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LOCAL THERAPY WITH IMIQUIMOD AS A POSSIBLE MEDICAL TREATMENT OF VULVAR INTRAEPITHELIAL NEOPLASMS

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Imiquimod is a local immunomodulator with antiviral effects. Vulvar intraepithelial neoplasia is a chronic precancerous condition of the skin of the vulva, with different malignant potential and clinical course. The aim of the paper was to determine therapeutic effects of Imiquimod in treating different types and grades of vulvar intraepithelial neoplasms. The study enrolled 17 patients with vulvar pre-cancerous conditions of different grade and histological type. The patients were treated with combined medical therapy oral systemic immunomodulatory and antiviral drug - inosine acedoben dimepranol and 5% Imiquimod cream locally applied to the lesion area using cotton swabs. Complete remision (CR) had 41.18% of patients, partial remission (PR) was seen in 47.06%, and 11.76% of patients had no response (NR). Out of these patients, response distribution for usual type was: CR 80%, 20% NR, and for differentiated type the response distribution was: 8.3% NR, 66.67% PR, while 25% of patients had CR. The use of imiquimod for conservative treatment of vulvar intraepithelial neoplasia is a beneficial alternative to surgical treatment. The best results of imiquimod treatment are achieved in younger patients with usual type of vulvar neoplasia, while the treatment effects are limited to partial response in older patients with differentiated VIN.
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Key words: *medical treatment, local therapy, vulvar intraepithelial neoplasia*

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Introduction

Imiquimod is a local immunomodulator with antiviral effects. Its application has been approved for the topical treatment of vulvar skin disorders and local manifestations of vulvar human papilloma virus (HPV) infections (condylomas) by the US "Food and Drug Administration" (FDA). Recommended duration of the treatment is 12-20 weeks, with colposcopic examinations at intervals of 4-6 weeks.

Imiquimod effects are demonstrated as an increase in the number of immune cells: CD1+ dendritic cells, CD8+ T cells and CD94+ natural killer cells, present not only in the area of vulvar intraepithelial neoplasia (VIN), but also in healthy surrounding tissue where skip lesions may be present. Therapeutic response rate is 73%, with complete remission in 35%, and partial remission in 38% of patients (1).

This drug modifies immune response by activating Toll-like receptors (TLR)-7 and TLR-8 cascade. It affects superficial receptors of immature plasmacytoid dendritic cells inducing their maturation and activation. Activated dendritic cells produce cytokines, mediators that activate T-cell immune response which is believed to be directly responsible for HPV 16 elimination.

Imiquimod possesses a direct pro-apoptotic activity against tumour cells and antiviral activity against HPV. Therapeutic efficacy of imiquimod can be seen not only in the area of VIN, but also in adjacent areas where skip lesions may be present. Such an extended immunological reaction and immune-stimulating effects that are present even after cessation of Imiquimod application are certainly one of the reasons for lower rate of recurrence in comparison to surgical treatment (2, 3).

Vulvar intraepithelial neoplasia is a chronic precancerous condition of the skin of the vulva, with different malignant potential and clinical course. VIN demonstrates a proliferation of abnormal keratinocytes of the vulvar epidermis without invasion of the basement membrane.

In the last 100 years there have been many changes in the classification of vulvar pathological conditions due to different etiopathogenic pathways. In 1967 Richart proposed the term 'intraepithelial neoplasia' for the classification of cervical conditions, and in 1982 Crum used the term not only for lesions of the cervix, but also for vulvar lesions. In 1986 International Society for the Study of Vulvovaginal Disease (ISSVD) introduced the term 'vulvar intraepithelial neoplasia' which was graded as VIN I, II and III (4).

The College of American Pathologists (CAP) and American Society for Colposcopy and Cervical Pathology (ASCCP) published the Lower Anogenital Squamous Terminology (LAST) guidelines in 2012, by which all HPV lesions involving the cervix, vulva, vagina, anus, perineum and penis are classified into two groups:

1. low-grade squamous intraepithelial lesion (LSIL), and

2. high-grade squamous intraepithelial lesion (HSIL).

LSIL is equivalent to uVIN I, and HSIL involves uVIN II and III. The World Health Organization (WHO) and ISSVD in 2014 and 2015 respectively accepted this classification with addition of differentiated VIN as separate category (5).

Characteristics of VIN, usual type (uVIN), are:

• It occurs in young women in the third and fourth decades of life, more commonly in female smokers and immunosuppressive ones with multiple sexual partners;

• Low malignant potential (3 - 5%);

• 9% of the untreated and 3.3% of treated uVIN cases progress to vulvar squamous cell carcinoma cancer (VSCC) (6);

• Asymptomatic or present with itching and dysuria;

• It is manifested as erythematous macules or papules, verrucous plaques; about 10% of the lesions may be pigmented, and approximately 66 % lesions are multifocal (7).

Differentiated VIN (dVIN) is less common, accounting for 2 to 10 % of all VIN.

• In older patient it may be associated with chronic dermatoses, lichen, chronic oxidative stress and ischemia;

• Clinical manifestations more commonly involve unifocal grey-white lesion that may be in the form of rough, vaguely defined, nodular white plaques, or red lesions and ulcerations;

• HPV negativity (only 28.6% of VSCC cases are HPV positive) – alternative mechanism of etiopathogenesis (8);

• High malignant potential: 32.8% dVIN and 5.7% uVIN progress to VSCC. About 23.7% uVIN and 85.7% dVIN are present prior or at the time of VSCC diagnosis (9);

•In 86.7% of uVIN cases HPV has been found positive, and HPV type 16 has been detected in 77.2% of cases (10);

•18 – 52% of patients have associated lesions at other anogenital locations (11).

Aim of the paper

The aim of the paper was to determine therapeutic effects of Imiquimod in treating different types and grades of vulvar intraepithelial neoplasms.

Patients and methods

This paper gives initial results of a larger, prospective study that investigates medical management of VIN. The study enrolled 17 patients with vulvar pre-cancerous conditions of different grade and histological type.

The patients in our study were treated with combined medical therapy – oral systemic immunomodulatory and antiviral drug - inosine acedoben dimepranol 50 tablets of 500 mg, 3 x 2 tablets daily and 5% Imiquimod cream locally applied to the vulvar lesion site and to healthy skin within 1cm of the lesion using cotton swabs once daily, late in the evening, three times a week (every second day) for 12 weeks, with regular vulvoscopic evaluation every 4 weeks.

Side effects of the medical therapy applied in this study were mild, including mild skin irritation, itching and burning. These symptoms disappeared after the completion of the treatment and during the treatment the intensity of side effects was tolerable, so the cessation or a decrease in the number of application was not required.

All the patients underwent HPV typing prior to medical treatment. The study was conducted in accordance with the principles of good clinical practice and ethical standards of scientific research. All the patients signed informed consent to participate in the study.

Statistical data analysis was performed with SPSS version 15.0 statistics software package.

Results

Therapeutic response was described as complete response (CR), partial response (PR) with minimum reduction of 25%, and no response (NR) category for the patients with no improvement and no therapy response.

All the patients from the first age group were HPV positive, and those from the second age group were HPV negative.

Table 1 illustrates the number of patients according to the age groups and therapeutic response.

Statistical significance was tested by χ^2 test, the results are shown in the Graph 1 ($\chi^2 = 6.335$, p = 0.38 (p < 0.05)).

Table 1. Distribution of patients according to age groups and in relation to therapeutic response

Age group of patients	Complete response	Partial response	Non response	Total
Adolescent girls	4	0	1	5
Postmenopausal women	3	8	1	12
Total	7	8	2	17
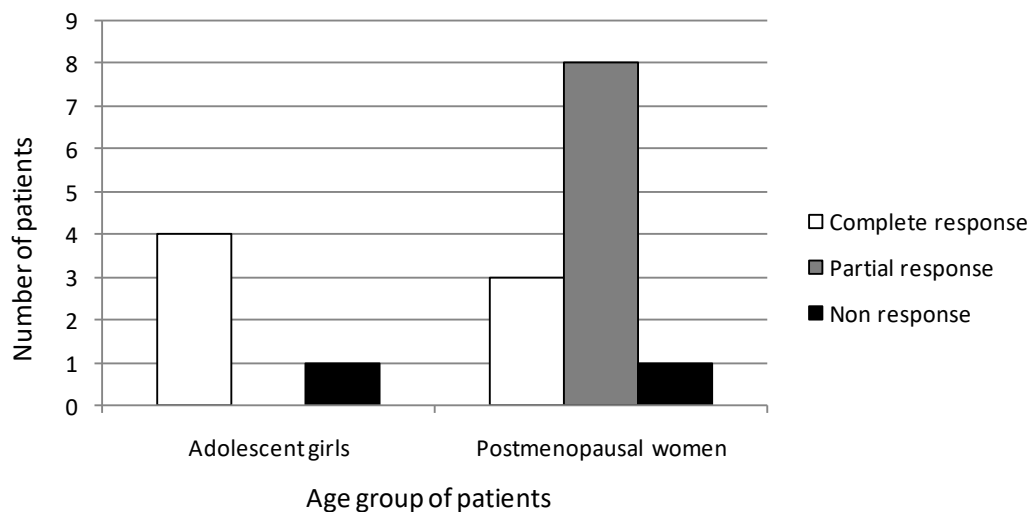
$\chi^2 = 6{,}335$, p = 0,38 (p < 0,05)

Graph 1. Statistical significance of the therapeutic response between the age groups

Graph 2 shows percentage of patients from the first age group based on the response. The same data for the second age group of patients are shown in Graph 3.

