Introduction. Spontaneous coronary artery dissection (SCAD) is a very rare disease with poor prognosis. It mainly affects young women free of risk factors for coronary artery disease (CAD) and women during the peripartum period. The prognosis for myocardial infarction caused by SCAD is poor, management is often difficult and guidelines still missing. **Case report.** We presented a woman with acute myocardial infarction of anterior wall of the left ventricle, caused by spontaneous dissection of medial segment of the left anterior descending coronary artery. We treated the patient with thrombolytic therapy and performed coronary angiography after that. Finally we decided to do nothing more. Two years later we performed coronary angiography again and founded the coronary artery normal. We also analyzed 19 cases publiched from 1996 to 2012 when coronary artery dissection had been treated with thrombolytic agent. Analysis revealed only one case of 19, with complication after treating SCAD with thrombolysis. **Conclusion.** Sometimes, regarding myocardial infarction in young women with no risk factors for CAD, especially in young women in peripartum, we should think about SCAD. The presented case, like eight others, demonstrates that good clinical outcomes can be achieved with thrombolysis. In spite of all this, we still need more data to verify that thrombolysis does not have to harm the therapy for SCAD. For the time being thrombolytic therapy could be an option.

Key words:
acute coronary syndrome; aneurysm dissecting; myocardial infarction; fibrinolytic agents; treatment outcome.

Apstrakt

Uvod. Spontana disekcija koronarne arterije (SDKA) predstavlja veoma retku bolest sa lošom prognozom. Obično se javlja kod mladih žena bez faktora rizika od koronarne arterijske bolesti (KAB) i kod žena za vreme periporođajnog perioda. Prognoza infarkta miokarda uzrokovanog SDKA je loša, lečenje je često teško i preporuke još uvek ne postoje. **Prikaz bolesnika.** Prikazali smo ženu sa akutnim infarktom miokarda prednjeg zida leve komore, prouzrokovanog spontanom disekcijom medijalnog segmenta prednje descendentne arterije. Lečenje smo započeli trombolitičkom terapijom i urađena je koronarna angiografija nakon toga. Dve godine kasnije na ponovljenoj koronarnoj angiografiji nađene su normalne koronarne arterije. Takođe, analizirani su prikazi 18 bolesnika objavljeni u periodu od 1996. do 2012. godine, zajedno sa prikazom našeg bolesnika, kod kojih je disekcija koronarne arterije lečena trombolitičkom terapijom. Ustanovljeno je da je samo kod jednog bolesnika, od njih 19, opisana komplikacija nakon primene trombolitičke terapije u lečenju SDKA. **Zaključak.** Ponekad, kad imamo infarkt miokarda kod mladih žena bez faktora rizika od KAB, posebno kod žena u periporođajnom periodu, treba misliti na SDKA. Primer naše bolesnice, kao i osam drugih, pokazuje da se dobri klinički rezultati mogu postići trombolizom. Uprkos svemu tome, još uvek je potrebno više podataka kako bi se potvrdilo da tromboliza ne šteti u terapiji SDKA. Za sada, trombolitička terapija može biti jedna od opcija.

Ključne reči:
akutni koronarni sindrom; aneurizma, disekantna; infarkt miokarda; fibrinolitici; lečenje, ishod.

Introduction

Spontaneous coronary artery dissection (SCAD) is a rare, underdiagnosed pathology with a very poor prognosis. The first report on SCAD was by Pretty in 1931, while the first angiographic diagnosis was made in 1978. Fewer than 400 cases have been reported in the literature [1]. SCAD is a poorly understood cause of myocardial infarction. It occurs in relatively young persons and represents a tiny proportion (0.07–1.1%) of patients undergoing angiography in most registries and series.

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Among reported case series ranging from 3 to 47 cases, there is the approximate 2 : 1 female predominance. About one third of the cases in women occur in the peripartum period. The clinical presentation of SCAD depends on the extent and the flow limiting severity of the coronary dissection, and ranges from asymptomatic to unstable angina, acute myocardial infarction, and ventricular arrhythmias to sudden cardiac death, and may be responsible for as many as 1 of 10 episodes of acute coronary syndrome in women younger than 50 years [2, 3, 4]. Currently, clinical recognition of SCAD has increased as coronary angiography is utilised frequently in the clinical evaluation of patients with acute coronary syndromes. Moreover, intracoronary imaging techniques such as intravascular ultrasound (IVUS) and optical coherence tomography (OCT) have enabled a more detailed clinical assessment of SCAD [3, 4].

The etiology of SCAD is unclear. Approximately one third of women with SCAD are pregnant or peripartum. Hormonal effects in the vessel wall such as reduced collagen synthesis, smooth muscle cell proliferation, and abnormalities in the proteoglycan matrix are implicated and may also explain cases of SCAD seen with oral contraceptive pill use [4–6]. Dissection is caused when there is bleeding into the media of the artery, separating the vessel layers with subadventitial hematoma in the false lumen, compressing the true lumen to varying degrees [7].

The left anterior descending (LAD) coronary artery is the most frequently involved vessel in autopsy and angiographic series of the LAD artery accounts on average for 60% of cases [3, 6, 7]. Patients have been treated successfully with medical therapy, coronary stenting, and coronary artery bypass grafting, depending on the extent and location of disease. In patients who have completed infarctions without residual ischemic symptoms medical therapy has been associated with good long-term outcomes. The role of thrombolysis in patients with ST elevation myocardial infarction (STEMI) is controversial [3, 4, 8].

In this article, we reported the case of STEMI caused by spontaneous coronary artery dissection, showing regression after conservative medical treatment. The management options and complications were discussed.

Case report

A 48-year-old female was admitted to the Emergency Department because of intense retrosternal pain of one hour duration and an electrocardiographic pattern of acute ST elevation anterior myocardial infarction (Figure 1). The patient was admitted to the intensive care unit and treated with intravenous thrombolysis (t-PA). We also administrated glycoprotein IIb/IIIa inhibitor (tirofiban), aspirin, clopidogrel, heparin, nitrates, and beta-blocker, and the patient's clinical status progressively improved. After the given therapy there was more than 50% resolution of ST segment elevation in leads V3, V4 and V5. Serial measurement of biochemical markers was consistent with myocardial necrosis. One day after admission we performed coronary angiography and found SCAD of the medial part of the LAD artery (Figure 2) with TIMI I-II flow in distal part of LAD. Neither were there atherosclerotic lesions in the affected vessel nor in the other coronary arteries and we decided to do nothing except medical therapy. After 5 days

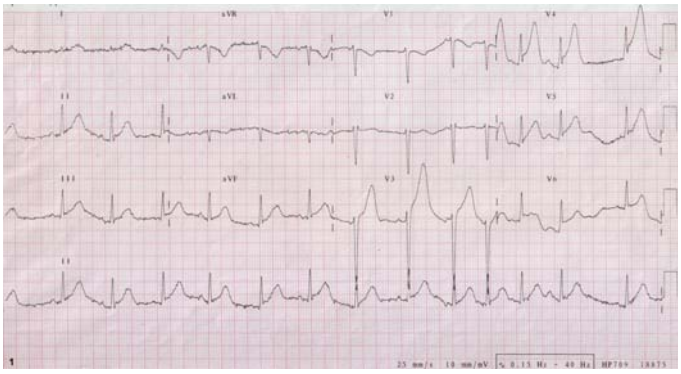
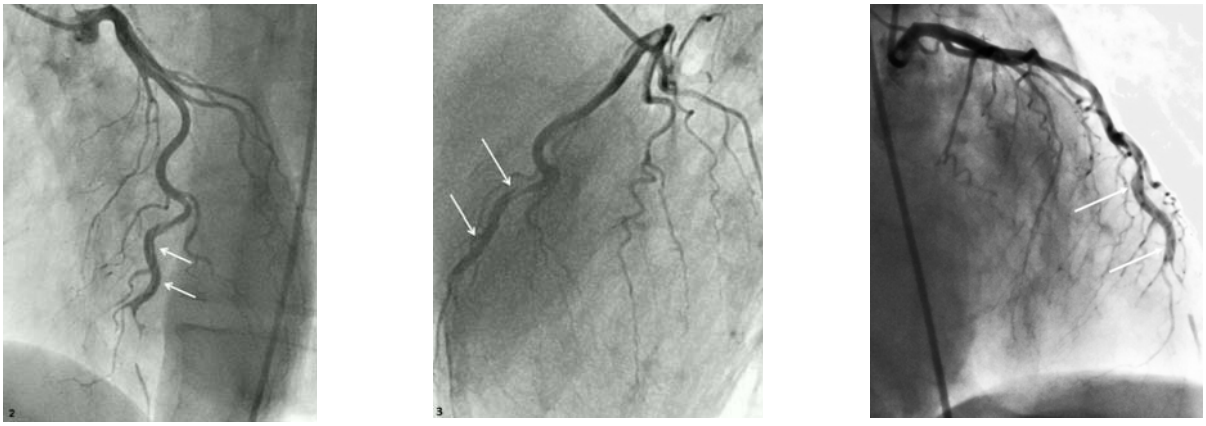


Fig. 1 – ECG record during myocardial infarction.

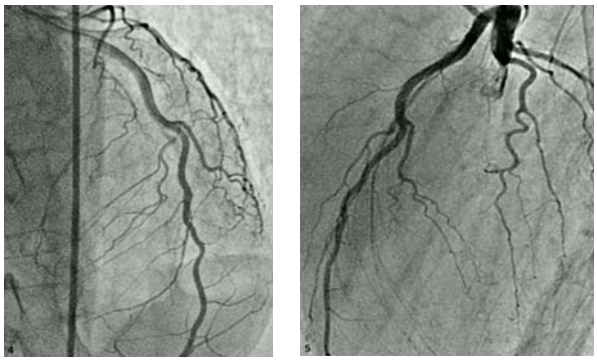


Figs. 2 – Coronary angiography of the left anterior descending coronary artery during myocardial infarction (arrows point dissection).

brain (B-type) natriuretic peptide (BNP) was highly elevated. Echocardiography performed two weeks post-admission revealed the akinetic apex and apical segments of the anterior and the inferior wall of the left ventricle. The global systolic performance of the left ventricle was satisfactory with the estimated ejection fraction of 45–50%. The patient was a smoker without any other conventional cardiovascular risk factor for coronary artery disease. In the thirteenth year of life she had infective endocarditis. She suffered from obsessive compulsive disorder and regularly visited the psychiatrist. Her past medical history was unremarkable and she denied any use of vasoconstricting or recreational drugs. She took no oral contraceptives (estrogen plus progestin) and had no a history of thromboembolic disease. All laboratory investigations performed in order to assess the inflammatory risk, coagulation abnormalities, as well as autoimmune disorders were found to be within normal limits. The patient had an uneventful clinical course and was discharged on a beta-blocker, aspirin, clopidogrel, statin and an ACE inhibitor.

The patient remained asymptomatic and 2 years later was subjected to second coronary angiography, which showed complete healing of the previous LAD dissection (Figure 3). There was normal sinus rhythm on ECG, with micro R wave in V2 and V3 with slightly negative T wave in D2, D3, aVF and from V4 to V6 (Figure 4).

We also retrieved literature (source PubMed) dealing with trombolytic therapy of SCAD. The retrieving process is presented in Figure 5. We identified 18 case reports in which



Figs. 3 – Coronary angiography of the left anterior descending corronary artery 2 years after myocardial infarction.

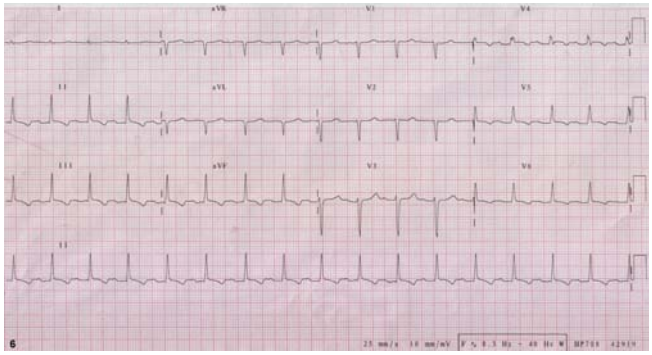


Fig. 4 – ECG record 2 years after myocardial infarction.

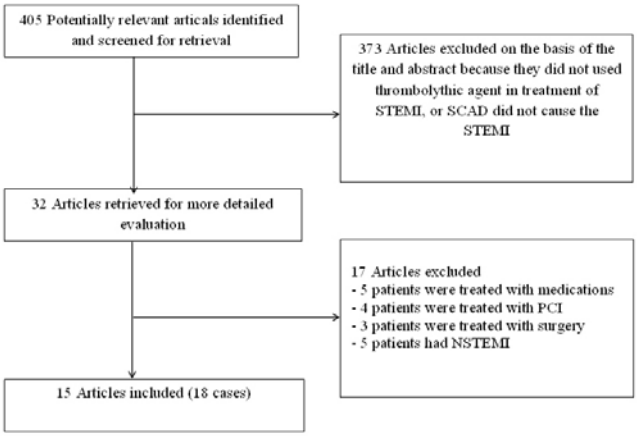


Fig. 5 – Flow chart of the case selection process.
SCAD – spontaneous coronary artery dissection; STEMI – ST segment elevation myocardial infarction; NSTEMI – non ST segment elevation myocardial infarction.

trombolytics had been used in treatment of SCAD. Analysis of these cases including own one (this case) revealed only one case with complications as a consequence of thrombolytic use (Table 1).

Discussion

SCAD is a very rare disease with poor prognosis occurring more commonly in women, particularly in the peripartum period [1, 6–8]. The majority of affected individuals (approximately 75%) are young women without risk factors for atherosclerosis, of whom approximately 30% are in the peripartum period. SCAD usually involves a single vessel (*ie*, the LAD artery in women and the right coronary artery in men) [1–6, 8]. There is no consensus on the treatment of SCAD. All three, medical, percutaneous coronary interventions and surgical approaches have been employed, but no randomized control trials have compared the three approaches. However, coronary dissection may regress spontaneously [1, 8–10].