The results obtained demonstrate that a total of 41.18% of patients achieved CR, PR was seen in 47.06%, and 11.76% of patients had no response. Out of these patients, response distribution for usual type was: CR 80%, 20% NR, and for differentiated type the response distribution was: 8.3% NR, 66.67% PR, while 25% of patients had CR.

Discussion

Radical surgical intervention – simplex vulvectomy – was used in treating VIN from early 1960s until 1970s. Such a surgical treatment resulted in severe psychological, physiological and sexual impairments. After assessing etiopathogenesis and clinical course of VIN, some less aggressive surgical treatments became available, such as local excision, laser ablation, photodynamic treatment, or medical treatment with "topical imiquimod and cidofovir". Conservative non-surgical treatment is performed only in patients with no evidence of invasive disease (12).

The excision includes the margins of 0.5 to 1cm of normal skin tissue without visible disease with scalpel, laser, or electrosurgical excision and is related to differentiated type of VIN, as well as to all the patients with VIN with suspected invasive disease, multifocal changes and immunocompromised patients. The recurrence rate is higher in these patients and ranges between 20% and 40%, with psychosexual dysfunction commonly reported (13).

The result of excision margins from the healthy skin is a significant prognostic and therapeutic parameter important for treatment outcomes.

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Adolescent girls
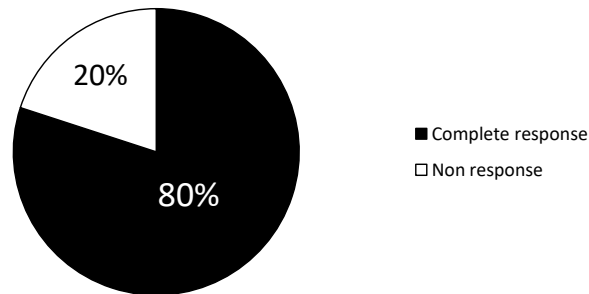
Graph 2. Percentage of patients from the first age group dependent on therapeutic response

Postmenopausal women
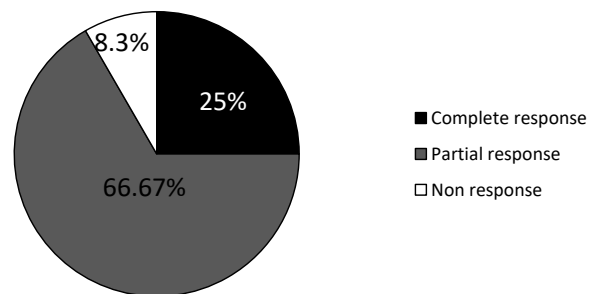
Graph 3. Percentage of patients from the second age group dependent on therapeutic response

The percentage of positive margins after surgical excision is high and ranges from 24-68%. The reason for such a high percentage should be sought in non-specific clinical manifestation of VIN, undefined margins of the pathological process of the skin of the vulva, and subclinical manifestation of HPV infection in the surrounding tissue. A recurrence rate over 50% was registered in treated patients with positive surgical margins (14).

Medical treatment is usually reserved for usual type of HPV positive VIN. Apart from imiquimod, antiviral drugs such as cidofovir, indole-3-carbinol, 5-fluorouracil, interferon-α/γ, podophyllotoxin, inosine acedoben dimepranol are also used. Recurrence rate of 9% was registered in patients with complete response to imiquimod, 17% after local excision, 23 - 40% after laser ablation. Generally speaking, the percentage of recurrences of VIN after imiquimod cream treatment is lower than after surgical excision (15% vs 42%). Side effects of the treatment are fewer with topical immunomodulators and include local irritation, burning, redness, or rarely systemic adverse effect such as headache (15).

Conclusion

The use of imiquimod for conservative treatment of vulvar intraepithelial neoplasia is a beneficial alternative to surgical treatment.

The best results of imiquimod treatment are achieved in younger patients with usual type of vulvar neoplasia, while the treatment effects are limited to partial response in older patients with differentiated VIN.

Further larger investigations are mandatory for the implementation of the drug into standard administration procedure regarding the therapeutic approach for certain histological types of VIN.

Side effects of imiquimod are mild and withdraw soon after cessation of treatment.

Acknowledgement

References

1. Committee Opinion No. 675. Management of vulvar intraepithelial neoplasia. American College of Obstetricians and Gynecologists. Obstet Gynecol 2016; 128: e178-8. [CrossRef] [PubMed]
2. Terlou A, van Seters M, Ewing PC, Aaronson NK, Gundy CM, Heijmans-Antonissen C, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod: seven years median follow-up of a randomized clinical trial. Gynecol Oncol 2011; 121(1): 157-62. [CrossRef] [PubMed]
3. Van Esch EM, Dam MC, Osse ME, Putter H, Trimbos BJ, Fleuren G, et al. Clinical characteristics associated with development of recurrence and progression in usual-type vulvar intraepithelial neoplasia. Int J Gynecol Cancer 2013; 23(8): 1476-83. CrossRef] [PubMed]
4. Wilkinson EJ, Kneale B, Lynch PJ. Report of the ISSVD Terminology Committee. J Reprod Med 1986; 31: 973-4.
5. Bornstein J, Bogliatto F, Haefner HK, Stockdale CK, Preti M, Bohl TG, et al. The 2015 International Society for the Study of Vulvovaginal Disease (ISSVD) Terminology of Vulvar Squamous Intraepithelial Lesions. J Low Genit Tract Dis 2016; 20(1): 11-4. [CrossRef] [PubMed]
6. del Pino M, Rodriguez-Carunchio L, Ordi J. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. Histopathology 2013; 62(1): 161-75. [CrossRef] [PubMed]
7. Hampl M, Sarajuuri H, Wentzensen N, Bender HG, Kueppers V. Effect of human papillomavirus vaccines on vulvar, vaginal, and anal intraepithelial lesions and vulvar cancer. Obstet Gynecol 2006; 108(6): 1361-8. [CrossRef] [PubMed]
8. van de Nieuwenhof HP, Massuger LF, van der Avoort IA, Bekkers RL, Casparie M, Abma W i sar. Vulvar squamous cell carcinoma development after diagnosis of VIN increases with age. Eur J Cancer 2009; 45(5): 851-6. [CrossRef] [PubMed]
9. Maniar KP, Ronnett BM, Vang R, Yemelyanova A. Coexisting high-grade vulvar intraepithelial neoplasia (VIN) and condyloma acuminatum: independent lesions due to different HPV types occurring in immunocompromised patients. Am J Surg Pathol 2013; 37(1): 53-60. [CrossRef] [PubMed]
10. Léonard B, Kridelka F, Delbecque K, Goffin F, Demoulin S, Doyen J, et al. A clinical and pathological overview of vulvar condyloma acuminatum, intraepithelial neoplasia, and squamous cell carcinoma. Biomed Res Int 2014; 2014: 480573. [CrossRef] [PubMed]
11. van Beurden M, ten Kate FJ, Smits HL, Berkhout RJ, de Craen AJ, van der Vange N, et al. Multifocal vulvar intraepithelial neoplasia grade III and multicentric lower genital tract neoplasia is associated with transcriptionally active human papillomavirus. Cancer 1995; 75(12): 2879-84. [CrossRef] [PubMed]
12. Chafe W, Richards A, Morgan L, Wilkinson E. Unrecognized invasive carcinoma in vulvar intraepithelial neoplasia (VIN). Gynecol Oncol 1988; 31(1): 154-65. [CrossRef] [PubMed]
13. Likes WM, Stegbauer C, Tillmanns T, Pruett J. Pilot study of sexual function and quality of life after excision for vulvar intraepithelial neoplasia. J Reprod Med 2007; 52(1): 23-7. [PubMed]
14. van Seters M, van Beurden M, ten Kate FJ, Beckmann I, Ewing PC, Eijkemans MJ, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod. N Engl J Med 2008; 358(14): 1465-73. [CrossRef] [PubMed]
15. Hillemanns P, Wang X, Staehle S, Michels W, Dannecker C. Evaluation of different treatment modalities for vulvar intraepithelial neoplasia (VIN): CO(2) laser vaporization, photodynamic therapy, excision and vulvectomy. Gynecol Oncol 2006; 100(2): 271-5. [CrossRef] [PubMed

LOKALNA TERAPIJA IMIKVIMODOM KAO MOGUĆI VID MEDIKAMENTOZNOG TRETMANA VULVARNIH INTRAEPITELNIH NEOPLAZIJA

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Imikvimod je lokalni imunomodulator sa antivirusnim dejstvom. Vulvalna intraepitelna neoplazija (VIN) predstavlja hronično prekancerozno kožno oboljenje vulve, koje ima različiti potencijal maligne progresije i klinički tok. Cilj rada bio je da se utvrdi terapijski efekat imikvamida u terapiji različitih tipova i stadijuma vulvarnih intraepitelnih neoplazija. U istraživanje je uključeno 15 bolesnica sa vulvarnim prekancerozama različitog stadijuma bolesti i histološkog tipa. Ispitanice su uzimale kombinovanu medikamentoznu terapiju, oralno sistemski imunomodulator i antivirotik - inozin acedoben dimepranol i lokalno 5% krema imikvimod koja se aplikuje štapićem sa vatom na mestu lezije. Kompletnu remisiju (CR) imalo je ukupno 41,18% bolesnica, parcijalnu (PR) ukupno 47,06% i bez odgovora (NR) je bilo 11,76% bolesnica. Od toga, kod uobičajnog tipa distribucija odgovora bila je sledeća: CR 80%, a 20% je bilo NR, a kod diferentovanog: 8,3% NR, 66,67% PR, a kod 25% bolesnica CR. Upotreba imikvamida u konzervativnom tretmanu vulvarnih intraepitelnih neoplazija predstavlja dobru alternativu hirurškom tretmanu. Najbolji rezultati u lečenju imikvimodom su dobijeni kod mlađih bolesnica sa uobičajnim tipom vulvarnih neoplazija, dok je kod starijih bolesnica sa diferentovanim tipom VIN-a efekat bio ograničen na parcijalnu remisiju bolesti.

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Ključne reči: *medikamentozni tretman, lokalna terapija, vulvarna intraepitelna neoplazija*

ASTRAND PROGRESSIVE LOAD TEST IN ASSESSING AEROBIC CAPACITY OF ATHLETS

Ljubiša M. Lilić, Sladjana Milošević, Božidar Stojiljković

The most accurate picture of physical preparation is given by aerobic capacity, which is an important factor in planning and dosage of physical load.

The aim of this research was to determine the value of the aerobic capacity of athletes of different sport branches, as well as possible differences in relation to non-athletes.

We analyzed 60 respondents in total, divided into three groups of 20 each. The first group included footballers, the second handball players, and the third- control group were non-athletes.

The maximum aerobic capacity was determined by Astrand's 6 - minute test.

The highest value of VO_{2max} is recorded with football players 4.26 L/min and this value is statistically significantly higher p < 0.001 compared to the other two groups.

Somewhat lower VO_{2max} values were found at handball players 4.01 L/min.

The lowest values of VO_{2max}, both in absolute and relative values, have non-athletes and these values are statistically significantly lower than in the previous two groups p < 0.001.

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Key words: *physical preparation, aerobic capacity, maximum oxygen consumption, physical activity, submaximal test*

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Introduction

Aerobic ability is an important parameter in the dosage of physical load in health people as well as in the treatment of cardiovascular patients. At the same time, this parameter gives us a picture of physical preparation.

Physical activity of the aerobic type is an integral part of primary and secondary prevention of cardiovascular diseases (1-3).

Individual values of maximum oxygen consumption are the most precise parameters for dosing of physical activity (4).

Aerobic capacity is the main indicator of physical fitness of athletes, and it is limited by maximum oxygen intake (VO_{2max}).

Maximum oxygen intake is a factor that limits metabolic processes that transform chemical energy into mechanical ones (5-7).

Proper dosage of physical activity for the purpose of either therapy or training is achieved by using individual values of maximum oxygen consumption.

The functional ability of the cardiovascular system, the respiratory system, and the ability of the tissue to utilize the delivered oxygen, most accurately determines the maximum consumption of oxygen.

The maximum consumption of oxygen is the maximum amount of oxygen the organism consumes in the unit of time at load of progressive intensity, which does not change significantly during the further increase of the intensity of the load (4).

The maximum consumption of oxygen is expressed in liters or milliliters per minute (liters / min, ml / min). This expressed value is the absolute value (8-10).

A more objective and more precise expression of the maximum oxygen consumption is in relative values, since the body weight (ml/TM kg/min) signi-

ficantly influences the maximum oxygen consumption (1, 4).

Aerobic ability is most easily and accurately determined in laboratory conditions by the tests of maximum physical load, and can be carried out either on Thread mill or ergometer bicycle (11).

When the test is to be performed on a large number of subjects, the bench step is an ergometer of choice (11).

By determing the aerobic capacity according to Astrand test our goal is to establish if the type of sport and training affects this important functional parameter that determines the energy capacity.

Material and methods

The survey included 40 active male respondents (age 21.2 ± 0.8) who are actively engaged in sports, 20 football players and 20 handball players. As a control group, 20 male persons of similar age (20.9 ± 3) who do not actively engage in sports, are tested.

The tests were carried out in the specially prepared room of the Sports Center in Leposavić, always at the same time, with the same instruments, using the same technique according to the standard procedure.

We determined the body mass - an anthropological variable with a decimal beam scale. The subjects were barefoot and in sports equipment.

The value of maximum oxygen consumption was determined by the progressive continuous submaximal test according to Astrand established protocol.

The test started by measuring the heart rate, pulse and blood pressure in peaceful seating position. Each respondent, after entering the laboratory, sat on a chair for 5 minutes, then approached the measurements. The pulse was measured by the palpable method.

After measuring the pulse and pressure, the participants sat on an ergometer bike, adjusted the seats according to their height, and then started the test with the rhythmic turn of the pedal, at the beginning it was 50 rpm. After 6 minutes the load was increased by 50 Watts. In each 6 - minute cycle, the pulse was measured every minute for the last 30 seconds, and the resulting value was multiplied by 2 to calculate the rate of heart frequency. The blood

pressure was measured at half of each 6 - minute cycle with tensiometer.

The test was interrupted at the moment of the maximum heart rate when we measured a frequency that did not differ for more than five beats per minute in two successive measurements due to the entry of the heart into the stationary state. The test was usually interrupted at a frequency of 130-140 beats per minute. The obtained values were used to read the absolute maximum oxygen consumption from the normograms.

By dividing absolute oxygen consumption with body weight in kilograms, we obtained a relative consumption of oxygen (ml/kg/min).

The statistical processing of all parameters was done by calculating the mean value and the standard deviation, and the obtained parameters are shown in tables and graphics. The statistical significance of the differences is determined by Student's t-test.

Results

The maximum consumption of oxygen represents the physical capacity of athletes and is often compared to his physical ability.

The highest value of the maximum oxygen consumption expressed in absolute units (l/min) was recorded in the footballers 4.26 l/min and this value is statistically significantly higher (p < 0.001) compared to the other two groups (Table 1), as demonstrated by the Student's t-test.

The results of the relative maximum consumption of the oxygen are shown in Table 2. The highest value (51.78 ml/kg/min) is determined in footballers and this value is statistically significantly different from the other two groups (p < 0.001). Such high values of the absolute and the relative maximum oxygen consumption of a football players show that football is a demanding sport with quite a lot of aerobic periods during the game. Handball players compared to non-athletes have statistically significantly higher values of these two parameters (absolute and relative maximum consumption of oxygen) which shows that quality and controlled daily training significantly elevates the aerobic power of the respondents.

Table 1. Mean values of aerobic capacity in tested groups l / min.

Examinees	VO$_{2max}$/l/min	SD
Football players	4.26	0.15
Handball players	4.01	0.21
Non-athletes	3.21	0.18

Table 2. Average values of aerobic capacity in examined groups, relative value per ml/kg/min.

Examinees	VO$_{2max}$/ml/kg/min	SD
Football players	51.78	1.22
Handball players	46.12	1.71
Non-athletes	40.93	1.20

Discussion

High values of aerobic capacity (absolute and relative) are necessary for achieving good results in sport, so their determination is an important parameter of fitness and physical ability (4, 5, 8, 10).

Aerobic capacity is the most important indicator of the functional capabilities of all systems involved in the supply, transport and energy transformation of oxygen (4).

The damage of any of these links will affect the athletes' aerobic abilities (5, 12). The maximum values of aerobic capacity in athletes are observed around the age of 20-22. nevertheless, the system of training and work of athletes in later years, can also lead to a slight increase of maximal aerobic abilities of athletes.

Analyzing our experimental groups, we see that the highest values of the absolute maximum oxygen consumption (51.78 ml/kg/min) and the relative maximum oxygen consumption are met with the football players.

Diaz also came to similar results (11) by measuring the maximum consumption of oxygen in Mexican footballers (53.8 ml/kg/min).

Vishoff analyzed Norwegian national team players (60 ml/kg/min), and found significantly higher average values of maximum oxygen consum-ption.

This great difference between our respondents and the respondents interviewed by the authors mentioned above can be explained by the ranking of the participants' competition.

Generally speaking, the high values of the maximum aerobic capacity of the football players in relation to the handball players and control group can be explained by the efforts and reinforced training that football requires from the player. In order to meet these high energy demands that sport requires, athletes must have a more efficient energy system.

Because of this, handball players have lower values of maximum oxygen consumption compared to football players, and statistically significantly higher than non-athletes.

All this supports the claim that training is the most important factor in the development of aerobic capacity.

Conclusion

The values of maximum oxygen consumption are important parameters for assessing the physical fitness of athletes.

By analyzing this parameter, it is possible to determine the level of fitness with certainty, check the quality of the training and evaluate the possible results. The higher values of the maximum oxygen consumption are a predisposition of greater success.

In our work, the highest value of absolute and relative maximum oxygen consumption is encountered among the football players, and the reason should be sought in the type of sport and mode of training.

The values of absolute and relative maximum oxygen consumption are significantly higher among athletes compared to non-athletes, which is a sure sign that the value of this parameter is strongly influenced by training.