The role of thrombolytic therapy is controversial, but there are more cases in the literature with successful thrombolysis than with complications from thrombolysis. There are several different outcomes when treating a SCAD with thrombolytic agent: firstly, thrombolytics have been accused of favoring intramedial hematoma extension and compression of the true lumen [1, 11]. Secondly, thrombolysis treatment may be effective in lysis thrombi in the false lumen, allowing the true lumen to re-expand [7, 12, 13] and finally, thrombolysis may aggravate bleeding and the dissection [4, 6, 12].

We analyzed 19 cases reports published from 1996 untill 2012 with coronary artery dissection treated with thrombolytic agent. Successful use of thrombolytic agents have been published in eight cases including our [6–8, 13–15]. In 11 cases [1, 4, 11, 16, 17], thrombolytic agents did not help to re-establish coronary flow, but only in one case complications were described that may be associated with thrombolytic therapy [18]. Two patients died, not from the effects of thrombolytic therapy, but from large myocardial infarction [1, 16].

Table 1

Thrombolytic treatment in patients with coronary artery dissection

Case report	Year	Journal	Thrombolytic	Successful	Complication
Leclercq F, et al. [13]	1996	Eur Heart J.	rt-PA	yes	no
Mahenthiran J, et al.	2000	J Natl Med Assoc	rt-PA	yes	no
S. Narasimhan, et al.	2004	IJTCVS	Streptokinase + rt-PA	no	yes? (dead)
Maeder M. et al. [4]	2005	Intern Journal of Cardiology	Reteplase	no	no
Maeder M, et al. [4]	2005	Intern Journal of Cardiology	rt-PA	no	no
Evangelou D, et al. [6]	2006	Intern Journal of Cardiology	Tenecteplase	yes	no
Pierre-Justin G, et al.	2007	Intern Journal of Cardiology	rt-PA	yes	no
Cano O, et al. [11]	2009	Intern Journal of Cardiology	Tenecteplase	no	?
T. Karaahmet T, et al. [15]	2009	Anadolu Kardiyol Derg	?	yes	no
Saadat H, et al. [21]	2009	Int J Angiol	?	no	?
Andreou AY, et al. [22]	2009	Exp Clin Cardiol	?	no	?
Andreou AY, et al. [22]	2009	Exp Clin Cardiol	?	no	?
Motreff P, et al. [1]	2010	Cardiology	?	no	yes?(dead)
Motreff P, et al. [1]	2010	Cardiology	?	no	no
Almafragi A, et al. [18]	2010	Cardiol J	?	no	?
Ito H, et al. [8]	2011	Am J Cardiol	?	yes	no
Ito H, et al. [8]	2011	Am J Cardiol	?	yes	no
Hidalgo-Urbano RJ, et al. [17]	2011	Rev Esp Cardiol	?	no	yes
Jović Z, et al.	2015	Vojnosanit Pregl (this issue)	rt-PA	yes	no

rt-PA – recombinant tissue plasminogen activator

The presented case was conservatively managed, including thrombolysis [4, 6], glycoprotein IIb/IIIa inhibitor [9, 19] and clopidogrel [4, 13, 20–23], and the patient had no recurrence of chest pain in a long-term follow-up of two years. Also, control coronary angiography after two years was normal.

Conclusion

SCAD dissection is a rare and uncommon cause of acute coronary syndrome that should be considered in young patients, particularly women, which is presented with myocardial infarction. It is known that thrombolytic therapy can

lead to complications when applying for SCAD, but there is still no clear evidence of this. In the analysis that we conducted, there was only one case of 18 previously published and this our case, which demonstrated complications after treating SCAD with thrombolysis. The presented case, like eight others, confirm that good clinical outcomes can be achieved with thrombolysis, glycoprotein IIb/IIIa inhibitors and long-term dual antiplatelet therapy. In spite of all this, we still need more data to verify that thrombolysis does not harm the therapy for SCAD.

REFERENCES

1. *Motreff P, Souteyrand G, Dauphin C, Eschalier R, Cassagnes J, Lusson JR.* Management of spontaneous coronary artery dissection: review of the literature and discussion based on a series of 12 young women with acute coronary syndrome. Cardiology 2010; 115(1): 10−8.
2. *Tweet MS, Gulati R, Aase LA, Hayes SN.* Spontaneous coronary artery dissection: a disease-specific, social networking community-initiated study. Mayo Clin Proc 2011; 86(9): 845−50.
3. *Vrints CJ.* Spontaneous coronary artery dissection. Heart 2010; 96(10): 801−8.
4. *Maeder M, Ammann P, Angelrn W, Rickli H.* Idiopathic spontaneous coronary artery dissection: incidence, diagnosis and treatment. . Int J Cardiol 2005; 101(3): 363−9.
5. *McCann AB, Whitbourn RJ.* Spontaneous coronary artery dissection: a review of the etiology and available treatment options. Heart Vessels 2009; 24(6): 463−5.
6. *Evangelou D, Letsas KP, Korantzopoulos P, Antonellis I, Sioras E, Kardaras F.* Spontaneous coronary artery dissection associated with oral contraceptive use: a case report and review of the literature. Int J Cardiol 2006; 112(3): 380−2.
7. *Mahenthiran J, Revankar R, Koka V, Hoo J, Shenoy M.* Spontaneous coronary artery dissection presenting as acute myocardial infarction. J Natl Med Assoc 2000; 92(2): 87−90.
8. *Ito H, Taylor L, Bowman M, Fry ET, Hermiller JB, van Tassel JW.* Presentation and therapy of spontaneous coronary artery dissection and comparisons of postpartum versus nonpostpartum cases. Am J Cardiol 2011; 107(11): 1590−6.
9. *Erdim R, Gormez S, Aytekin V.* Spontaneous healing of spontaneous coronary artery dissection: a case report. J Invasive Cardiol 2008; 20(8): E237−8.
10. *Kalra N, Greenblatt J, Ahmed S.* Postpartum spontaneous coronary artery dissection (SCAD) managed conservatively. Int J Cardiol 2008; 129(2): e53−5.
11. *Cano O, Almenar L, Chirivella M, Martínez L.* Idiopathic spontaneous coronary artery dissection. Clinical and pathological correlate. Int J Cardiol 2009; 133(1): e18−9.
12. *Irani F, Colyer WR Jr, Tinkel J.* Spontaneous coronary artery dissection: to treat or not to treat-2 atypical cases and a review of the literature. Am J Ther 2012; 19(1): e62−5.
13. *Leclercq F, Messner-Pellenc P, Carabasse D, Lucke N, Rivalland F, Grolleau R.* Successful thrombolysis treatment of a spontaneous left main coronary artery dissection without subsequent surgery. Eur Heart J 1996; 17(2): 320−1.
14. *Pierre-Justin G, Pierard LA.* Spontaneous coronary artery dissection in an antilles man with acute inferior myocardial infarction. Int J Cardiol 2007; 118(2): 237−40.
15. *Karaahmet T, Tigen K, Gürel E, Cevik C, Mutlu B, Başaran Y.* Spontaneous dissection of the left main coronary artery regressed with thrombolytic therapy: evaluation with multislice computed tomography angiography. Anadolu Kardiyol Derg 2009; 9(1): E2−3.
16. *Narasimhan S.* Spontaneous coronary artery dissection. IJTCVS 2004; 20: 189−91.
17. *Hidalgo-Urbano RJ, Almendro-Delia M, Villar-Rodríguez JL.* Haemopericardium in a fibrinolysis in acute myocardial infarction secondary to a spontaneous coronary artery dissection. Rev Esp Cardiol 2011; 64(6): 539−40.
18. *Almafragi A, Convens C, van den Heuvel P.* Spontaneous healing of spontaneous coronary artery dissection. Cardiol J 2010; 17(1): 92−5.
19. *Choi JW, Davidson CJ.* Spontaneous multivessel coronary artery dissection in a long-distance runner successfully treated with oral antiplatelet therapy. J Invasive Cardiol 2002; 14(11): 675−8.
20. *Cheung S, Mithani V, Watson RM.* Healing of spontaneous coronary dissection in the context of glycoprotein IIB/IIIA inhibitor therapy: A case report. Cathet Cardiovasc Interv 2000; 51(1): 95−100.
21. *Saadat H, Taherkhani M, Safi M, Vakili H, Namazi MH, Poorhoseini HR,* et al. Percutaneous treatment of spontaneous left main coronary artery dissection extending to the left anterior descending and circumflex arteries possibly triggered by thrombolytic therapy. Int J Angiol 2009; 18(3): 151−4.
22. *Andreou AY, Georgiou PA, Georgiou GM.* Spontaneous coronary artery dissection: Report of two unsuspected cases initially treated with thrombolysis. Exp Clin Cardiol 2009; 14(4): 89−92.
23. *Dakik HA, Nader GA, Arja WA, Sawaya J, Gharzuddine W.* Asymptomatic spontaneous coronary artery dissection. Clin Cardiol 2010; 33(7): E40−2.

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CASE REPORT

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Hemangioma of uterine cervix associated with high-grade squamous intraepithelial lesion

Hemangiom grlića materice udružen sa skvamoznom intraepitelnom lezijom visokog stepena

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Abstract

Introduction. Benign, especially polypoid, lesions of the cervix are common in everyday work. Rare tumors of this region are vascular ones, particularly capillary and/or cavernous hemangiomas. Cervical hemangiomas are especially rare, usually asymptomatic and only one third of the cases is clinically manifested as vaginal bleeding, polyps, etc. **Case report.** We reported a 54-year-old postmenopausal female patient who had undergone conization due to high-grade squamous intraepithelial lesion (H-SIL). Microscopic analysis of slices confirmed the existence of dysplastic changes in the endocervical epithelium and in small part in the epithelium of the gland of H-SIL type. In 2 of 15 slices, within the walls of the cervix, dilated, amplified and abnormal blood vessels lined with endothelium were observed, vaguely limited by the surrounding connective tissue of lamina propria and smooth muscle wall of the cervix. According to the pathological characteristics, the change corresponded to the hemangioma. Both changes were completely removed. **Conclusion.** In comparison with the available literature data, the presented case is the first to describe the association of hemangioma and dysplastic changes in the endocervical epithelium. Hemangioma was incidentally discovered in the histological sections of the material after the conization in a postmenopausal women.

Key words:
cervix uteri; hemangioma; uterine cervical dysplasia; comorbidity.

Apstrakt

Uvod. Benigne, posebno polipoidne, lezije grlića materice česte su u svakodnevnom radu. Ređi tumori ove regije su vaskularni tumori, i to kapilarni i/ili kavernozni hemangiomi. Hemangiomi grlića materice posebno su retki, obično su asimptomatski, a tek u trećini slučajeva klinički se mogu manifestovati kao vaginalna krvarenja, polipi i dr. **Prikaz bolesnika.** Prikazana je bolesnica, stara 54 godine, kojoj je zbog skramozne intraepitelne displazije visokog stepena (H-SIL) urađena konizacija. Mikroskopskom analizom isečaka potvrđeno je postojanje displatičnih promena u endocervikalnom epitelu i malim delom u epitelu žlezda tipa H-SIL. U 2 od 15 isečaka unutar zida grlića materice, uočeni su dilatirani, umnoženi i nepravilni krvni sudovi obloženi endotelom, nejasno ograničeni od okolnog veziva lamine proprije i glatke muskulature zida grlića materice. Prema patohistološkim karakteristikama promena je odgovarala hemangiomu. Obe promene su uklonjene u celosti. **Zaključak.** Prema nama dostupnoj literaturi, naš prikaz slučaja je prvi opisani slučaj udruženosti hemangioma i displastičnih promena endocervikalnog epitela. Hemangiom je slučajno ustanovljen na patohistološkim isečcima materijala dobijenog konizacijom kod žene u postomenopauzi.

Ključne reči:
grlić materice; hemangiom; grlić materice, displazija; komorbiditet.

Introduction

Benign vascular tumors and tumor-like lesions include a broad spectrum of clinical and pathological entities. There are numerous histological classifications of hemangiomas according to the histological type of vascular spaces (capillary, cavernous, venous), localization (cutaneous, intramuscular, etc.), the dominant view of cells (epithelial, spindle cells, etc.), the patient's age

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(juvenile, senile) and the biological behavior (true neoplasms, malformations, telangiectasia, reactive changes) [1]. This group of tumors does not include neoplasms with prominent vascular components (e.g. vascular leiomyoma).

Hemangiomas, along with lipomas, are the most common tumors of the skin and soft tissues. In the pediatric age, hemangiomas make more than 7% of all soft tissue tumors [2]. The tumors of the vascular origin in the female genital system are rare. Cavernous hemangioma of the cervix are extemely rare, benign lesions. To date, fewer than 50 cases have been reported [3]. Hemangioma of the uterine body in comparing of the cervix is more often and until 1988, 88 such cases had been described in professional literature [4].

Typically, the histological picture of hemangiomas shows that they are composed of proliferated, irregularly shaped and well-differentiated blood vessels lined by the endothelium and surrounded by pericytic cells [1, 2, 4].

Case report

We presented a 54-year-old postmenopausal women with cervical hemangioma incidentically discovered after conisation proveded by high grade squamous intraepithelial lesion (H-SIL).

On routine systematic examination, conducted one year before conization, the patient was inspected by the gynecologist. At the time of examination, the patient denied the existence of any symptoms. According to the patient's medical history she had two vaginal deliveries, two miscarriages and at that moment she was postemenopausal. After gynecological examination and colposcopy, suspected changes in the cervix was only observed which were iodine negative. For these fields, the gynecologist took a biopsy and endocervical curettage was done. Pathological analysis of the first material confirmed the existence of mild and moderate dysplasia of the endocervical epithelium and accompanying inflammation.

The bioptic material and curettage did not reveal another histopathological changes. In the upcoming period the patient was on control twice with repeated cytological analysis.

After the last gynecological examination the biopsy of cervical portion and endocervical curettage were performed again, at that moment the patient was 54-year-old, and the histopathological analysis pointed to a dysplasia, H-SIL of the surface epithelum of endocervix. Four months later, a cone biopsy was done and the material was sent to histopathological analysis.