References

1. Fletcher G. How to implement physical activity in primary and secondary prevention. A statement for healthcare-professionals from the Task Force on Risk-reduction, American Heart Association. Circulation 1997; 96(1): 355-7. [CrossRef] [PubMed]
2. American College of Sports Medicine. Gudelines for exercise testing and prescription. 4th ed. Lea and Febiger; 1991.
3. Twisk J, Kemper C, Van Mechelen W. Tracking of activity and fitness and the relationship with cardiovascular disease risk factors. Med Sci Sports Exerc 2000; 32(8): 1455-61. [CrossRef] [PubMed]
4. Ranković G, Mutavdžić V, Toskić D, Preljević A, Kocić M, Nedin-Ranković G, et al. Aerobic capacity as an indicator in different kinds of sports. Bosn J Basic Med Sci 2010; 10(1): 44-8. [CrossRef] [PubMed]
5. Živanić S, Životić-Vanović M, Mijić R, Dragojević R. Aerobna sposobnost i njena procena Astrandovim testom na bicikl-ergometru. Beograd: Udruženje za medicinu sporta Srbije; 1999: 16.
6. Živanić S. Morfo-funkcionalne karakteristike prvoligaš-kih fudbalera u SCG. Sportska Medicina 2003; 1(03): 89.
7. Životić-Vanović M. Fiziološke osnove za procenu aerobne sposobnosti pripadnika OS kod primene ergometrijskog step-testa [Doktorska disertacija]. Beograd: Vojnomedicinska akademija; 1991.
8. Janz K, Dawson J, Mahoney L. Tracking physical fitness and physical activity from childhood to adolescence: the Muscatine study. Med Sci Sports Exerc 2000; 32(7): 1250-7. [CrossRef] [PubMed]
9. Fleg JL, Pina IL, Balady GJ, Chaitman BR, Fletcher B, Lavie C, et al. Assessment of functional capacity in clinical and research applications: An advisory from the Committee on Exercise, Rehabilitation, and Prevention, Council on Clinical Cardiology, American Heart Association. Circulation 2000; 102(13): 1591-7. [CrossRef] [PubMed]
10. Živanić S, Životić VM, Malićević S, Mijić R, Ostojić S, Bogdnović S. Morfo-funkcionalne karakteristike prvoligaškik fudbalera u SCG. Zbornik sažetaka Prvi srpski kongres sportskih nauka i medicine sporta; Beograd: 2003.
11. Mazić S, Životić-Vanović M, Igrački I, Živanić S, Velkovski S. A simple and reliable step-test for indirect evaluation of aerobic capacity. Med pregl 2001; 54 (11-12): 522-9. [PubMed]
12. Fletcher GF, Balady G, Froelicher VF, Hartley LH, Haskell WL, Pollock ML. Exercise standards. A statement for healthcare professionals from the American Heart Association. Writing Group. Circulation 1995; 91(2): 580-615. [CrossRef] [PubMed]

ASTRANDOV PROGRESIVNI TEST OPTEREĆENJA U PROCENI AEROBNOG KAPACITETA SPORTISTA

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Najpreizniju sliku fizičke pripremljenosti daje aerobni kapacitet. On je važan faktor u planiranju i doziranju fizičkog opterećenja.

Cilj ovog istraživanja bio je određivanje vrednosti areobnog kapaciteta sportista različitih sportskih grana, kao i eventualnih razlika u odnosu na nesportiste.

Ukupno smo analizirali 60 ispitanika podeljenih u tri grupe od po 20. Prvu grupu sačinjavali su fudbaleri, drugu rukometaši, a treću kontrolnu grupu nesportisti.

Maksimalni aerobni kapacitet određivan je Astrandovim šestominutnim testom.

Najveću vrednost VO_{2max} konstantovan je kod fudbalera 4,26 L/min. Ova vrednost je statistički značajno veća p < 0,001 u odnosu na ostale dve grupe.

Nešto niže vrednosti VO_{2max} su konstatovane kod rukometaša 4,01 L/min.

Najniže vrednosti VO_{2max}, kako u apsolutnim tako i relativnim vrednostima, imaju nesportisti i one su statistički značajno manje nego kod prethodne dve grupe p < 0,001.

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Ključne reči: *fizička pripremljenost, aerobni kapacitet, maksimalna potrošnja kiseonika, fizička aktivnost, submaksimalni test*

A JOINPOINT REGRESSION ANALYSIS OF LONG-TERM TRENDS IN LEUKEMIA INCIDENCE AND MORTALITY IN CENTRAL SERBIA AND NIŠAVA DISTRICT (1999-2014)

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Leukemia contributes 2.3% to the overall cancer incidence in Serbia and 2.9% in the total mortality, while the estimated incidence and mortality rate for males and females were 8.7 and 6.3 ‰, respectively and occupies 13th place among both sexes in Central Serbia.

The objective of our study was to examine the time trends of leukemia in Central Serbia, with a focus on Nišava district, from 1999-2014, using a Joinpoint regression analysis and compare them with the trend in other populations, and identify possible changes.

The standardised incidence and mortality were obtained from the Serbian Cancer Registry of Central Serbia. Time trends for incidence and mortality of leukemia were assessed using the annual percent change, estimated through Joinpoint regression analysis (age period cohort models – APC) using the Joinpoint Regression Software.

Our results demonstrate a stable trend of the age-adjusted leukemia incidence rate both in males and females in Central Serbia during the observed 1999-2014 period. However, statistically significant decreasing trend of leukemia incidence rate was found in men from Nišava district, while non-significant slightly increasing pattern was present in women. Joinpoint analysis in our research demonstrated favorable mortality declines until the 2002, and than stabile trend in Central Serbia in both sexes to the end of the observed period. Conversely, mortality among males in Nišava district shows a positive trend, but not statistically significant.

The results of the study suggest that leukemia profile in Central Serbia was stabile during the study period. It is particularly interesting that incidence is decreasing among male population from Nišava district.

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Key words: *Leukaemia, Central Serbia, Nišava region, Joinpoint regression analysis*

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Introduction

According to the International Agency for Research on Cancer (GLOBOCAN 2012), leukemia contributes 2.3% to the overall cancer incidence in Serbia and 2.9% in the total mortality, while estimated incidence and mortality rate for males and females were 8.7 and 6.3 ‰, respectively (1). Among all cancers in Serbia, leukemia occupies 13th place among both sexes.

This evidence is based on research conducted at Los Alamos National Laboratory, studies of nuclear workers at other sites, and others exposed to ionising radiation (2, 3). Occupational exposure to benzene and other solvents is one of the most consistently observed risk factors for myeloid malignancy usually used in manufacture of organic chemicals and chemical intermediates (4, 5). Previous cancer treatment regimens with known cytotoxic agents that have leukemogenic potential, although other factors, such as the intensity of treatment and use of growth factors, also may have played a role in leukemia occurrence (6). Besides different environmental exposures, cigarette smoking also directly affects the central and peripheral hematopoietic system and decreases the number of circulating CD34+ progenitor cells in healthy individuals leding to leukemia (7, 8). Likewise, other factors as genetic disorders (9) and socio economic status (10, 11) are confirmed as risk factors for leukemia incidence. Encouraging news in recent decades is that a significant improvement has been set up in the diagnosis and treatment of leukemia.

The objective of our study was to examine the time trends of leukemia in Central Serbia, with a focus on Nišava district, from 1999-2014, using a Joinpoint regression analysis and compare them with trend in other populations, and identify possible changes.

Patients and methods

Data sources

Standardised incidence and mortality for the period 1999-2014 were obtained from the Serbian Cancer Registry of Central Serbia. The Registry, founded in 1985, covers the population of Central Serbia (approximately 5.2 million persons according to the Census of 2011). This Cancer Registry has been collecting data since 1996 when it was reorganised by the Agency for Research on Cancer – IACR, and the European Network of Cancer Registries – ENCR. According to the International Classification of Diseases Tenth Revision (ICD-10) and the Third Edition of International Classification of Diseases for Oncology, leukemia is defined as ICD-10 codes C91-C95 (12).

Time trends for incidence and mortality of leukemia were assessed using the annual percent change, estimated through Joinpoint regression analysis (age period cohort models – APC) using the Joinpoint Regression Software. This software has been developed by the United States National Cancer Institute for the analysis of data from the Surveillance Epide-

miology and End Results Program (SEER). This method covers changes in data trends by connecting several different line segments on a log scale at "joinpoints". The analysis starts with the minimum number of joinpoints (i.e., 0 joinpoint, representing a straight line) and tests for model fit with a maximum of 4 joinpoints. Monte Carlo permutation method is used to test the significance. Additionally, an annual percent change (APC) for each line segment is estimated. The APC is used to determine whether a difference exists from the null hypothesis of no change (0%). In the final model, each joinpoint shows a statistically significant change in trends (increase or decrease) and each of those patterns is described by an APC. In the case of non-statistically significant trends ($p > 0.05$), we used the term "stable".

Results

In the period from 1999 to 2014, the total number of new leukaemia cases in Central Serbia was 7035, among which 523 (7,4%) cases were in Nišava district. Leukemia was diagnosed in 4158 males and 2877 females (male-to-female ratio 1.5:1) in Central Serbia, while in Nišava district the male:female ratio was 1.8:1 (337 males, 186 females).

The total number of fatal cases was 5922 (3383 males and 2539 females died of leukemia with a male to female ratio 1.3:1). In Nišava district the total number of deaths was 336, of which 218 were males and 118 were females (ratio 1.8:1).

Table 1. Age-standardized incidence of leukaemia in Serbia and Nišava district in the period from 1999 to 2014.