It consisted of a cone clip from the wall of the cervix, 2 cm high and 2.8 cm in the diameter of the vaginal portion. The entire material was taken and divided into 15 slices. In the serial sections, the cervical wall had the usual macroscopic characteristics.

After the routine processing of the material [fixed in 10% formalin, cut to the thickness of 5 microns and stained with the hematoxylin-eosin (HE) method], it was histopathologically analyzed.

In just 2 from 15 specimens, there was a lesion unclearly distinguishable from the surrounding tissue and situated in the middle part of the cervical wall and with any contact by surface area and margins (Figure 1).

The lesion was made of proliferated and dilated blood vessels with a thin wall of a cavernous shape and by the erythrocytes into the lumen. The blood vessels covered by endothelial cells without atypical elements (Figure 2). Around the blood vessels, there was a proliferated and partly hyalinizated connective tissue After the analysis of the HE-stained histological sections, staining after the periodic acid schiff (PAS) and Mason methods was performed, which additionally visualized abnormal vessels and clearly defined their shape and size (Figure 3). Based on the described histological features, the diagnosis of cavernous hemangioma was made.

Each of the described changes, dysplastic epithelium and hemangioma, completely removed by conization.

In the surface epithelium of the endocervical epithelium and glands, there was a stratified sqamous epitelium with moderate and severe dysplasia (H-SIL) (Figure 4). A part of the surface endocervical epithelium was eroded.

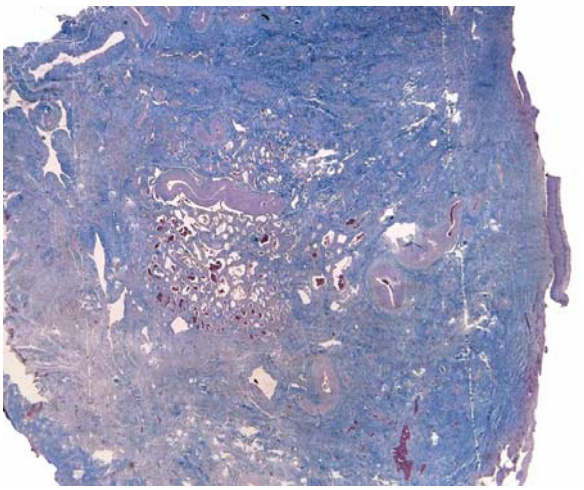

Fig. 1 – Overview of the cervical wall and hemangioma (Masson, ×20).

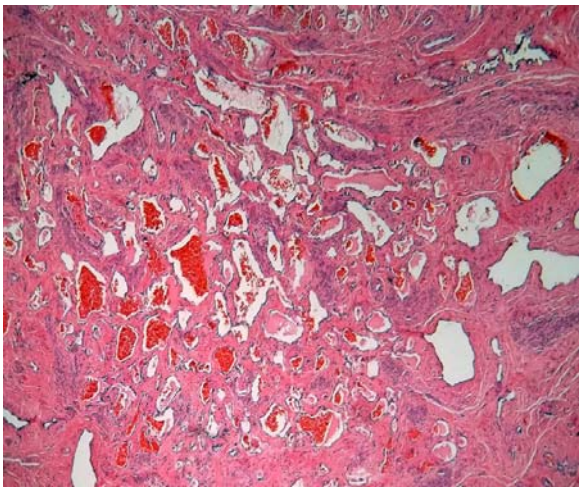

Fig. 2 – Abnormal blood vessels in the cavernous shape (HE, ×40).

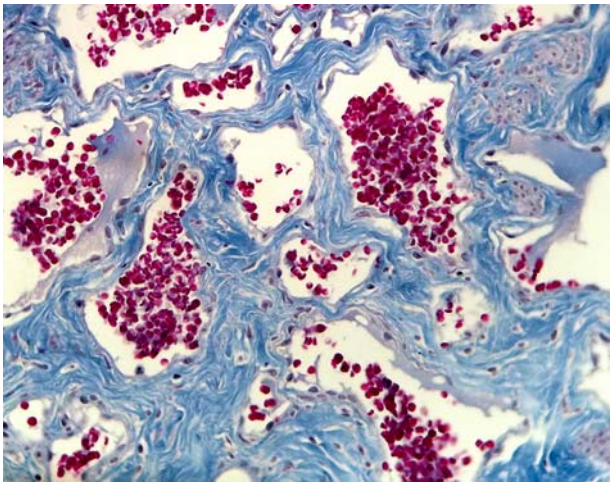

Fig. 3 – Abnormal blood vessels in the cavernous shape (Masson, ×40).

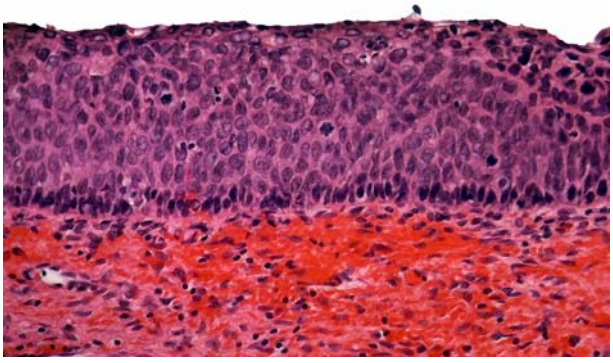

Fig. 4 – Dysplastic stratified squamous epithelium (HE, ×40).

Discussion

Hemangioma could involve either the uterine corpus and/or the cervix. The uterine body appears to be the more favored site [5].

In the retrospective study of Gupta et al. [6] they presented 10 cases of vascular tumors of the female genital tract, and just one of them was cervical cavernous hemangioma wich clinically have been presented as endocervical polip. In another study, Andola and Andola [7] presented very similar results.

The first case of diffuse uterine hemangioma was described in 1897 as an incidental finding in a young woman after the delivery of twins [8].

Compared with the hemangiomas of the uterine body, the isolated hemangioma of the cervix are even more uncommon [9–12] and were first described by Weed in 1948.

The traditional hemangiomas, including those of the female genital tract, are divided after their morphological characteristics into: capillary, cavernous and venous. Capillary hemangiomais composed of numerous intertwining capillary sized vessels lined by the endothelium. When in lesions vascular channels are considerably englared, then term cavernous hemangioma been used. They differ capillary hemangioma in that it is less well circumscribed, is larger and is usually deeper in submucosal tissues, and because asymptomatic do not cause any deformation of the cervix (as in the presented case). Histologically, according to the literature, the cervical hemangiomas are usually of the cavernous type, as in our case [13].

Hemangioma of the uterus is an mesenchymal tumor [14]. The origine of uterine hemangioma cells possibly represent pluripotent, embryonic mesodermal cells within the uterus [8].

Hemangioma is also classiffied into congenital and acquired [15, 16]. Kasznica and Nisar [17] in their paper showed a case of congenital vascular malformation of the uterus in autopsy of stillborn [17]. It was noted that approximatly 73% vascular lesion are detectable at the birth and 85% are present in first year of life [18].

Congenital hemangioma to be associated with hereditary diseases: Kippel-Trenaunay syndrome, hereditary hemorrhagic teleangiectasia, tubesous sclerosis, blue rubber bleb nevus syndrome, Maffucci syndrome and Kasabach-Merritt syndrome [15]. There is a higher incidence of some vascular tumors caused by hereditary or genetic disorders, but in some hemangiomas their genetic basis cannot be determined [10, 18].

The cause of the development of acquired vascular tumors is still unclear. Some authors believe that hemangiomas of the female genital tract occur under the influence of hormonal contraceptives or due to pregnancy [10].

Jung et al. [14] described a case of hemangima of the cervix with focal nodular hyperplasia of the liver supported the view that uterine hemangioma is associated with exogenous hormone use that causes congenital vascular tumor. In another study, Boneti et al. [12] reported that the presence of estrogen receptors on the endothelial cells was related to the development of hemangioma. Typical acquired cases of uterine hemangioma are associated with previous pelvic surgery, endometrial curettage, trophoblastic disease, endometrial carcinoma and maternal ingestion of diethylstilbestrol [8,14].

All hemangiomas have a limited power of proliferation [1, 2]. Chou and Chang [15] have suggest that different imunophenotypic profiles may also be used to classify a hemangioma in differnet phases. Congenital hemangima is usually in the proliferative phase. In this stage the endothelium is immature, plumper and SKI (v-ski sarcoma oncogene homolog) perinuclear positive. In acquired hemangioma, the endothelium is in the involuted phase.

The majority of hemangiomas of the uterine cervix are small and asymptomatic, so they are mostly discovered incidentally, during routine histopathological examination of the organ removed surgically, in the absence of gross lesion (such as in the case reported), or when this lesion is traumatized and/or ulcerated leading to clinical symptoms such as beading [6].

Just in about 35% of the published papers, the cervical hemangiomas have clinical manifestations, such as: intermenstrual spotting, menometroragia, post-menpoausal metrorrhagia, postcoital metrorrhagia, dyspareunia – gynecological simptoms or obstetrical complications: premature rupture of membrane, the fetal death *in utero*, the postpartal haemorrhage and disseminated intravascular coagulation [19].

Clinically, most cases has been reported in young pregnant women and the condition is very rare in postmenopausal pa-

tients, such is the reported case [10, 14]. Mostly, symptomatic hemangioma was discovered in young and pregnant women, probably quick growth of the lesion in pregnancy may generate complications and symptoms [20].

The differential diagnosis of hemangioma includes a cervical malignanat and benign tumors and tumor-like lesions.

Appropriate treatment form of hemangioma of the female genital tract remains unclear. Few cases in literature described conservative treatments, such as carbon dioxide laser excision, knife excision, cryotherapy, radiotherapy, electrocauterization, and uterine artery embolization, having been tried [11].

Treatment of most hemanagioma is generally surgical: hysterectomy in 38% of cases was done or conservative therapy and local excision [15, 21].

Conclusion

In comparison with the available literature data, the presented case is the first to describe the association of hemangioma and dysplastic changes in the endocervical epithelium. Hemangioma was incidentally discovered in the histological sections of the material after the conization in a postmenopausal women.

R E F E R E N C E S

1. *Nucci MR, Oliva E*. Gynecologic Pathology. London: Churchill Livingstone Elsevier; 2009.
2. *Tavassoli FA, Devilee P*. WHO Pathology and Genetics. Tumors of the Breast and Female Genital Organ. Lyon: IARC Press; 2003.
3. *Ozyer S, Uzunlar O, Gocmen M, Bal S, Srvan L, Mollamahmutoğlu L*. Cavernous hemangioma of the cervix: a rare cause of vaginal bleeding. J Low Genit Tract Dis 2006; 10(2): 107−8.
4. *Mastilović K, Rajović J, Žikić D, Stoiljković B*. Hemangioma of the uterus. Arch Oncol 2005; 13(3−4): 148−9.
5. *Virk RK, Zhong J, Lu D*. Diffuse cavernous hemangioma of the uterus in a pregnant woman: report of a rare case and review of literature. Arch Gynecol Obstet 2009; 279(4): 603−5.
6. *Gupta R, Singh S, Nigam S, Khurana N*. Benign vascular tumors of female genital tract. Int J Gynecol Cancer 2006; 16(3): 1195−200.
7. *Andola US, Andola SK*. Vascular tumours of the female genital tract: A clinicopathologic Study of 11 cases. J Clin Diagnostic Res 2011; 5(6): 1241−6.
8. *Johnson C, Reid-Nicholson M, Deligdisch L, Grinblat S, Natarajan S*. Capillary hemangioma of the endometrium: a case report and review of the literature. Arch Pathol Lab Med 2005; 129(10): 1326−9.
9. *Weed JC*. Hemangioma of the cervix. AJOG 1948; 56(5): 991–3.
10. *Gan AM, Durdi GS, Sherigar B, Patted SS, Malur PR*. Hemangioma of the cervix: a rare cause of postcoital bleeding. South Afr J Gynecol Oncol 2011; 3(1): 43−5.
11. *Benjamin MA, Yaakub H, Telesinghe P, Kafeel G*. A rare case of abnormal uterine bleeding caused by cavernous hemangioma: a case report. J Med Case Rep 2010; 4(1): 136.
12. *Boneti LR, Boselli F, Lupi M, Bettelli S, Schirosi L, Bigiani N,* et al. Expression of estrogen receptor in hemangioma of the uterine cervix: reports of three cases and review of the literature. Arch Gynecol Obstet 2009; 280(3): 469−72.
13. *Dahiya N, Dahiya P, Kalra R, Marwah N, Jain S*. Cavernous hemnagioma of uterine cervix - a rere cause of postcoital bleeding. IJPSR 2011; 2(5): 1209−11.
14. *Jung HR, Cho CH, Kwon SH, Kwon SY*. Cavernous hemangioma of the uterus in a postmenopausal woman - a case report. Korenian J Pathol 2011; 45(5): 520−2.
15. *Chou WY, Chang HW*. Uterine hemangioma. Arch Pathol Lab Med 2012; 136(5): 567−71.
16. *Djunic I, Elezovic I, Ljubic A, Markovic O, Tomin D, Tadic J*. Diffuse cavernous hemangioma of the left leg, vulva, uterus, and placenta of a pregnant woman. Int J Gynaecol Obstet 2009; 107(3): 250−1.
17. *Kasznica J, Nisar N*. Congenital vascular malformation of the uterus in a stillborn: a case report. Hum Pathol 1995; 26(2): 240−1.
18. *Shanberge JN*. Hemangioma of the uterus associated with hereditary hemorrhagic telangiectasia. Obstet Gynecol 1994; 84(4 Pt 2): 708−10.
19. *Elkhateb S, Idrissi MA, Laabadi K, Chbani L, Chaara H, Melhouf A*. Cavernous hemangioma of the uterine cervix and pregnancy: a case report. Open J Obstet Gynecol 2011; 1: 221−4.
20. *Mahapatra S, Das R, Sethy S*. A cavernous hemangioma of the uterine cervix during pregnancy. Soth Afr J Gynecol Oncol 2012; 4(2): 63−5.
21. *Mahapatra S, Das BP, Kar A, Das R, Hazra K, Sethy S*. Cavernous hemangioma of uterine cervix in pregnancy mimicking cervical fibroid. J Obstet Gynaecol India 2013; 63(4): 288−90.