	Serbia				Nišava district			
	male		female		male		female	
year	No of cases	ASR	No of cases	ASR	No of cases	ASR	No of cases	ASR
1999.	222	6	160	4.2	9	3.3	10	2.6
2000.	233	7.1	153	3.2	26	26	12	2.9
2001.	269	7.5	196	4.8	29	10.6	12	3.3
2002.	276	7.7	212	5.5	25	9.7	13	3
2003.	306	14	207	5.5	21	8.1	8	2.6
2004.	258	7	187	2.3	20	9.5	14	5.5
2005.	256	6.7	191	4.8	17	4.3	14	5
2006.	225	6.2	165	4	19	7	15	3.9
2007.	315	7.7	210	4.5	20	6.4	15	3.3
2008.	211	5	117	2.7	30	8.5	8	2.8
2009.	233	6.4	148	3.6	22	9.3	16	9.4
2010.	260	6.9	148	3.7	19	6.1	/	/
2011.	322	8.4	239	5.8	18	7.5	12	4.4
2012.	207	5.3	158	4	22	6.1	15	5.6
2013.	280	6.7	182	4.6	25	6.2	14	3.5
2014.	285	7.3	204	4.7	15	4.1	8	3.2

* ASR age-standardised rate per 100 000 (using standard world population)

Table 1 shows the age-standardised incidence rates (per 100 000 population) of leukemia in male

and female population of Central Serbia and Nišava district for the period 1999-2014. For the whole

analysed period the average age-standardised incidence for men was 7.24 and for females 4.24 in Central Serbia, and situation with Nišava district is as follows: 8.29 for men and 4.07 for women. The highest age-standardised incidence in Central Serbia in males was in 2003, in females in 2011, and the lowest values of those rates were in 2008 (1, 2.7 respectively).

The findings from the Joinpoint analysis for leukemia incidence in Central Serbia and Nišava district are shown in Graphs 1 and 2.
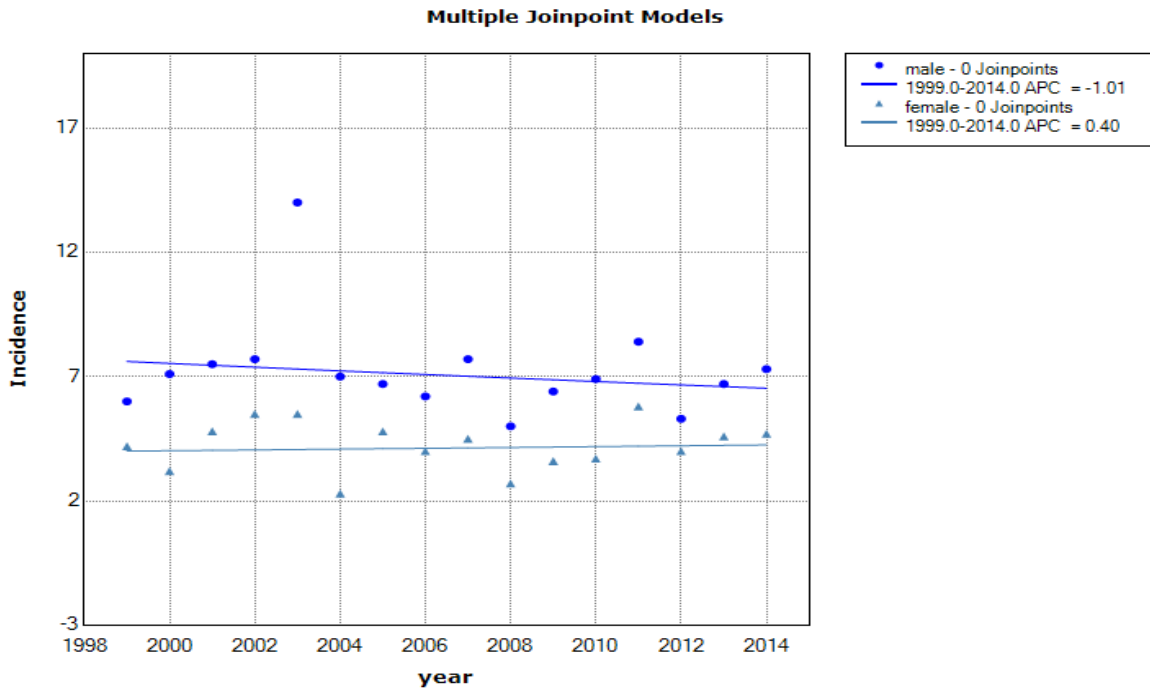
Graph 1. Incidence trend of leukemia in Central Serbia in the period 1999-2014.
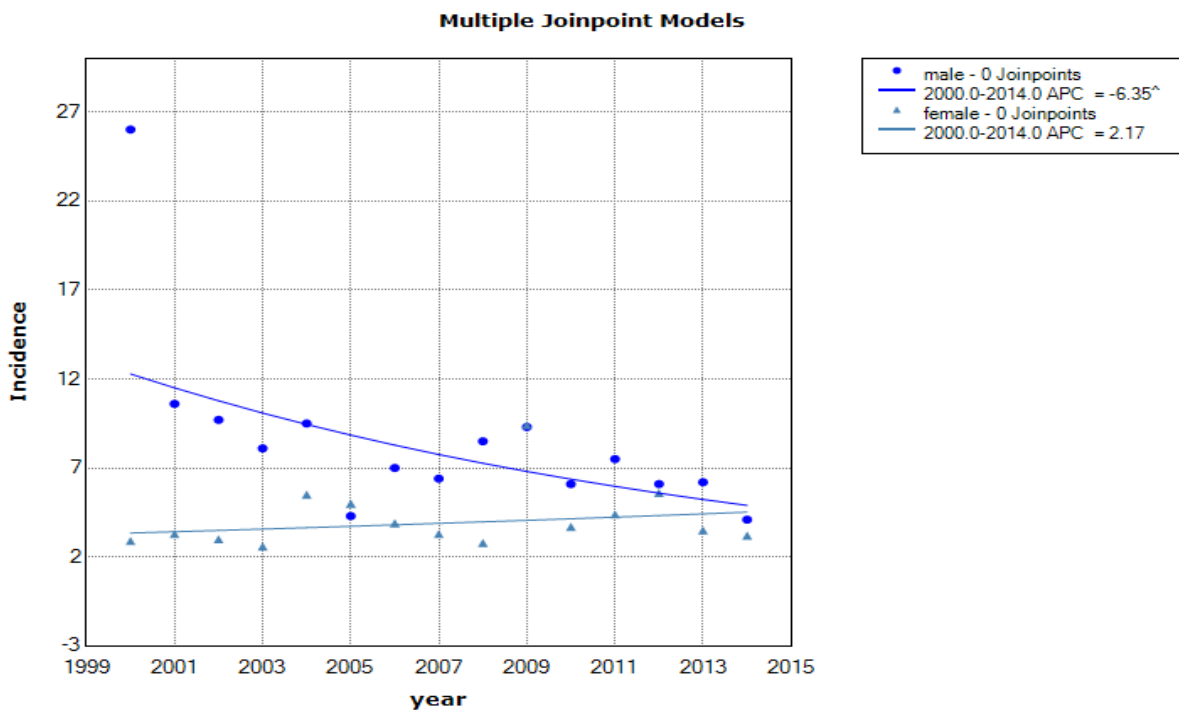
Graph 2. Incidence trend of leukemia in Nišava district in the period 1999-2014.

Table 2 reports the distribution of age-standardised mortality leukemia cases by gender in Central Serbia and Nišava district from 1999 to 2014. (per 100 000 population). The average age-standardised mortality for men was 4.90 and for females 3.38 in Central Serbia, while in Nišava district it was: 4.12 for men and 1.87 for women, twice less compared to the women in Central Serbia. In Central Serbia, ASR of the mortality rate for both sexes was the highest in 1999 (6.6, 5.2, respectively). In Nišava district we did not find available data for 1999 and 2000, as well as for females in 2012-2014.

The results from the Joinpoint analysis for leukemia age-standardised mortality in Central Serbia and Nišava district are presented in Graphs 3 and 4.