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CASE REPORT

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Bilateral hippocampal infarction and amnesia: A case report

Bilateralni hipokampalni infarkt i amnezija

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Abstract

Introduction. The hippocampus along with other structures of the medial temporal lobe plays an important role in the process of learning and memory consolidation. Bilateral hippocampal lesions lead to persistent anterograde amnesia while unilateral damage results in milder, content-specific forms of amnesia. Hippocampus may be affected by an acute or chronic pathologic process from a wide spectrum of neurological disorders. **Case report.** A 61-year-old female patient with a long history of hypertension, glucose intolerance, hypercholesterolemia and depression was hospitalized for acute anterograde amnesia, which led to repeated excessive drug taking. By further examinations that included laboratory tests, electroencephalography, Doppler sonography of intra- and extracranial vessels and neurovisualization methods [multislice computed tomography (MSCT) and magnetic resonance imaging (MRI) of the brain] bilateral hippocampal ischemia that occurred at different times was detected. Cognitive and neuropsychological evaluation revealed an isolated severe damage of episodic memory with the inability of retention of new information which persisted at the control examination three months later. The assumed mechanism of occurrence of ischemia in this case could be arterio-arterial embolism. **Conclusion.** Although ischemic stroke is one of the most common neurological diseases, ischemic stroke of the hippocampus is rare, the isolated bilateral presentation with clinical signs of severe amnestic syndrome in particular. Timely recognition and modern therapeutic approach could have a favorable impact on the recovery from severe neurological, cognitive deficit. It could be suggested that in patients with the clinical image of acute anterograde amnesia and vascular risk factors the MSCT examination of the brain with computed tomography perfusion and angiography is performed immediately upon hospitalization.

Key words:
hippocampus; brain infarction; amnesia, anterograde; magnetic resonance imaging.

Apstrakt

Uvod. Hipokampus zajedno sa ostalim strukturama medijalnog temporalnog režnja ima značajnu ulogu u procesu učenja i konsolidacije pamćenja. Bilateralne lezije hipokampusa dovode do perzistentne anterogradne amnezije dok unilateralno oštećenje rezultuje blažim, sadržajno određenim oblicima amnezije. Hipokampus može biti pogođen akutnim i hroničnim patološkim procesom u sklopu širokog spektra neuroloških oboljenja. **Prikaz bolesnika.** Bolesnica, stara 61 godinu, sa istorijom dugogodišnje arterijske hipertenzije, intolerancije glukoze, hiperholesterolemije i depresije, hospitalizovana je zbog akutne anterogradne amnezije koja je dovela do ponavljanog prekomenrog konzumiranja lekova. Daljim ispitivanjima koja su uključila laboratorijska ispitivanja, elektroencefalografiju, doplersonografiju intra- i ekstrakranijalnih krvnih sudova, neurovizuelizacione tehnike [multislajsnu kompjuterizovanu tomografiju (MSCT) i magnetsku rezonancu (MR) mozga] ustanovljena je bilateralna ishemija hipokampusa različitog vremena nastanka. Kognitivno i neuropsihološko ocenjivanje pokazalo je izolovano teško oštećenje epizodičnog pamćenja, sa nemogućnošću pamćenja novih informacija koje se održavalo i nakon tromesečne kontrole. Predpostavljeni mehanizam nastanka ishemije u ovom slučaju mogao bi biti arterijsko-arterijski embolizam. **Zaključak.** Iako je ishemički moždani udar jedna od najčešćih neuroloških bolesti, ishemički infarkt hipokampusa je redak, posebno izolovana bilateralna prezentacija sa kliničkom slikom teškog amnestičkog sindroma. Blagovremeno prepoznavanje i savremeni terapijski pristup bi mogli uticati povoljno na oporavak teškog neurološkog, kognitivnog deficita. Izdvaja se sugestija da se kod bolesnice sa ovakvom kliničkom slikom akutne anterogradne amnezije i vaskularnim faktorima rizika odmah pri prijemu uradi MSCT pregled mozga, po mogućstvu za CT perfuzijom i angiografijom.

Ključne reči:
hipokampus; mozak, infarkt; amnezija, anterogradna; magnetna rezonanca, snimanje.

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Introduction

The role of the hippocampus (HC) in the process of learning and memory consolidation is important and undeniable. In conjunction with the surrounding limbic system structures the hippocampus participates in the creation of emotional behavior, endocrine stress regulation and has the potential for adult neurogenesis [1].

The HC is located in the limbic gyrus on the medial surface of the cerebral hemispheres and together with the amygdaloid complex, the surrounding entorhinal, perirhinal and parahippocampal cortex, with which it creates multiple connections, makes the medial temporal lobe (MTL) [2].

A key shift in understanding the role of the hippocampus happened with the case of Henry Molaison. In 1953, in an attempt to cure severe epilepsy, a Canadian neurosurgeon Scoville surgically removed anterior two-thirds of both temporal lobes: apical part of the temporal lobe, uncus and amygdala, *formatio hippocampi* and adjacent part of the parahippocampal gyrus [3].

The patient was cured of epilepsy but later it was established that he suffered from a form of memory impairment, anterograde amnesia, which was widely studied over the next 30 years on the case of this patient. He lost the ability to retain information from the recent past, immediately before and after the surgery, while information from the distant past were preserved, even more if they were more distant. The patient's short-term memory, cognitive and other intellectual abilities was mostly preserved [4]. In this case, as well as in many other ones, it was shown that the severe forms of amnesia are connected to bilateral hippocampal lesions, whereas unilateral damage results in milder, mostly content-specific determined forms of amnesia.

The hippocampus can be affected by acute and chronic pathological processes in a wide spectrum of neurological disorders.

Although acute ischemic cerebral infarction is one of the most common neurological diseases, ischemic stroke of the hippocampus is rare [5, 6]. This phenomenon is partly explained by less frequent rate of ischemic brain infarction in the posterior cerebral circulation, i.e. 15% of the total number. Isolated hippocampal infarction which does not involve the existence of ischemia in other areas of the posterior circulation is particularly rare [6, 7]. Vascularisation of the hippocampus is mainly provided by the posterior cerebral artery (PCA) through its branches, anterior, middle and posterior hippocampal arteries. Vascularisation of the front part, the head of the hippocampus, is also provided, in greater or lesser extent, by the branch of anterior choroidal artery [8].

In the available medical literature we can find a scarce number of cases of hippocampal infarction, especially those that include its clinical presentation and magnetic resonance imaging (MRI) features [9–11].

In this paper we presented a patient with bilateral ischemic infarction of the hippocampus and a type of memory disorder. It is an extremely rare case in terms of stroke localization, as well as the etiology of bilateral hippocampal damage.

Case report

A 61-year-old female patient with the years-long medical history of hypertension, glucose intolerance, chronic thyroiditis, and depression, treated for two years, was admitted to the toxicology outpatient clinic in the emergency center of the Military Medical Academy for suspected acute self-poisoning with drugs.

The patient's family said that the previous evening she took a large number of tablets of propranolol, repeatedly, and that the next day she took more drugs uncritically. It was noted that she "acted strangely", she seemed confused and repeated the same questions and actions over and over again.

During examination the patient was conscious, afebrile, slightly disoriented in time and space, with slow heart rate, 50/min, and 96% oxygen saturation, measured using a pulse oximeter. Blood pressure was 175/80 mmHg with normal physical findings. Electrocardiogram (ECG) showed sinus rhythm, frequency 49/min, intermediate axis, QTc 422 ms with no changes in ST and T segment.

Laboratory analyses are shown in Table 1.

Clinical image and toxicological and chemical analysis on admission revealed overdose with drugs from the group of beta blockers, anxiolytics and serotonin reuptake inhibitors which was manifested in bradycardia, mild confusion and disorientation.

The patient was hospitalized for the application of nonspecific detoxication treatment, which led to normalization of the heart rate and toxicological parameters. During the treatment at the Clinic of Toxicology (National Poison Control Centre in the Military Medical Academy, Belgrade), the mild confusion was present in the form of constant repetition of the same questions as well as the temporal and spatial disorientation.

Table 1

The values of laboratory and toxicological parameters that deviated from the normal

Parameters	Initially	Control	Normal
Glucose (mmol/L)	9.8	6.9	4.1–5.9
Sodium (mmol/L)	136	144	136–145
Potassium (mmol/L)	3.5	4.1	3.5–5.1
Cholesterol (mmol/L)	–	6.4	< 5.2
LDL holesterol (mmol/L)L	–	4.64	< 3.5
AST (U/L)	71	18	0–37
ALT (U/L)	150	25	10–49
Propranolol (mg/L)	0.04		0.02–0.3
Bromazepam (mg/L)	0.07		0.05–0.2
Fluoxetine (mg/L)	0.23		0.12–0.5

LDL – low density lipoprotein; AST – aspartat aminotransferase; ALT – alanine aminotrasferase.

Neurological consultative examination showed a discrete pyramidal deficit of the right side and uncertainty regarding the events connected to the circumstances of admission to the hospital. Multislice computed tomography (MSCT) of the brain was indicated. It showed bilateral ischemic lesions of the hippocampus and parahippocampal gyrus. Left lesion was somewhat older, 50 × 20 mm in diameter, and right lesion in the subacute phase was 45 × 18 mm in diameter (Figure 1).

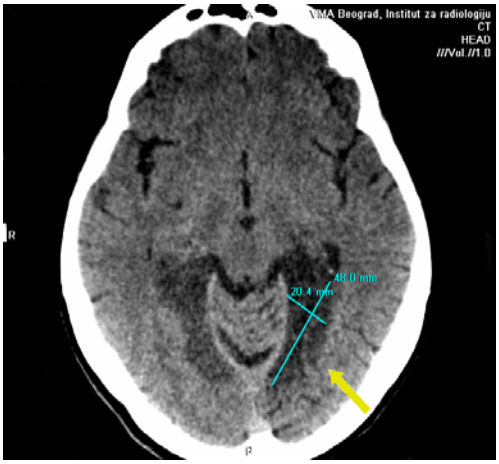


Fig. 1 – Axial noncontrast multislice computed tomography (MSCT) brain scan: bilateral areas of hypodensity in the hippocampus and parahippocampal gyrus demonstrate ishemic lesions. Left in the chronic, right in the subacute phase.

The patient was transferred to the Clinic of Neurology, Military Medical Academy.

The interviews with the patient revealed spatial and temporal disorientation regarding the date and the name of the hospital to which she was admitted. The patient was communicative, in normal mood, found filling crosswords in her spare time. She provided autobiographical data adequately, but she had no recollection of the events that occurred on the day of admission to the hospital, or the events which took place during her stay at the Clinic of Toxicology. A period of 3–4 days prior to the onset of the symptoms was also not available to the memory. National Institutes of Health Stroke Scale (NHSS) score was 2.

Control laboratory tests showed marginally elevated levels of serum glucose, cholesterol and LDL cholesterol, and normal HbA1c, coagulation factors, prothrombin time, C reactive protein, homocysteine, tumor markers, thyroid hormones and serum enzymes values. Ophthalmic examination and chest X-ray were normal. Cardiac examination confirmed arterial hypertension, which was corrected by antihypertensive therapy. Echocardiographic findings were normal, except the mild mitral regurgitation. Atrial fibrillation was excluded by holter ECG monitoring.

Electroencephalography (EEG) examination showed no presence of epileptic activity.

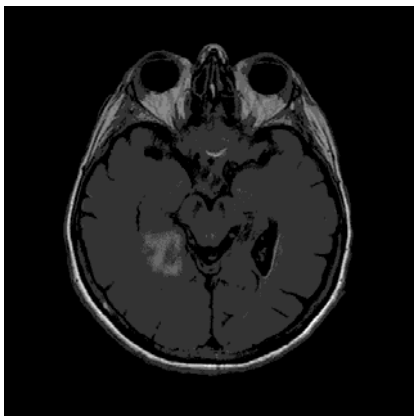
Doppler sonography of blood vessels of the neck showed the presence of fibrolipid plaques bilaterally in the region of bifurcation of common carotid arteries, with lumen reduction of about 50% in the first part of the right carotid artery. Left vertebral artery had a narrower lumen with reduced flow, with the characteristics of high resistance (RI 0.93).

Transcranial Doppler of cerebral blood vessels indicated high resistance in the vertebrobasilar territory. In all three blood vessels Doppler signal had a flattened tip and pulsatility indexes were above expected. Left anterior carotid artery (ACA) showed significantly higher flow rate, which indirectly suggested stenosis.

Contrast Transcranial Doppler (TCD) did not register the existence of spontaneous microemboli or the signs of right-to-left shunt.

MRI of the brain, performed on the sixth day after the onset of the symptoms, showed the area of hyperintensity in T2 fluid-attenuated inversion recovery (FLAIR) sequences with hypointensity in T1 sequences and signs of diffusion restrictions on diffusion weighted imaging (DWI) sequence in the region of the right hippocampus and parahippocampus, which corresponded to ischemia in the subacute phase. The signs of petechial hemorrhage were present in the specified zone cortically. Contralaterally in the same region, more extensive in posterior, a zone of encephalomalacy with hemosiderin deposits was observed representing the sequelae of chronic infarction lesion with hemorrhagic transformation. Multiple lacunar ischemic lesions were revealed within the deep cerebral white matter and subcortically bilaterally fronto-parietally (Figures 2a, 2b).