Table 2. Age adjusted mortality of leukemia in Serbia and Nišava district in the period from 1999 to 2014.

year	Serbia				Nišava district			
	male		female		male		female	
	No of cases	ASR	No of cases	ASR	No of cases	ASR	No of cases	ASR
1999.	181	6.6	148	5.2	/	0	/	0
2000.	166	6.1	135	4.8	/	0	/	0
2001.	195	4.7	156	3.2	15	4.3	10	3.5
2002.	167	3.9	144	2.8	13	5.5	14	3.7
2003.	203	4.9	141	3.2	9	2.6	8	2.5
2004.	167	3.8	140	2.8	6	2.2	8	2.2
2005.	215	5	130	2.6	14	4	6	1.1
2006.	205	4.3	181	3.8	19	5.7	16	3.8
2007.	247	5.6	166	2.9	19	5.8	9	2
2008.	216	4.4	162	2.9	14	5.5	10	2.3
2009.	233	5.2	179	3.1	17	5.3	14	3.3
2010.	252	5.2	189	3.4	19	5.2	14	3.5
2011.	230	4.8	170	3	16	4.5	9	2
2012.	232	4.9	173	4.6	15	3.6	/	0
2013.	241	4.6	162	3.1	22	6.3	/	0
2014.	233	4.5	163	2.7	20	5.4	/	0

** ASR - age-standardised rate per 100 000 (using standard world population)
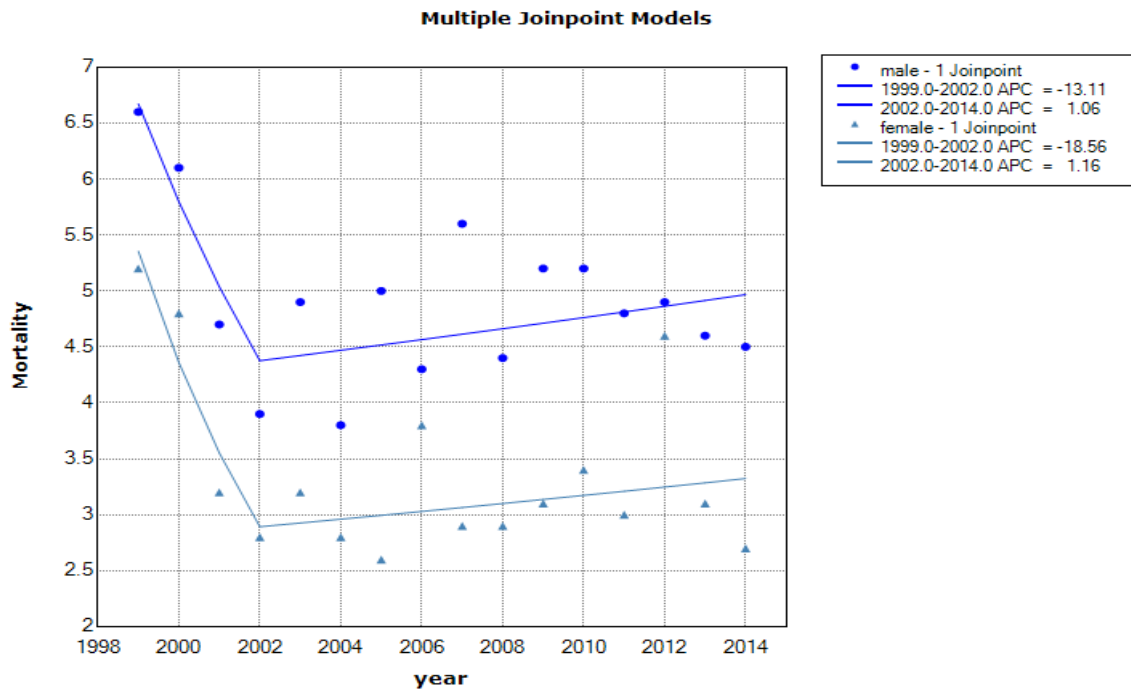
Graph 3. Mortality trend of leukemia in Central Serbia in the period 1999-2014.

Multiple Joinpoint Models
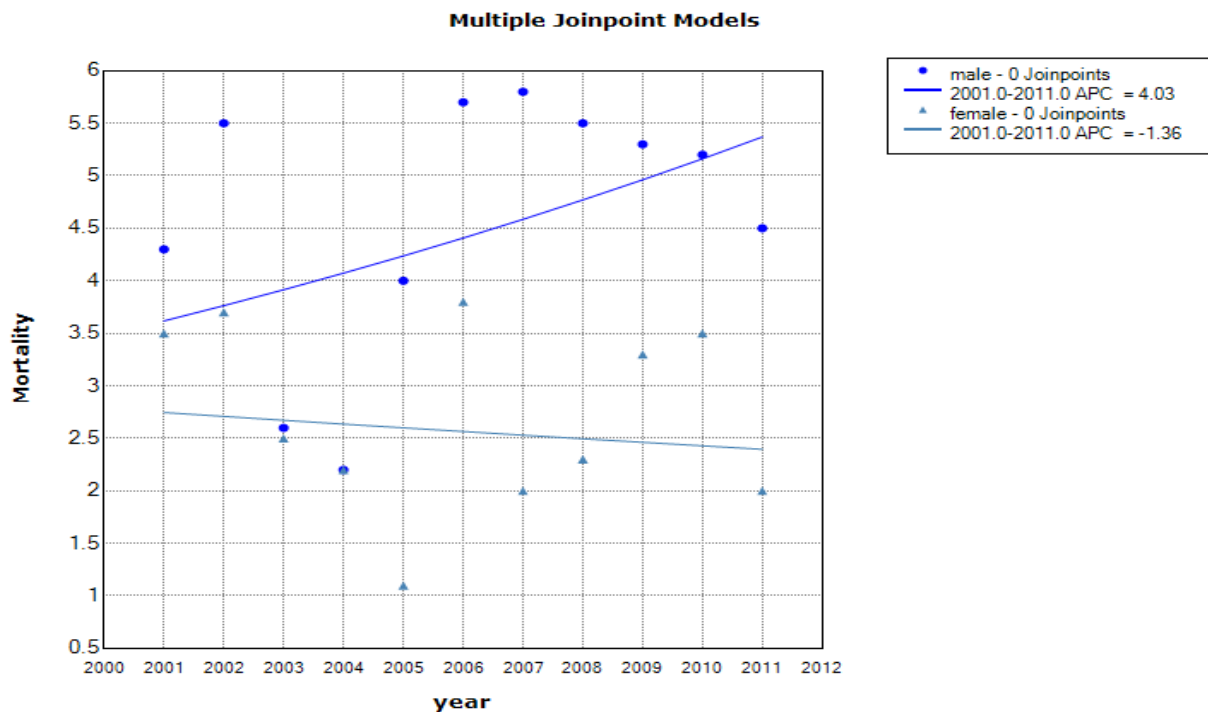
Graph 4. Mortality trend of leukemia in Nišava district in the period 1999-2014.

Discussion

According to our knowledge, this study offers the first nationwide analysis of leukemia incidence and mortality trends over time using cancer registration in Serbia. The use of Joinpoint analysis has allowed statistical testing of trends in incidence and mortality rates for leukemia, detecting some significant changes. Therefore, Joinpoint analysis provides a much clearer picture of what is happening during a particular period in specific terms (identifying the years in which significant changes in trends occurred) than a single summary trend statistic (13).

According to the GLOBOCAN estimates for the year 2012, the age-standardised incidence rate of leukemia in Serbia was 8.6/100 000 in men and 5.7/100 000 in women, which refers Serbia to the 18th place in Europe. The highest incidence was recorded in Australia and New Zealand and the lowest in Middle and Western Africa (1). The standardized mortality rate in Serbian men is similar to the European average of 8.5/100 000, while the incidence rate in women is slightly higher than the European average of 5.4/100 000. The Northern Europe has the lowest rate in both sexes. The age-standardised mortality in Serbian men was 2.9/100 000, which is slightly less than average European mortality rate (3.1/100 000). The mortality rate in women in Serbia was 2.9/100 000, also marginally less compared to the European average (3.2/100 000).

Our results demonstrate a stable trend of the age-adjusted leukemia incidence rate both in males and females in Central Serbia during the observed 1999-2014 period. However, statistically significant decreasing trend of leukemia incidence rate was

found in men from Nišava district with APC -6.3% (-10.3 - -2.2; p=0,005), while non-significant slightly increasing pattern was present in women. Leukemia incidence profile in Nišava district is consistent with The United Kingdom findings (14). Several studies showed continued declines in incidence trends, similar to our results (15, 16). In addition to the various risk factors included in the etiology of leukemias, it is hard to hypothesise possible reasons for the observed incidence trends. In addition to specific changes of incidence, they might be caused by changes of diagnostic procedures or increased surveillance due to screening programs, as well as changes of classification or reassignment of one diagnostic entity to the other. Generally speaking, the average incidence rate was higher in men compared to women, both in Central Serbia and Nišava region. It is observed that Nišava region has higher average age-standardised incidence in men in comparison to the rest of Central Serbia (7.24: 8.29 per 100 000) but without statistical significance (p = 0.4).

The incidence of childhood leukemia in the world is increasing. The reason for this should be sought in ionising radiation to which children are more prone. Considering Serbia was bombarded and exposed to ionizing radiation during 1999, from the given incidence trend we can conclude that radiation, at least, has not yet been reflected in the incidence pattern.

Malvezzi and associates review appropriate leukemia mortality trends in all countries of the EU as a whole, giving predicted rates for 2016 between 4 (Spain and UK) and 5/100 000 (Poland and Italy) (17). Joinpoint analysis in our research demonstrated favorable mortality declines until the 2002, and

then stabile trend in Central Serbia in both sexes to the end of the observed period. Conversely, mortality among males in Nišava district shows a positive trend, but not statistically significant. Mortality trends represent the reflection of improvements of performance status of patients at diagnosis, treatment and improved supportive care. The results of decreased mortality rates are coherent with previously published data (18, 19). In recent decades we have witnessed several major novelties in the treatment of leukemia, such as the initiation of an allogeneic hematopoietic stem cell transplantation (usually limited to patients younger than 65 years) and the recent introduction of tyrosine kinase imatinib inhibitor (20) and monoclonal antibodies (rituximab)(21). Leukemia survival is more noticeable in younger patients in whom improvements are better due to more adequate diagnosis, management and therapy treatment (22).