Angiography on a 3D (time of flight magnetic resonance angiography – TOF MRA) did not register the signal in the in-


a)

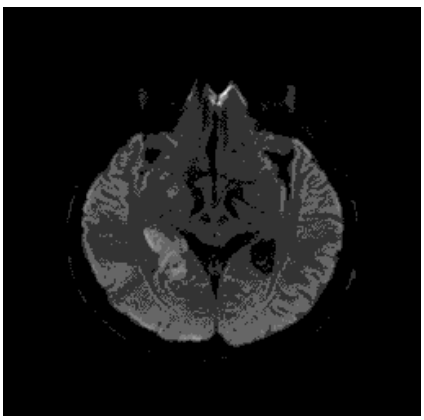

b)

Fig. 2 – a) T2 fluid-attenuated inversion recovery (FLAIR) shows hyperintensity of the signal in the region of the hippocampus and parahippocampally on the right. On the left side hypointensity in the same region; b) DWI shows diffusion restriction in the hippocampus and parahippocampal gyrus on the right.

tracranial segment of the left vertebral artery, which corresponded to occlusion. In the right vertebral artery, at the transition from extracranial to intracranial segment atherosclerotic plaques could be seen, which caused stenosis of about 50%. Basilar artery with its branches was displayed normally. As an incidental finding, the existence of two aneurysms in the medial ophthalmic segment of the right *arteria cortis interna* (ACI), small in dimensions, was observed (Figure 3).

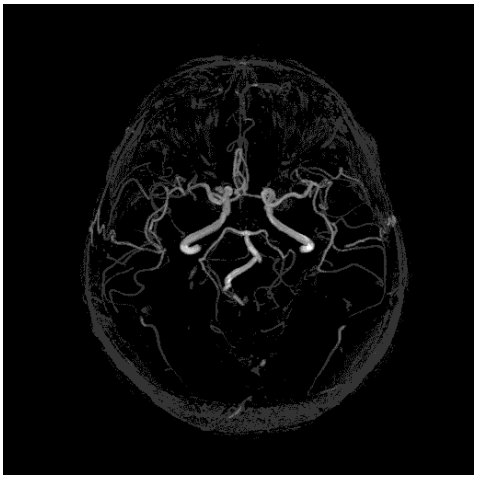


Fig. 3 –Angiography does not visualize the left AVwhile the right one is stenosed at the transition from the extra to intracranial AV segment.

Cognitive evaluation, performed on the fifth day after the onset of the symptoms, showed normal attention span and working memory, preserved vocabulary of previously acquired knowledge, preserved semantic memory, normal speech in expressive and receptive sense, preserved praxis, structural and visuospatial capability. There was mild to moderate executive dysfunction in terms of disrupted initiation and set shifting, as well as slightly reduced information processing speed. Severe anterograde amnesic syndrome, related to the domain of episodic memory, dominated. The ability of immediate memory was preserved within the average. In the area of declarative memory, verbal and visual learning severe deficit was registered, along with the inability to retain information. The patient was unable to recall any of the previously presented information. The effect of reminders was insufficient and recognition was disturbed.

The manifested form of cognitive impairment, with predominant and relatively limited focus on the syndrome of anterograde amnesia, suggested bilateral mediotemporal localization of the pathological process. Executive dysfunction and mild slowing of the information processing speed could be explained by chronic subcortical white matter vascular lesions with frontal distribution.

Cognitive examination was repeated after three months, revealing cognitive deficit maintained at the same intensity and the same pattern.

On cognitive screening test errors were registered predominantly in delayed recall tasks and phonemic fluency at both the initial and the control examination. Uncertainty regarding time and space orientation was more pronounced at the initial examination and to a lesser extent at the control examination. Detailed neuropsychological testing was then focused specifically on the domain of memory, executive functions and speed of information processing. The results of tests are shown in detail in Table 2.

Table 2

Cognitive screening and neuropsychological tests scores

Cognitive test	Initial evaluation	3-month follow up	Normative values (SD)
Screening test baterries			
MMSE	23	25	26.1 (3.1)
MoCA	20	22	27.4 (2.2)
Memory tests			
DSF	6	6	6 ± 1
DSB	5	5	5 ± 1
SRT			
SRT LTS	6	2	33.54 (18.30)
SRT CLTR	0	0	27.25 (21.61)
SRT DR	0	0	6.25 (2.80)
BVMT-R			
Trial 1	4	4	5.38 (2.2)
Trial 2	4	5	7.93 (2.07)
Trial 3	0**	2**	9.94 (2.00)
Total recall	8**	10**	22.35 (5.44)
Delayed Recall	0**	0**	8.48 (2.17)
Learning	0**	0**	3.76 (1.83)
Percent retained	0**	0**	90.66 (9.54)
Executive functions tests			
TMT A	52*	48*	33.22 (9.10)
TMT B	120*	119*	74.55 (19.55)
VFT	33	35	< 30
SDMT	43	47	37.71 (11.51)

MMSE – Minimental state examination [12]. MoCA – Montreal cognitive assessment [13]. DSF – Digit span forward; DSB – Digit span backward working memory; immediate memory [14]. SRT – Selective reminding test, version of 6 attempts, for estimate of verbal learning and memory [15,16]. LTS – Long-term storage;CLTR – Consistent long-term retrievel; DR – Deley recall. BVMT-R – The brief visuospatial memory test – revised, for the evaluation of visuospatial learning [17]. TMT A, TMT B – Trial making test A and B provides information on visual search, scanning, speed of processing, mental flexibility, and executive functions [18]. VFT – Verbal fluency test executive functions [17]. SDMT – Symbol digit modalities test – speed of information processing [17].

*** Scores that indicate mild to moderate damage**

**** Scores that indicate severe damage > than 3 standard deviation (SD).**

The patient was treated with statins, antithrombotic, and antihypertensive therapy. An antidepressant and a small dose of an anxiolytic were reintroduced with the advice regarding taking of medication under supervision. Cognitive rehabilitation therapy was recommended.

Discussion

The paper by Szabo et al. [6] analized the patients with ischemic stroke in the vascular territory of the PCA, which accounted for 5.25% of all ischemic strokes. The hippocampus was affected in 21% of the cases, most commonly the lesion of one hippocampus, while only 5% of the patients suffered bilateral hippocampal infarction.

In all the cases of hippocampal ischemia extrahippocampal regions in the ipsilateral vascular territory of the PCA were affected, as the well as thalamopeduncular region, occipital lobe and lesions of the cerebellum. There were no isolated hippocampal lesions in any of the cases [19].

In the case of the presented patient a bilateral lesion of the hippocampus was identified. Ischemias occurred at different times. The left hippocampus, where the described ischemia was in a chronic phase, was affected first and afterwards the right hippocampus with subacute ischemia. In both cases the lesions appeared mainly in dorsal part of the hippocampus and the parahippocampal region. They were isolated, without other evident lesions in the posterior cerebral circulation outside the hippocampus.

This model of ischemia could be explained by distal vascular pathology, primarily by occlusion of the middle or posterior hippocampal artery, branches of the P2 segment of the PCA.

The pathogenetic mechanism of ischemia occurrence in the posterior circulation, according to the findings in studies, was mainly associated with cardiogenic embolism, arterio-arterial embolisation due to atherosclerosis of large blood vessels, the basilar and vertebral arteries [20, 21]. More recent findings, based on the studies that have used modern MRI techniques (angiography, DWI) suggest large-artery atherosclerosis as a leading cause, whereby three different scenarios can be distinguished: *in situ* atherothrombosis, arterio-arterial embolism and consequent occlusion of the ostium of perforating branches on the ground of the atherosclerosis of a larger arterial tree [5].

The estimated, more likely the mechanism of ischemia in the case of the presented patient, considering the existing occlusion of the left and the stenosis of the right AV, could be arterio-arterial embolism with remote ischemic lesion in the vascular region of the middle and posterior hippocampal artery, first on the left and then on the right. This is supported by the presence of hemosiderin deposits in the area of ischemia, caused by hemorrhagic transformation, which is more common in the case of the embolic stroke. Another possible reason is the occlusion of orifice of middle and posterior hippocampal artery on the ground of the already existing atherosclerotic stenosis of the PCA, the segment P2. Bilateral presentation in the latter case could be caused by anatomic variation of the arterial branching where the branches of both hippocampuses originate from the one arterial tree [22].

Differentially diagnosed isolated lesions of the hippocampus are often associated with another type of pathology such as herpes simplex, paraneoplastic limbic encephalitis, complex partial seizures, transient global amnesia and brain tumors [23, 24].

Ischemic etiology of hippocampal damage in the presented case is supported by the existence of vascular risk factors and the presence of atherosclerosis of extracranial and intracranial vessels.

A sudden outset of symptoms, the absence of clinical, laboratory signs of infection and the characteristic presentation of lesions in DWI and T2 FLAIR MRI sequences made the differential diagnosis of encephalitis unlikely. EEG examination showed normal brain activity during the existence of anterograde amnestic syndrome, which also excludes non-convulsive status *epilepticus* as a potential cause.

Regarding the cognitive deficit, cases of persistent anterograde amnesia as sequelae of ischemic damage of the hippocampus were described in the literature and it was confirmed in the presented case.

In 1900 Bechterew described a patient with the characteristic form of amnesia. The post-mortem study revealed bilateral hippocampal infarction [25].

Later, in similar cases which were neuropsychologically analyzed in more detail, it was observed that unilateral infarctions in the vascular territory of PCA which include the hippocampus result in a milder form of amnesia compared to the bilateral, both in terms of duration as well as the degree and the type of memory damage [10, 11, 26]. Lesions of the left hippocampus are connected to difficulties in verbal content memorizing, while in the case of the right sided lesion remembering of visual content, orientation, spatial relationships and faces was more affected [6, 27]. These conclusions correlate to findings that emerged in the era of excessive surgery of severe epilepsy, at the beginning of the last century. They opened a new chapter in the understanding of the memory processes and the role of the hippocampus and the structures of medial temporal lobe.

Ischemia of the left hippocampus that occurred earlier in the presented patient did not lead to noticeable impairment of memory and everyday activities. The image of profuse amnestic syndrome developed only after the occurrence of ischemia of the right hippocampus.

Researches in the field of memory, especially in the last years of the previous century, led to significant findings. First of all, the existence of various types of memory, mediated by different mechanisms and engaging of the various neural structures, were determined. Declarative or explicit memory, consisting of episodic and semantic memory, refers to the conscious recollection of events and facts and depends on the integrity of the hippocampus, the structures of the medial temporal lobe, diencephalon and the basal forebrain nuclei [28].

Nondeclarative or implicit memory is independent of the limbic system structures and temporal neocortex and involves knowledge and skills acquired on the unconscious

level during the lifetime. It is believed that in the process of the implicit memory, motor learning, the key role is played by the basal ganglia, posterior neocortex and the cerebellum. Hence, it is understandable that in patients with severe amnesia implicit memory remains intact [14, 29].

In case of amnesia manifested in the presented patient the damage of episodic memory was severe and isolated while the semantic and implicit memory were preserved. This fully correlates with the lesion localization.

The representative neural structures of semantic memory are located outside the hippocampus and medial temporal region. Recent evidence indicate to the anterior and lateral temporal neocortex, mainly the left, as a key integrative region [14].

Dissociation regarding the form of amnesia where, in the presented patient, dominated the anetrograde over the retrograde, was described in cases of ischemic lesions of the hippocampus. The degree of retrograde amnesia depends on the size of the lesion [29]. Preservation of the more distant memory is explained by the fact that the recall of older information is carried out by other structures too, independently of the hippocampus.

According to the standard model of memory consolidation the hippocampus is initially dominant in memory traces formation. With the prolongation of the consolidation process the neocortical component in the memory traces gradually strengthens and over time they become independent of the hippocampus and medial temporal lobe [30]. According to the model of multiple memory traces the hippocampus is the mediator in the process of creating a memory trace. There is no prolonged consolidation, but with each recall of previous information new hippocampal/medial temporal lobe (HC/MTL) memory traces are formed, thus achieving their stronger representation. Therefore, more distant memories are less susceptible to damage in comparison to the more recent ones [31, 32].

Conclusion

In neurological practice ischemic stroke is frequently seen. Ischemia in the posterior cerebral circulation is a less common and isolated bilateral hippocampal damage with clinical signs of severe amnestic syndrome is an unusual manifestation which may initially lead to dilemmas in the differential diagnosis. In addition, these infarcts are not well presented by National Institutes of Health Stroke Scale score. All that can affect timely implementation of the therapy, primarily thrombolytic therapy, in which early recanalization can lead to a favorable outcome in terms of recovery from this severe functional neurological deficit. Hence, it could be suggested that to patients with the clinical image of acute anterograde amnesia and vascular risk factors multislice computed tomography examination of the brain with computed tomography perfusion and angiography, or, if possible, magnetic resonance imaging of the brain according to the protocol for stroke should be performed immediately at admission.

R E F E R E N C E S

1. *Kitamura T, Inokuchi K.* Role of adult neurogenesis in hippocampal-cortical memory consolidation. Mol Brain 2014; 7: 13.
2. *Duvernoy HM.* The human hippocampus. Berlin: Springer; 2004.
3. *Scoville WB, Milner B.* Loss of recent memory after bilateral hippocampal lesions. 1957. J Neuropsychiatry Clin Neurosci. 2000; 12(1): 103−13.
4. *Scoville WB, Milner B.* Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 1957; 20(1): 11−21.
5. *Lee E, Kang DW, Kwon SU, Kim JS.* Posterior cerebral artery infarction: diffusion-weighted MRI analysis of 205 patients. Cerebrovasc Dis 2009; 28(3): 298−305.
6. *Szabo K, Förster A, Jäger T, Griebe M, Kern R, Hennerici MG,* et al. Hippocampal lesion patterns in acute posterior cerebral artery stroke: clinical and MRI findings. Stroke 2009; 40(6): 2042−5.
7. *Tao WD, Kong FY, Hao ZL, Lin S, Wang DR, Wu B,* et al. One-year case fatality and disability after posterior circulation infarctionin a Chinese hospital-based stroke study. Cerebrovasc Dis 2010; 29(4): 376−81.
8. *Erdem A, Yasargil MG, Roth P.* Microsurgical anatomy of the hippocampal arteries. J Neurosurg 1993; 79(2): 256−65.
9. *Victor M, Angevine JB Jr, Mancall EL, Fisher CM.* Memory loss with lesions of hippocampal formation. Report of a case with some remarks on the anatomical basis of memory. Arch Neurol 1961; 5: 244−63. (Italian)
10. *Mohr JP, Leicester J, Stoddard LT, Sidman M.* Right hemianopia with memory and color deficits in circumscribed left posterior cerebral artery territory infarction. Neurology 1971; 21(11): 1104−13.
11. *Takahashi S, Higano S, Kurihara N, Mugikura S, Sakamoto K, Nomura H,* et al. Correlation of lesions in the hippocampal region noted on MR images with clinical features. Eur Radiol 1997; 7(2): 281−6.
12. *Tombaugh TN, McDowell I, Kristjansson B, Hubley AM.* Mini-Mental State Examination (MMSE) and the Modified MMSE (3MS): A Psychometric Comparison and Normative Data. Psychol Assess 1996; 8: 48−59.
13. *Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I,* et al. The Montreal Cognitive Assessment (MoCA©): A Brief Screening Tool For Mild Cognitive Impairment. J Am Geriatr Soc 2005; 53(4): 695−9.
14. *Hodges JR.* Cognitive assessment for clinicians. Oxford: Oxford University Press; 2007.
15. *Buschke H.* Selective reminding for analysis of memory and learning. J Verb Learn Verb Behav 1973; 12: 543−50.
16. *Obradovic D, Petrovic M, Antanasijevic I, Marinkovic J, Stojanovic T, Obradovic S.* The Brief Repeatable Battery: psychometrics and normative values with age, education and gender corrections in a Serbian population. Neurol Sci 2012; 33(69: 1369−74.
17. *Ralph H, Benedict B.* Brief Visuospatial Memory Test- Revised. Odessa, FL: Psychological Assessment Resources, Inc; 1997.
18. *Tombaugh TN.* Trail Making Test A and B: Normative data stratified by age and education. Arch Clin Neuropsychol 2004; 19(2): 203−14.
19. *Förster A, Gass A, Kern R, Wolf ME, Hennerici MG, Szabo K.* MR imaging-guided intravenous thrombolysis in posterior cerebral artery stroke. AJNR Am J Neuroradiol 2011; 32(2): 419−21.
20. *Živković M, Šternić N, Kostić VS.* Ishemic disease of the brain. Belgrade: Zavod za udžbenike i nastavna sredstva; 2000. (Serbian)

21. *Brandt T, Steinke W, Thie A, Pessin MS, Caplan LR*. Posterior cerebral artery territory infarcts: clinical features, infarct topography, causes and outcome. Multicenter results and a review of the literature. Cerebrovasc Dis 2000; 10(3): 170−82.
22. *Cachia D, Swearer J, Ferguson W, Moonis M*. Selective cognitive patterns resulting from bilateral hippocampal ischemia. Arch Med Sci 2011; 7(1): 168−72.
23. *Sedlaczek O, Hirsch JG, Grips E, Peters CN, Gass A, Wohrle J*, et al. Detection of delayed focal MR changes in the lateral hippocampus in transient global amnesia. Neurology 2004; 62(12): 2165−70.
24. *Szabo K, Poepel A, Pohlmann-Eden B, Hirsch J, Back T, Sedlaczek O,* et al. Diffusion weighted and perfusion MRI demonstrates parenchymal changes in complex partial status epilepticus. Brain 2005; 128(Pt 6): 1369−76.
25. *Bechterew WW*. Demonstration eines gehirns mit Zerstörung der vorderen und inneren theile der hirnrinde beider schlafenlapen. Neurol Zentralbl 1900; 19: 990−1.
26. *Insausti R, Annese J, Amaral DG, Squire RL*. Human amnesia and the medial temporal lobe illuminated by neuropsychological and neurohistological findings for patient E. P. Proc Natl Acad Sci USA 2013; 21: 110−21.
27. *Damasio AR, Geschwind N*. The Neural Basis of Language. Annu Rev Neurosci 1984; 7: 127−47.
28. *Moscovitch M, Rosenbaum RS, Gilboa A, Addis DR, Westmacott R, Grady C*, et al. Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. J Anat 2005; 207(1): 35−66.
29. *Ocić G*. Clinical neuropsychology. Belgrade: Zavod za udžbenike i nastavna sredstva. 1998. (Serbian)
30. *Frankland PW, Bontempi B*. The organization of recent and remote memories. Nat Rev Neurosci 2005; 6(2): 119−30.
31. *Moscovitch M*. Theories of memory and consciousness. Oxford: Oxford University Press; 2000.
32. *McGaugh JL*. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 2004; 27: 1−28.

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CASE REPORT

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Massive retroperitoneal hematoma as a complication of anticoagulation therapy in a patient treated in a pulmonary intensive care unit

Masivni retroperitoneumski hematom kao komplikacija antikoagulantne terapije kod bolesnika lečenog u pulmološkoj jedinici intenzivne nege

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Abstract

Introduction. Retroperitoneal hematoma may occur as a result of trauma, but also from rupture of arterial aneurysms (aortic or iliac), surgical complications, tumors or anticoagulation therapy. **Case report.** We presented a patient on permanent anticoagulation therapy. On the day of admission to our institution, the patient had the value of his INR 5.57 which required immediate suspension of the therapy. The main symptom in this patient was pain in the right inguinal canal with propagation along the right leg, which was indicated in clinical picture of spontaneous retroperitoneal haematoma. After three days the fall of hemoglobin occurred, so the additonal diagnostics was done. A computed tomography of the abdomen was performed showing well limited, large retroperitoneal hematoma (213 × 79 × 91 mm). Transfusion of concentrated red blood cells was performed twice with satisfactory correction of hemoglobin level, and four units of fresh frozen plasma. The patient was hemodynamically stabilized and discharged after a two-month long intensive care unit treatment, with the advice to use low-molecular weight heparin 2 × 0.4 mg subcutaneusly, due to persistent arrhythmia. **Conclusion.** In patients on anticoagulation therapy regular monitoring of the anticoagulant status is extremely important, because of the possibility of fatal complications development, such as retroperitoneal hematoma.

Key words:
anticoagulants; drug toxicity; hemorrhage; hematoma; retroperitoneal space; treatment outcome.

Apstrakt

Uvod. Retroperitoneumski hematom može nastati kao rezultat traume, rupture aneurizme arterije (aortne ili ilijačne), hirurške komplikacije, tumora ili antikoagulantne terapije. **Prikaz bolesnika.** Prikazali smo bolesnika na trajnoj antikoagulantnoj terapiji koji je na dan prijema u našu ustanovu imao vrednost INR 5,57. Odmah je obustavljena antikoagulantna terapija. Bolesnik je od tegoba navodio i bolove u desnom ingvinalnom kanalu koji su zračili duž desne noge, što se uklapalo u kliničku sliku spontanog retroperitonumsku hematoma. Posle tri dana došlo je do sniženja nivoa hemoglobina, tako da je primenjena dopunska dijagnostika. U nalazu dobijenom kompjuterizovanom tomografijom abdomena opisan je dobro ograničen retroperitoneumski hematom velikih dimenzija (213 × 79 × 91 mm). Primenjena je transfuzija koncentrovanih eritrocita koja je dovela do zadovoljavajuće korekcije nivoa hemoglobina. Bolesnik je dobio i četiri jedinice sveže smrznute plazme. Nakon što je hemodinamski stabilizovan, otpušten je iz jedinice intenzivne nege, posle dvomesečnog lečenja. Savetovano mu je da zbog perzistentne artimije koristi niskomolekulski heparin 2 × 0,4 mg potkožno. **Zaključak.** Kod bolesnika na antikoagulantnoj terapiji izuzetno je značajno redovno praćanje njihovog antikoagulantnog statusa, upravo zbog razvoja ponekad i fatalnih komplikacija, kao što je spontani retroperitonealni hematom.

Ključne reči:
antikoagulansi; lekovi, toksičnost; krvarenje; hematom; retroperitonealni prostor; lečenje, ishod.

Introduction

Retroperitoneal hematoma (or retroperitoneal bleeding) refers to accumulation of blood in the retroperitoneal space. It is most frequently seen after femoral artery catetherisation or pelvic and lumbar trauma [1]. Also, retroperitoneal hemorrhage can be the result of ruptured abdominal aortic aneurysm or some kidney or adrenal gland conditions. Spontane-

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ous retroperitoneal hemorrhage refers to bleeding without trauma or retroperitoneal pathology. Spontaneous retroperitoneal hematoms is rare but potentially life threatening condition and it is almost always seen in association with anticoagulation therapy, coagulopathies and in patients on hemodialysis [2].

The incidence of retroperitoneal hematoma has been reported to be 0.6–6.6% in patients on oral anticoagulant therapy [3].

Coumarins are vitamin K antagonists that produce their anticoagulant effect by interfering with the cyclic interconversion of vitamin K and its 2,3 epoxide (vitamin K oxide). Vitamin K antagonists inhibit carboxylation of the regulatory anticoagulant protein C and S and therefore have the potential to exert a procoagulant effect [4].

Drugs may influence the pharmacodynamics of warfarin by inhibiting synthesis or increasing clearance of vitamin K-dependent coagulation factors or by interfering with other pathways of hemostasis.

Drugs such as aspirin, nonsteroidal anti-inflammatory drugs, high doses of penicillins and moxolactam increase the risk of warfarin-associated bleeding by inhibiting platelet function. The mechanisms by which erythromycin and some anabolic steroids potentiate the anticoagulant effect of warfarin are unknown. Sulfonamides and several broad-spectrum antibiotic compounds may augment the anticoagulant effect of warfarin by eliminating bacterial flora and aggravating vitamin K deficiency in patients whose diet is deficient of vitamin K [4].

It would be reasonable to monitor the prothrombin time (PT) more frequently when any drug therapy is added or withdrawn from the regimen of a patient treated with an oral anticoagulant drug.

According to the literature when the value of international normalised ratio (INR), that is standardized measure of protrombin time, ≥ 5, the risk of bleeding increases 3.6 times compared to the referent range of 2.0–3.0 [5]. Patients on oral anticoagulant therapy with INR ≥ 6 are faced with the risk of major, life-threatening bleeding [6].

We presented a patient treated in the pulmonary intensive care unit, with a large retroperitoneal hematoma, as the result of complication of anticoagulant therapy.

Case report

A 73-year-old male patient was transferred to the Pulmonary Intensive Care Unit from the Coronary Care Unit, Clinical Center of Serbia, because of acute partial respiratory insufficiency. He complained of exertional fatigue lasting for the last year and ten days before admission he got tired in peace. During the last five days the patient also felt pain in the right inguinal canal emitting in the right leg. The day before admission at the Coronary Care Unit strong fever, chills and dyspnea appeared and on the day of admission, his general condition deteriorated and the patient became drowsy and was hospitalized in the Coronary Care Unit with suspected pulmonary embolism. Computed tomography (CT) scan of the thorax had not comfirmed the existence of pulmonary embolism or other pathological processes in the chest. At the Coronary Care Unit echocardiography of the heart was made: dilated aortic root was found (4.0 cm), normal size of the left ventricle, thick concentric walls, ejection fraction rate of 50%, enlarged left atrium (4.6 cm), enlarged right ventricle (3.3 cm) while without free wall motion abnormalities with tricuspid regurgitation 2–3 +; indirectly measured systolic pressure in the right ventricle was 46 mm.

In his personal history we found that the patient had myocardial infarction 15 years ago, triple coronary artery bypass grafting (ACBG) was made and in February 2007 redo triple ACBG was performed and also a biological valve was implanted because of aortic valve insufficiency. The patient also had diabetes for about five years, then permanent absolute arrhythmia and was treated from gout, and was on continuous oral anticoagulant therapy. In the Coronary Care Unit the patient was treated with warfarin sodium 3.75 mg and 5 mg alternately, isosorbid-5-mononitrate 2 × 20 mg, metformine 1,000 mg 2 × 1, enalapril 10 mg, furosemid 40 mg, spironolacton 50 mg and bisoprolol 5 mg.

On the day of admission the patient was conscious, oriented, somnolent, afebrile with cyanosis and dyspnea, and decompensated heart failure with pedal edema, without sings of hemorrhagic syndrome. Through auscultation of the lung we found bilateral lower breath sounds and arrhythmic heart rate, clear tones, systolic ejection murmur of intensity grade 2. Chest radiography showed enlarged cardiac silhouette and the voluminous hilum (Figure 1). Electrocardiogram described atrial fibrilation with absolute arrhytmia of ventricules, negative T-waves in V2-V6. Blood presure was 140/90 mmHg, while the heart frequency was 125/beats *per* minutes. Arterial blood gas reflected significant partial respiratory insufficiency (pH 7.40, pO_2 5.5 kPa, pCO_2 4.9 kPa, saturation 83%).

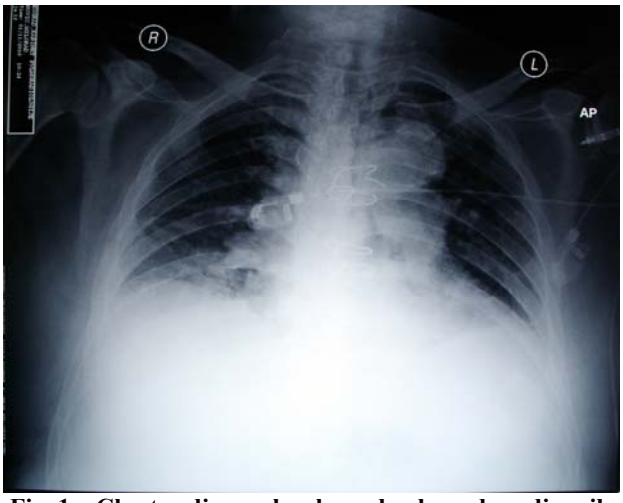

Fig. 1 – Chest radiography showed enlarged cardiac silhouette and the voluminous hilum.

Laboratory analyses were: leukocytes (Le) 14.5 × 10^9/L, sedimentation rate (SE) 84 mm/h, C-reactive protein (CRP) 120 mg/L, fibrinogen 7.0 g/L, urea 13.3 mmol/L (normal range 2.5–7.5 mmol/L), creatinine 169 umol/L (normal range 59–104), aspartate aminotransferase (AST)

108 U/L (normal range 0–37), alamine aminotransferase (ALT) 96 U/L (normal range 0–41), laetate dehydrogenase (LDH) 601 U/L (normal range 220–400), d-dimer 2.56 mg/L (normal range 0–0.55), INR 5.57. Other laboratory values were in the reference range. All the therapies prescribed by cardiologist were continued. Because of high INR values oral anticoagulant therapy was immediately discontinued and anticoagulant status of the patient was monitored every day.