Limitations of the study

Some possible limitations should be taken into account. Our research is a registry based study, therefore, possible incomplete data collection is probable. The World Health Organization labeled the quality of data related to the cause of death in Serbia as moderate (23). Also, GLOBOCAN 2012 categorized data from Serbia as B2 (high quality regional data for incidence and medium quality for complete vital registration for the mortality rates) (24). Futhermore, inability to distinguish between age specific groups of leukemia incidence and mortality, as well as histological types of leukemia and variations in leukemia classifications should be considered.

Conclusion

The results of the study suggest that leukemia profile in Central Serbia was stabile during the study period. It is particularly interesting that incidence is decreasing among males from Nišava district. Mortality in the investigated period is declining by 2002 in Central Serbia, followed by mild increase to the end of the observed period. The only exception presents the mortality among males from Nišava district, which is rising but without statistically significance.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer 2015; 136(5): E359-86. [CrossRef] [PubMed]
2. Daniels RD, Schubauer-Berigan MK. A meta-analysis of leukaemia risk from protracted exposure to low-dose gamma radiation. Occupational and environmental medicine 2011; 68(6): 457-64. [CrossRef] [PubMed]
3. Schubauer-Berigan MK, Daniels RD, Fleming DA, Markey AM, Couch JR, Ahrenholz SH, et al. Chronic lymphocytic leukaemia and radiation: findings among workers at five US nuclear facilities and a review of the recent literature. British journal of haematology 2007; 139(5): 799-808. [CrossRef] [PubMed]
4. Zhang L, Steinmaus C, Eastmond DA, Xin XK, Smith MT. Formaldehyde exposure and leukemia: A new meta-analysis and potential mechanisms. Mutation Research/Reviews in Mutation Research 2009; 681(2-3): 150-68. [CrossRef] [PubMed]
5. Poynter JN, Richardson M, Roesler M, Blair CK, Hirsch B, Nguyen P, et al. Chemical exposures and risk of acute myeloid leukemia and myelodysplastic syndromes in a population-based study. International journal of cancer 2017; 140(1): 23-33. [CrossRef] [PubMed]
6. Morton LM, Dores GM, Tucker MA, Kim CJ, Onel K, Gilbert ES, et al. Evolving risk of therapy-related acute myeloid leukemia following cancer chemotherapy among adults in the United States, 1975-2008. Blood 2013; 121(15): 2996-3004. [CrossRef] [PubMed]
7. Fircanis S, Merriam P, Khan N, Castillo JJ. The relation between cigarette smoking and risk of acute myeloid leukemia: an updated meta-analysis of epidemiological studies. American journal of hematology 2014; 89(8): E125-32. [CrossRef] [PubMed]
8. Qin L, Deng H-Y, Chen S-J, Wei W. Relationship between cigarette smoking and risk of chronic myeloid leukaemia: a meta-analysis of epidemiological studies. Hematology 2017; 22(4): 193-200. [CrossRef] [PubMed]
9. Stieglitz E, Loh ML. Genetic predispositions to childhood leukemia. Therapeutic Advances in Hematology 2013; 4(4): 270-90. [CrossRef] [PubMed]
10. Acharya S, Hsieh S, Shinohara ET, DeWees T, Frangoul H, Perkins SM. Effects of Race/Ethnicity and

Socioeconomic Status on Outcome in Childhood Acute Lymphoblastic Leukemia. Journal of pediatric hematology/oncology 2016; 38(5): 350-4. [CrossRef] [PubMed]

11. Knoble NB, Alderfer MA, Hossain MJ. Socioeconomic status (SES) and childhood acute myeloid leukemia (AML) mortality risk: Analysis of SEER data. Cancer epidemiology 2016; 44: 101-8. [CrossRef] [PubMed]

12. Organization WH. International statistical classification of diseases and related health problems: World Health Organization; 2004. [CrossRef]

13. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. Statistics in medicine. 2000; 19(3): 335-51. [CrossRef] [PubMed]

14. McNally RJ, Roman E, Cartwright RA. Leukemias and lymphomas: time trends in the UK, 1984-93. Cancer causes & control 1999; 10(1): 35-42. [CrossRef] [PubMed]

15. Xie Y, Davies SM, Xiang Y, Robison LL, Ross JA. Trends in leukemia incidence and survival in the United States (1973-1998). Cancer 2003; 97(9): 2229-35. [CrossRef] [PubMed]

16. Thygesen LC, Nielsen OJ, Johansen C. Trends in adult leukemia incidence and survival in Denmark, 1943-2003. Cancer causes & control 2009; 20(9): 1671-80. [CrossRef] [PubMed]

17. Malvezzi M, Carioli G, Bertuccio P, Rosso T, Boffetta P, Levi F, et al. European cancer mortality predictions for the year 2016 with focus on leukaemias. Annals of oncology: official journal of the European Society for Medical Oncology 2016; 27(4): 725-31. [CrossRef] [PubMed]

18. Chihara D, Ito H, Matsuda T, Katanoda K, Shibata A, Saika K, et al. Decreasing Trend in Mortality of Chronic Myelogenous Leukemia Patients After Introduction of Imatinib in Japan and the U.S. The Oncologist 2012; 17(12): 1547-50. [CrossRef] [PubMed]

19. Bertuccio P, Bosetti C, Malvezzi M, Levi F, Chatenoud L, Negri E, et al. Trends in mortality from leukemia in Europe: an update to 2009 and a projection to 2012. International journal of cancer 2013; 132(2): 427-36. [CrossRef] [PubMed]

20. Jiang Q, Yu L, Gale RP. Patients' and hematologists' concerns regarding tyrosine kinase-inhibitor therapy in chronic myeloid leukemia. Journal of cancer research and clinical oncology 2018; 144(4): 735-41. [CrossRef] [PubMed]

21. Salles G, Barrett M, Foa R, Maurer J, O'Brien S, Valente N, et al. Rituximab in B-Cell Hematologic Malignancies: A Review of 20 Years of Clinical Experience. Advances in therapy 2017; 34(10): 2232-73. [CrossRef] [PubMed]

22. Aricò M, Valsecchi MG, Conter V, Rizzari C, Pession A, Messina C, et al. Improved outcome in high-risk childhood acute lymphoblastic leukemia defined by prednisone-poor response treated with double Berlin-Frankfurt-Muenster protocol II. Blood 2002; 100(2): 420-6. [CrossRef] [PubMed]

23. Mathers CD, Ma Fat D, Inoue M, Rao C, Lopez AD. Counting the dead and what they died from: an assessment of the global status of cause of death data. Bulletin of the world health organization 2005; 83(3): 171-7. [CrossRef] [PubMed]

24. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh J-WW, Comber H, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. European journal of cancer 2013; 49(6): 1374-403. [CrossRef] [PubMed]

JOINPOINT REGRESIONA ANALIZA TRENDA INCIDENCIJE I MORTALITETA LEUKEMIJE U CENTRALNOJ SRBIJI I NIŠAVSKOM REGIONU U PERIODU 1999-2014. GODINE

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Leukemije su zastupljene sa 2,3% u ukupnoj učestalosti karcinoma u Srbiji i 2,9% u ukupnom mortalitetu, dok su incidencija i mortalitet za oba pola iznosili 8,7 i 6,3 ‰, na osnovu čega ih svrstavamo na 13. mesto među svim karcinomima registrovanim u Centralnoj Srbiji.

Cilj rada bio je da se utvrdi vremenski trend leukemija u Centralnoj Srbiji, sa posebnim osvrtom na Nišavski okrug, u periodu 1999-2014. godine, koristeći Joinpoint regresionu analizu, i uporediti ih sa trendom u drugim populacijama i identifikovati karakteristične promene.

Standardizovane stope incidencije i mortaliteta uzete su iz Registara za rak Centralne Srbije. Trend stope incidencije i mortaliteta je određivan Joinpoint analizom upotrebom Joinpoint Regresioni softvera.

Rezultati ukazuju na stabilan trend incidencije prilagođene za godine među oba pola u Centralnoj Srbiji za ispitivani period. Međutim, utvrđeno je statistički značajno smanjenje incidencije među muškarcima Nišavskog regiona, dok je među ženama iz istog regiona uočen blagi porast trenda, ali bez statističke značajnosti. Joinpoint analiza je u našem istraživanju pokazala pad trenda mortaliteta do 2002. godine, a potom stabilan trend za oba pola u Centralnoj Srbiji do kraja ispitivanog perioda. Suprotno tome, trend mortaliteta među muškarcima iz Nišavskog okruga je rastao, ali bez statističke značajnosti.

Rezultati studije ukazuju da je profil leukemije u Centralnoj Srbiji stabilan tokom perioda istraživanja. Posebno je interesantan pad incidencije kod muškaraca iz Nišavskog okruga.

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Ključne reči: *leukemija, Centralna Srbija, Nišavski region, Joinpoint regresiona analiza*

DETECTION OF BLOODSTAINS ON COTTON FABRIC AFTER WASHING

Ivan Stojanović

After a criminal act a perpetrator may try to wash the bloodstains, either by hand wash in cold water or using a regular washing machine. The washing procedure can alter original bloodstains on fabric. The current experiment was focused on determining an effect of blood drying time and various washing conditions (water temperature, detergent use, machine, and hand wash simulated washing) on presentation of bloodstains on washed cotton fabric. The result of this experiment suggests that regular hand wash procedure in cold water (30°C), regardless of drying time lapsed from the deposition of blood on fabric until washing, would not be sufficient to completely remove or destroy original contour of the bloodstain on cotton fabric. All tested samples washed at 60 and 95°C suggest that a machine washing procedure with the usage of detergent should be sufficient for removing visible bloodstains from cotton fabric, but not for latent bloodstains. As an advice for prosecutors, even in case of no obvious bloodstains, bloodstain pattern analysts should always examine clothing of the suspects in all criminal cases connected with bloodshed event.