Somnolence with hypoxemia despite oxygen therapy was a dominant sign of clinical features at the beginning of hospitalization, so non-invasive mechanical ventilation was applied which corrected arterial blood oxygenation. Three days later in blood analyses we registered the fall of hemoglobin level (123...94...82...68 g/L) and, at the same time, the occurrence of a large hematoma in the soft tissue of the lateral side of the right hemothorax. Ultrasound examination of the lateral chest soft tissues described hematoma in organization, with the dimension of 25 mm and the length of 35–40 mm. As there was no evident bleeding in the chest and the Adler-Weber test was negative, we decided to make the scan of abdomen. CT of the abdomen described: retroperitoneal hematoma localized between the liver ant the pelvis, size 213 × 79 × 91 mm, density 24–56 HU. The right *musculus iliopsoas* and right *musculus iliacus* were edematous with signs of intramuscular hematoma, hematoma in the soft tissues of the right abdominal wall. In the infrarenal part of the abdominal aorta the aortic wall had sclerotic aneurysm of the segment length 50 mm and wide 48 mm, with no signs of extravasation of blood (Figure 2).

Transfusion of concentrated red blood cells was performed twice with a satisfactory correction of hemoglobin level, and four units of fresh frozen plasma. After the consultation with the abdominal surgeon, he did not indicate surgical treatment. We also consulted the hematologist, who concluded that it was spontaneous hemorrhage as a complication of anticoagulant therapy in the application of standard dose of medication and for future treatment he sugested low-molecular weight heparin which should be suspended at INR higher than 3.

Two control scan examinations of the abdomen described a slightly lower retroperitoneal hematoma. A month later the dimesion of hematoma was 143 × 70 × 78 mm. Because of altered state of consciousness and the present long-term sleep disorders, a neurologist was consulted and he suggested endocranial CT scan which showed notable degenarative changes with no pathological density and polysomnographic testing.

After applying the therapy, the patient was hemodynamically stabilized, The INR value was within the referent range, with satisfactory gas exchange. The patient was discharged after two months of hospital treatment in the Intensive Care Unit, with the advice to use anticoagulant therapy (low molecular weight heparin, nodroparin calcium 2 × 0.4 mL *sc*) because of the persistence of arrhythmia and due to hematoma transition, oral anticoagulants were delayed because of difficult dosing. Frequent monitoring of INR values was adviced, to.

Discussion

Anticoagulant therapy such as warfarin, unfractionated heparin and low-molecular weight heparin are widely used in everyday practice in prevention and therapy of deep vein thrombosis, pulmonary embolism, acute ishemic stroke, valvular heart disease and atrial fibrilation. Bleeding is the most common complication, but literature recognises many other complications like subcapsular renal hematoma, retroperitoneal and intraperitoneal hemorrhage, hemothorax, spinal epidural hematoma, gastrointestinal and cutaneous hematoma [7, 8].

An individual patient's risk for major anticoagulant-related bleeding can be estimated on the basis of specific risk factors

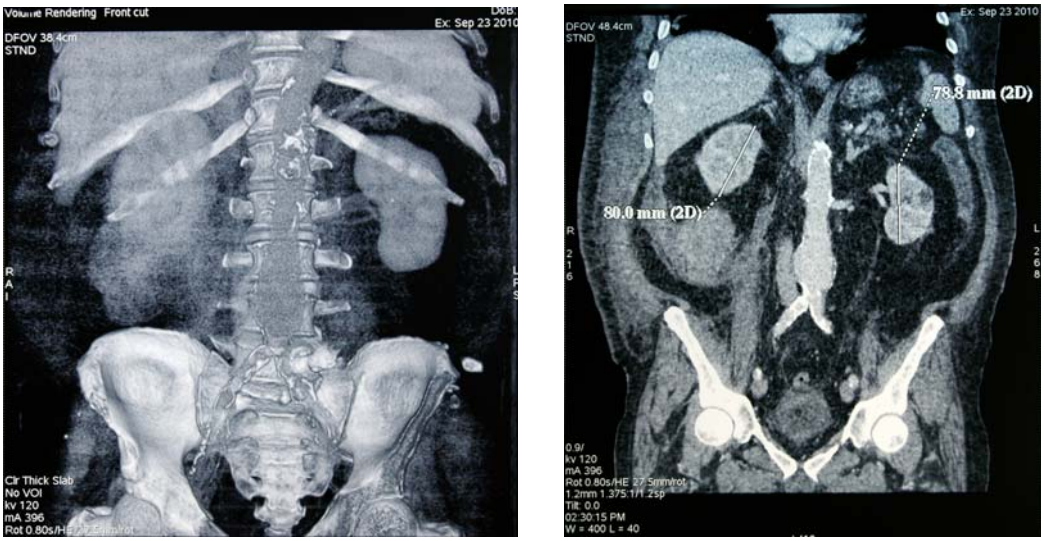

Fig. 2 – Computed tomography of the abdomen: retroperitoneal hematoma localized between the liver and the pelvis.

such as the intensity of the anticoagulant effect achieved and the presence of serious comorbid diseases, especially cerebrovascular, kidney, heart, and liver disease; older age and concurrent medicines may also be independent risk factors. The frequency of bleeding during warfarin therapy is reduced by less intense therapy achieving the prothrombin time with an INR of 2.0 to 3.0, which is efficacious for most indications [9].

A large study of Sasson et al. [10] showed that patients on anticoagulant therapy, even in therapeutical doses, should be monitored and checked regularly because of the possibility of complications of this therapy such as the development of spontaneous retroperitoneal hematoma. The most common symptoms are acute onset, persistent pain in the upper quadrant of the abdomen, in the groin or lumbal region with radiation to the scrotum. Pain and paresthesia are the result of pressure on the femoral nerve, the largest branch of the lumbal plexus.

We presented the patient who permanently used anticoagulant therapy. On the day of admission to our hospital he had the value of INR 5.57, so we immediately stopped this therapy. According to Naranjo et al. [11] adverse drug reaction probability scale, adverse event was definitely related to the drug.

The main symptom was the pain in the right ingvinal canal radiating down the right leg, which is comparable to the clinical features of spontaneous retroperitoneal hematoma.

The diagnosis of spontaneous retroperitoneal hematoma is a challenge even on high-resolution CT or nuclear magnetic resonance, because many benign and malignant lesions can mimic this condition. CT is superior to ultrasound in localization, extension and evaluation of the size of hematoma.

Good limited and large-scale retroperitoneal hematoma was described on CT in our patient [12].

Treatment of spontaneous retroperitoneal hematoma includes surgery and conservative treatment of anemia and correction of warfarin-associated coagulopathy [13]. Conservative treatrement depends on severity and location of hemorhage and the INR when bleeding occurs. Warfarin therapy should be withheld in all patients with bleeding during oral antigoagulant therapy. Patients should be treated with coagulation factor replacement and intravenous vitamin K [14]. Fresh frozen plasma provides rapid but partial reversal of coagulopathy providing replacement of coagulation factors II, VII IX and X. The usual dose of fresh frozen therapy is 15 mL/kg, although the optimal dose has not been established [15].

Vitamin K reverses the action of warfarin partially or wholly depending on the route of administration and the dose used. The most effective way of treatment is intravenous administration of vitamin K. Usual dose is 2–5 mg. Reduction of INR begins within 2 hours, and correction to normal range is expected within 24 hours [16].

Prothrombin complex concentrate depending on recommended doses ranges from 25 to 100 U/kg product used. Three-factor concentrates may not adequately correct the INR [9].

Recombinant factor VIIa, in usual dose of 10 to 90 μg⁄kg, has immediate effect [9].

A small hematoma with mild symptoms of neuropathy, without compression to surrounding structures and the need for transfusion and no signs of infection can be treated conservatively. On the other hand, surgical treatment is necessary when patients cannot be stabilized with conservative treatment, in unstable patients or due to constant compressive syndrome (femoral or ischiadical neuropathy) [17, 18].

The presented patient was successfully treated with the application of fresh frosen plasma and blood transfusion.

Conclusion

Spontaneous retroperitoneal hematoma as a rare complication is still a diagnostic challenge even for experienced clinicians. Acute abdominal pain, parestesia and laboratory signs of anemia in patients receiving oral anticoagulant therapy, should raise suspicion for intraabdominal bleeding. The early diagnosis is crucial because patients can be treated conservatively with good outcome. In patients who are on anticoagulant tretment it is extremely important to follow their status regularly, because of development of sometimes fatal complications such as spontaneous retroperitoneal hematoma

REFERENCES

1. *Panetta T, Sclafani SJ, Goldstein AS, Phillips TF, Shaftan GW*. Percutaneous transcatheter embolization for massive bleeding from pelvic fractures. J Trauma 1985; 25(11): 1021−9.
2. *Danaci M, Kesici GE, Kesici H, Polat C, Belet U*. Coumadin-induced renal and retroperitoneal hemorrhage. Ren Fail 2006; 28(2): 129−32.
3. *Pallea EX, Domingo P, Fontcubertta J, Felez J*. Spontaneus tertoperitoneal haemorrhage during oral anticoagulant therapy. Arch Inrern Med 1985; 145(8): 1531−4.
4. *Hirsh J, Dalen JE, Anderson DR, Poller L., Bussey H, Ansell J,* et al. Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. Chest 2001; 119: 8S−21S.
5. *The European Atrial Fibrillation Tril Study Group*. Optimal oral anticoagulant therapy in patients with nonrheumatic atrial fibrillation and recent cerebral ischemia. N Engl J Med 1995; 333: 5−10.
6. *Hylek EM, Chang YC, Skates SJ, Hughes RA, Singer DE*. Prospective study of outcomes of ambulatory patients with excessive Warfarin anticoagulation. Arch Inter Med 2000; 160(11): 1612−7.
7. *Balci NC, Sirvanci M, Tüfek I, Onat L, Duran C*. Spontaneous retroperitoneal hemorrhage secondary to subcapsular renal hematoma: MRI findings. Magn Reson Imaging 2001;19(8):1145-8.
8. *Kirazli Y, Akkoc Y, Kanyilmaz S*. Spinal epidural hematoma associated with oral anticoagulation therapy. Am J Phys Med Rehabil 2004; 83(3): 220−3.
9. *Crowther MA, Warkentin TE*. Bleeding risk and the management of bleeding complications in patients undergoing anticoagulant therapy: focus on new anticoagulant agents. Blood 2008; 111(10): 4871−9.
10. *Sasson Z, Mangat I, Peckham KA*. Spontaneous iliopsoas hematoma in patients with unstable coronary syndromes receiving

intravenous heparin in therapeutic doses. Can J Cardiol 1996; 12(5): 490−4.
11. *Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA,* et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 1981; 30(2): 239−45.
12. *Pless T, Loertzer H, Brandt S, Radke J, Fornara P, Soukup J.* Atraumatic retroperitoneal hemorrhage: interdisciplinary and differential diagnostic considerations based on a case report. Anaesthesial Reanim 2003; 28(2): 50−3. (German)
13. *Quartey B, Nelson J.* Massive spontaneous retroperitoneal hemorrhage induced by enoxaparinand subsequent abdominal compartment syndrome requiring surgical decompression: a case report and literature review. IJCRI 2011; 2(10): 14−8.
14. *Dentali F, Crowther M.* Management of excessive anticoagulant effect due to vitamin K antagonists. Hematology Am Soc Hematol Educ Program 2008; 2008: 266−70.
15. *Baglin T.* Management of warfarin (coumarin) overdose. Blood Rev 1998; 12(2): 91−8.
16. *Lubetsky A, Yonath H, Olchovsky D, Loebstein R, Halkin H, Ezra D.* Comparison of oral vs intravenous phytonadione (vitamin K1) in patients with excessive anticoagulation: a prospective randomized controlled study. Arch Intern Med 2003; 163(20): 2469−73.
17. *Ivascu FA, Janczyk RJ, Bair HA, Bendick PJ, Howells GA.* Spontaneous retroperitoneal hemorrhage. Am J Surg 2005; 189(3): 345−7.
18. *Daliakopoulos SI, Bairaktaris A, Papadimitriou D, Pappas P.* Gigantic retroperitoneal hematoma as a complication of anticoagulation therapy with heparin in therapeutic doses: a case report. J Med Case Rep 2008; 2(1): 162.

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CASE REPORT

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Transcatheter closure of atrial septal defect in a patient with Noonan syndrome after corrective surgery

Transkatetersko zatvaranje atrijalnog septalnog defekta kod bolesnice sa Nunanovim sindromom nakon hirurškog zatvaranja defekta

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Abstract

Introduction. Transcatheter atrial septal defect (ASD) closure is considered to be a gold standard for patients with the suitable anatomy as compared to cardiac surgery. Reocurrence of ASD after surgical closure is a very rare late complication which can be successfully managed with transcatheter procedure. **Case report.** We reported a female patient with Noonan syndrome who presented with hemodinamically significant ASD 37 years after the corrective cardiac surgery. Due to numerous comorbidities which included severe kyphoscoliosis, pectus excavatum and multiple surgeries we decided to perform transcatheter closure of ASD. The procedure itself was very challenging due to the patient's short stature and heart's orientation in the chest, but was performed successfully. The subsequent follow-up was uneventful and the patient reported improvement in the symptoms. **Conclusion.** Transcatheter closure of ASD in a patient with Noonan syndrome with the history of surgically corrected ASD can be performed successfully, despite challenging chest anatomy.

Key words:
noonan syndrome; heart septal defects; congenital abnormalities; cardiovascular surgical procedures; treatment outcome.

Apstrakt

Uvod. Transkatetersko zatvaranje atrijalnog septalnog defekta (ASD) smatra se zlatnim standardom kod bolesnika sa pogodnom anatomijom za ovaj pristup, u poređenju sa hirurškim zatvaranjem ovog defekta. Ponovna pojava ASD nakon hirurške korekcije defekta je retka komplikacija koja se uspešno može rešiti transkateterskom intervencijom. **Prikaz bolesnika.** Prikazali smo bolesnicu sa Nunanovim sindromom i hemodinamski značajnim ASD 37 godina nakon hirurške korekcije. S obzirom na brojne komorbiditete koji su uključivali tešku kifoskoliozu, *pectus excavatum,* kao i brojne hirurške intervencije, odlučili smo da izvedemo transkatetersko zatvaranje ASD. Sama procedura bila je veoma zahtevna zbog komorbiditeta, niskog rasta i poremećene orijentacije srca u grudnom košu, ali je uspešno izvedena. Period oporavka prošao je uredno, a bolesnica je navela i značajno poboljšanje prvobitnih tegoba. **Zaključak.** Transkatetersko zatvaranje ASD kod bolesnice sa Nunanovim sindromom može se uspešno izvesti, uprkos poremećenim anatomskim odnosima u grudnom košu.

Ključne reči:
nunanov sindrom; srce, atrijumski septumski defekti; anomalije; hirurgija, kardiovaskularna, procedure; lečenje, ishod.

Introduction

Noonan syndrome is an autosomal, dominant, variably expressed, multisystem disorder with an estimated prevalence of 1 in 1000–2500 newborns [1]. This syndrome occurs in both genders and is associated with the normal karyotype (46XX or 46XY) with identified missense mutations in the protein tyrosine phosphatase non-receptor type 11 gene (PTPN11), located on chromosome 12 [2, 3]. Characteristic findings include distinctive facial features, short stature, chest deformity and congenital heart disease [4]. It is the second most common syndromic cause of congenital heart disease, exceeded in the prevalence only by trisomy 21. The most common cardiovascular phenotypes of Noonan syndrome include pulmonary stenosis, hypertrophic cardiomyopathy and *secundum* atrial septal defect (ASD) [1].

Transcatheter closure of *secundum* ASD is a safe procedure with a high success rate and low morbidity as compared to cardiac surgery [5]. However, the outcomes of these procedures in patients with previous surgical closure of ASD, with reoccurrence of the same defect are unknown. We presented a patient with surgically corrected congenital heart

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disease and transcatheter closure of ASD, 37 years after the surgery.

Case report

A 39-year-old female with Noonan syndrome was referred to our Center because of the shortness of breath and New York Heart Association (NYHA) Functional Class 2, accompanied by palpitations. Her body height was 139 cm, and body weight 35 kg. Her medical history included surgically closed *secundum* ASD (direct suture with Tycron 4.0) and resection of the right ventricular infundibulum at the age of two, and percutaneous dilatation of pulmonary valve stenosis at the age of 16, multiple surgeries of her right foot because of congenital malformations, breast surgery for the fibroadenoma, kyphoscoliosis and pectus excavatum. Chest X-ray showed enlarged cardiac silhouette with severe kyphoscoliosis. Transthoracic echocardiogram (TTE) and subsequent transesophageal echocardiogram (TEE) revealed the preserved left and right ventricular systolic function, normal dimensions of the left ventricle and the atrium, severe pulmonary valve regurgitation, hypoplastic right ventricular outflow tract, dilated main pulmonary artery and its branches, as well as ASD with calculated Qp/Qs of 2 : 1. Injection of contrast into the cubital vein resulted in bubbles passing into the left atrium. Interestingly, cardiac magnetic resonance (CMR) imaging did not show any communication between the two atria (Figures 1 and 2).

The patient was presented to the international heart team for grown up congenital heart diseases and the decision to perform transcatheter closure of ASD was made, due to numerous comorbidities. It was estimated that the surgical risk of reoperation was unacceptably high for this patient, with a huge range of possible complications that could occur.

Right femoral venous access was obtained. A multipurpose catheter with a stiff guidewire passed through ASD and was placed into the left upper pulmonary vein. A lot of difficulties occurred in terms of heart`s orientation in the chest as the heart was rotated and the position of the interatrial septum was shifted towards the right side of the patient's chest, almost parallel to the diaphragm. Sizing was made with 25/40 mm balloon, which showed a defect of 10 mm in diameter, with continuous fluoroscopic and 3D TEE imaging. TEE probe passing through the esophagus was very demanding because of the almost 90 degrees bend in the proximal third, but was successful ultimately. Size 15 Figulla® Flex ASD (Occlutech®, Jena, Germany) occluder was delivered *via* 12 French introducer sheath with excellent stability and

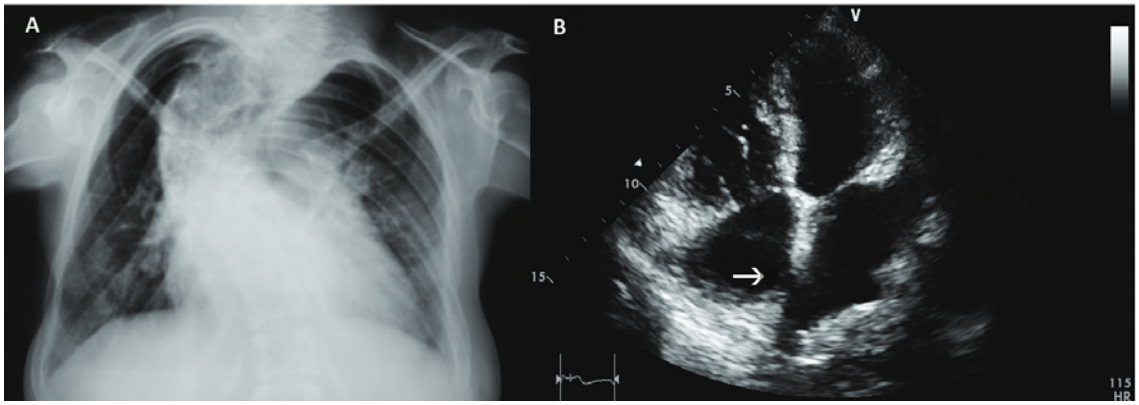

Fig. 1 – A) Chest x-ray showing the enlarged cardiac silhouette and severe kyphoscoliosis; B) Transthoracic echocardiogram showing atrial septal defect (arrow).

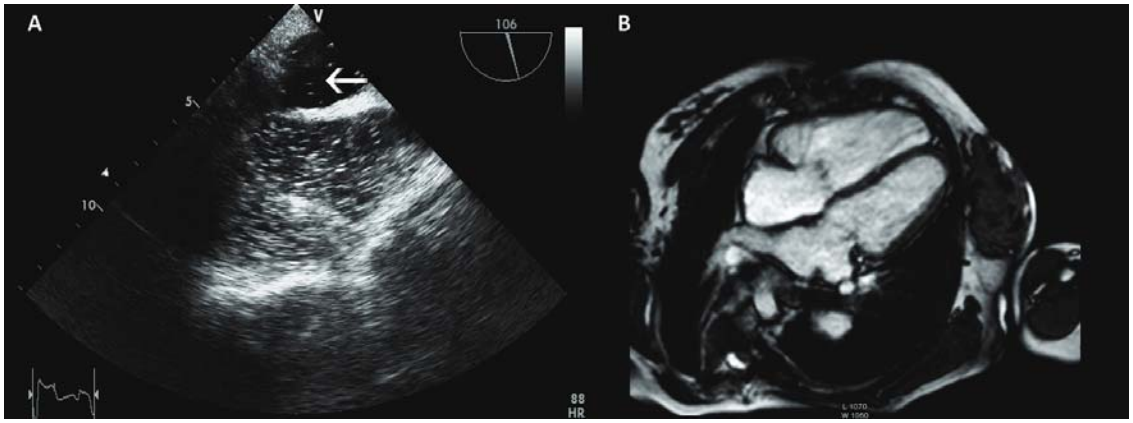

Fig. 2 – A) Transesophageal echocardiogram showing passage of the contrast from the right to the left atrium (arrow); B) Cardiac magnetic resonance imaging showing no visible communication between the two atria.

with exclusion of any shunt at the level of interatrial septum (Figure 3).

A 24 h-postprocedure TTE exam showed no shunt at the level of interatrial septum and the patient was discharged two days after the procedure in good condition, without complications and on dual antiplatelet therapy. One- and six-month clinical follow-ups were done and the patient reported significant improvement in her NYHA class from 2 to 1. TTE examination confirmed good apposition of the device without the evidence of residual shunt (Figure 4).

later with hemodinamically significant ASD, with calculated Qp/Qs of 2 : 1. Decision was made to perform transcatheter closure of ASD because of the patient's numerous comorbidities. The procedure itself was very demanding in terms of patient's short stature and low body weight as well as her kyphoscoliosis which was disturbing normal anatomy of the chest. Occluder device was successfully delivered, and complete closure of ASD was achieved, with excellent positioning and stability.

The follow-up period of 6 months was uneventful and the patient reported significant improvement in her symptoms.

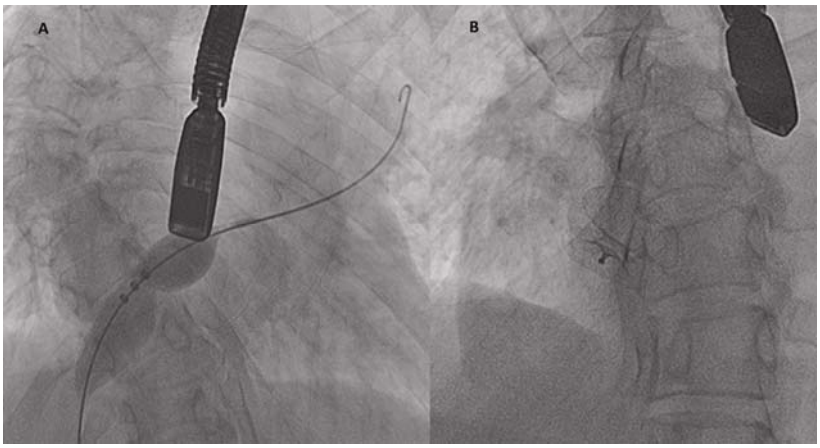

Fig. 3 – A) Fluoroscopy imaging showing the sizing of atrial septal defect with 25/40 mm balloon; B) Fluoroscopy imaging demonstrating atrial septal defect occluder positioned in the atrial septum.

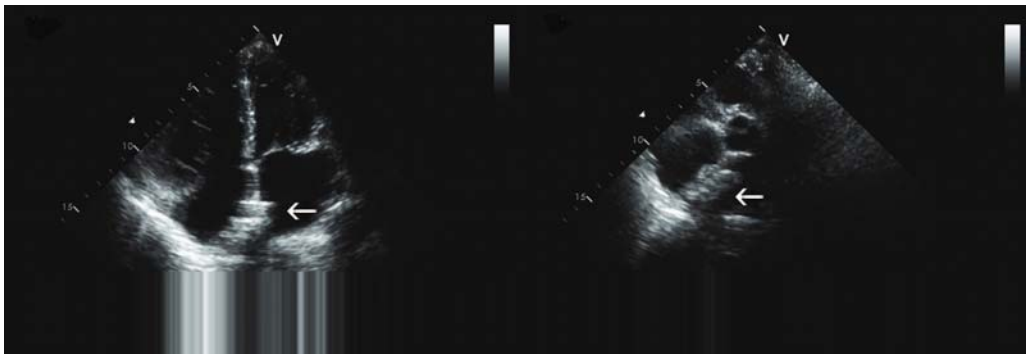

Fig. 4 – Transthoracic echocardiogram showing the good aposition of atrial septal defect occluder device on 6-month follow-up (arrows).

Discussion

Noonan syndrome is an autosomal dominant multisystem disorder which also affects the cardiovascular system. The most common cardiovascular phenotypes include pulmonary stenosis, hypertrophic cardiomyopathy and *secundum* ASD [1,4]. Some patients will require surgery or transcatheter procedures early in the childhood including surgery of the pulmonary valve, closure of ASD or resection of hypertrophied myocardium [6]. Reoccurrence of the defect after surgical closure of ASD is a rare complication occurring in less than 1% in the late postoperative period [7].

In this case report we presented a female patient diagnosed with Noonan syndrome in her early age, who underwent surgical closure of ASD and resection of the hypertrophied right ventricular infundibulum at the age of two. She presented 37 years

Conclusion

Transcatheter atrial septal defect closure in a patient with Noonan syndrome with the history of surgically corrected this defect can be performed successfully, despite the challenging chest anatomy. Although rare, significant atrial septal defect reoccurrence may potentially have deleterious effects on hemodynamics if not treated in a timely manner. Transcatheter closure of such defects could represent a reasonable treatment option in these patients.

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R E F E R E N C E S

1. *Roberts AE, Allanson JE, Tartaglia M, Gelb BD*. Noonan syndrome. Lancet 2013; 381(9863): 333−42.
2. *Opitz JM*. The Noonan syndrome. Am J Med Genet 1985; 21(3): 515−8.
3. *Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H*, et al. Mutations in PTPN11, encodes the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001; 29(4): 465−8.
4. *Romano AA, Allanson JE, Dahlgren J, Gelb BD, Hall B, Pierpont ME,* et al. Noonan syndrome: clinical features, diagnosis, and management guidelines. Pediatrics 2010; 126(4): 746−59.
5. *Cowley CG, Lloyd TR, Bove EL, Gaffney D, Dietrich M, Rocchini AP*. Comparison of results of closure of secundum atrial septal defect by surgery versus Amplatzer septal occluder. Am J Cardiol 2001; 88(5): 589−91.
6. *Webb GD, Smallhorn JF, Therrien J, Redington AN*. Congenital heart disease. In: *Bonow RO, Mann DL, Zipes DP, Libby P*, editors. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. Philadelphia, Pa: Saunders Elsevier; 2011. chap 65. Available from: http://www.nlm.nih.gov/medlineplus/ency/article/001114.htm
7. *Murphy JG, Gersh BJ, McGoon MD, Mair DD, Porter CJ, Ilstrup DM,* et al. Long-term outcome after surgical repair of isolated atrial septal defect. Follow-up at 27 to 32 years. N Engl J Med 1990; 323(24): 1645−50.

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

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

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

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