Key words: bloodstain, analysis, presumptive blood test

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Introduction

Criminal act connected with external hemorrhage of a victim usually leads to a deposition of bloodstains on a perpetrator's clothing. A perpetrator may try to wash the bloodstains, either by hand wash in cold water or using a regular washing machine. Washing procedure can alter original bloodstain on fabric, so bloodstain can become diluted or hard to see with the naked eye (1-6).

Washing experiments with bloodstained fabrics revealed that detergent brand Ariel Washing Powder (Procter & Gamble) removed bloodstains from washed fabrics most effectively compared with other tested detergents (3, 7). Based on literature data, our research was focused on two extremes: hand wash in cold water and machine wash with Ariel Washing Powder as one of the best household bloodstain removing detergent, also used for other real - life situations. Bloodstain pattern analysis on different fabrics must be held out based on a deeper charac-

terization of the textile structure, even down to the yarn level (8, 9).

Deoxyribonucleic acid (DNA) analysis is a very expensive forensic technique, so it is important to confirm the presence of the right stain prior to sending it for DNA analysis. Presumptive tests of blood are performed to identify blood in the stain (1-3, 10). Luminol and Leuco-crystal Violet (LCV) were commonly used chemicals to develop latent bloodstains on evidence and at crime scenes (11). Experiments revealed luminol chemical interference with bleach, but this effect dissipates after 8 hours (12). In recent years, Hemastix and Bluestar forensic tablets have been considered to be very useful, reliable and low - cost presumptive blood tests (11, 13). These two tests were used in this experiment for detection and visualization of latent bloodstains on washed fabrics.

Material and methods

Fabric: The white 100% cotton fabric (140 g/m^2) cut out into 100 square pieces 5x5 cm in diameter. The rest of the fabric of 3 kg was used to simulate a real situation washing procedure.

Stains: Blood was collected from the corpse of an autopsy conducted at the Institute of Forensic Medicine in Nis (case data: S-23/18; previously normal blood test results). Exactly 100 µl of blood was deposited onto every piece of fabric and 100 samples were obtained. Every sample was made in five replicates.

Washing machine: model Samsung, type WF80F5E0W2W/AD.

Washing: Three washing temperatures were used (30°C, 60°C and 95°C) under two main conditions: washing with water or using a standard detergent (Ariel Washing Powder, Procter & Gamble: composed of 5-15% active ionic and <5% non-ionic surfactant-detergent, phosphates, water softener (zeolites and polycarboxylates), enzymes, optical brighteners and perfumes).

Groups of samples: Samples were ordered into four groups: M95 (machine wash at 95°C with detergent Ariel; 2 hours 35 minutes washing time; centrifuge: 1200 cycles/minute), M60 (machine wash at 60°C with detergent Ariel; 2 hours 35 minutes washing time; centrifuge: 1200 cycles/minute), A (machine simulated hand wash at 30°C with detergent Ariel; 35 minutes washing time; 200 cycles/minute simulate drying out fabric by hands) and R (machine simulated hand wash at 30°C without detergent Ariel; 35 minutes washing time; 200 cycles/minute simulate drying out fabric by hands). Within every group, all samples were divided into 5 subgroups: 6h (6 hours from deposition of blood until washing procedure), 24h (24 hours from deposition of blood until washing procedure), 72h (72 hours from deposition of blood until washing procedure), 10d (10 days from deposition of blood until washing procedure) and 30d (30 days from deposition of blood until washing procedure).

Sample storage: Samples were stored in a dark place, without direct sunlight, at 20°C, relative humidity at about 35%, almost without air flow.

Examination: After washing procedure, all samples were left to dry at room temperature and examined on the next day. Examinations of samples were performed in the Biological traces laboratory of the Institute of Forensic Medicine in Nis. First, samples were examined visually under high - intensity white light. Subsequently, samples were treated with Bluestar forensic tablets solution and with Hemastix test strips.

Results

In all positive controls, blood was detected with Hemastix test strips and Bluestar forensic tablets. None of the negative controls gave positive results after washing in water or with the detergent.

A single simulated hand wash procedure in cold water (30°C) was not sufficient to remove the bloodstain from cotton fabric or completely destroy original contour of the bloodstain, regardless of drying time lapsed from the deposition of blood on fabric until washing. All of these samples had bloodstains on washed fabric with a partially faded contour of original stain. Some visible blood transfer from original bloodstain was noted onto fabric. The most of transfer staining was noted on the part of the fabric that was in close contact with the fabric containing original bloodstain, at the moment of extracting the fabric from the washing machine.

A machine washing procedure in hot water (60 and 95°C) with Ariel detergent was sufficient to remove visible bloodstains from cotton fabric, but all samples gave positive results on presumptive blood tests. There was no visible blood transfer on previously clean parts of the fabric, but Bluestar forensic tablets solution gave a diffuse positive result on the wide area of tested cotton fabric.

Table 1. Bloodstain examination test results on cotton fabric after washing

Washing procedure	Time lapsed from deposition of blood on cotton fabric until washing				
	6 hours	24 hours	72 hours	10 days	30 days
M95	0	0	0	0	0
M60	0	0	0	0	0
A	1	1	1	1	1
R	1	1	1	1	1

0 – no visible bloodstain and positive presumptive tests;
1 – visible bloodstain and positive presumptive tests;
M95 - machine wash at 95°C with detergent Ariel;
M60 - machine wash at 60°C with detergent Ariel;
A - machine simulated hand wash at 30°C with detergent Ariel;
R - machine simulated hand wash at 30°C without detergent Ariel.

Discussion

A washed cotton fabric with bloodstains presents several challenges for the bloodstain pattern analyst. First and the most important, the visible bloodstains and staining fabric with Bluestar reagent bore a little overall resemblance to the initial blood staining. Initial bloodstain patterns could be seen in most of the samples washed in cold water with or without detergent, but they were often intermingled with the "background noise" staining. Analysts must be very careful when interpreting these diffused stains. In the literature, some authors suggest that diffused bloodstains on washed fabric must not be

interpreted as a result of any action other than wash-ing (14). The result of this experiment suggests that regular hand wash procedure in cold water (30°C), regardless of drying time lapsed from the deposition of blood on fabric until washing, would not be suf-ficient to completely destroy original contour of the bloodstain on cotton fabric.

In the current research, Ariel was used as the most reliable detergent for removing bloodstains from fabric (3, 7). All tested samples washed at 60 and 95°C suggest that a machine washing procedure with the usage of detergent should be sufficient for removing visible bloodstains from cotton fabric. Sub-sequently, Hemastix strip test and Bluestar forensic tablets reagent were applied to all of these tested samples. Clear positive result registered on all tested samples. This result suggests that s single household machine washing cycle, regardless of washing prog-ram and usage of detergent, is not sufficient for com-pletely removing visible and latent bloodstains from cotton fabric.

According to the result of this experiment, in real criminal cases, the analysts could provide very useful information to prosecutors about bloodshed event if the data about washing procedure were re-liable and well known.

Conclusion

Regardless of washing program, a single hou-sehold machine washing cycle is not sufficient for removing both visible and latent bloodstains from cotton fabric. The data revealed after performing the current study encourage analysts that there is a high probability of finding bloodstains on cotton fabric af-ter washing with detergent. As an advice for prose-cutors, even in case of no obvious bloodstains, blood-stain pattern analysts should always examine cloth-ing of the suspects in all criminal cases connected with bloodshed event. Future systematic research in this field could provide more reliable data about the effect of washing procedure on bloodstained fabrics.

References

1. Bevel T, Gardner RM. Bloodstain Pattern Analysis with an Introduction to Crime Scene Reconstruction. 3rd ed. The United States: CRC Press; 2008. [CrossRef]
2. James SH, Kish PE, Sutton TP. Principles of bloodstain pattern analysis – theory and practice. Boca Raton FL: CRC Press Taylor and Francis Group; 2005. [CrossRef]
3. Mushtaq S, Rasool N, Firiyal S. Detection of dry blood-stains on different fabrics after washing with comer-cially available detergents. Aust J Forensic Sci 2016; 48(1): 87-94. [CrossRef]
4. Attingera D, Moore C, Donaldson A, Jafari A, Stone HA. Fluid dynamics topics in bloodstain pattern analy-sis: Comparative review and research opportunities. Forensic Sci Int 2013; 231(1-3): 375-96. [CrossRef] [PubMed]
5. Gifford WD. Bloodstain Survival in Water. IABPA News 1999; 15(2): 1-6.
6. Adair TW. Casting two-dimensional bloody shoe prints from concrete, fabric, and human skin: a review of several methods with recommendations. IABPA News 2005; 12: 4-8.
7. Arjun RI, Ashish P. Identification of bloodstains on dif-ferent fabrics after washing with routinely used deter-gents in India. Aust J Forensic Sci 2016; 1(1): 1-5.
8. Li X, Li J, Michielsen S. Effect of yarn structure on wicking and its impact on bloodstain pattern analysis (BPA) on woven cotton fabrics. Forensic Sci Int 2017; 276: 41-50. [CrossRef] [PubMed]
9. Ramsthaler F, Schmidt P, Bux R, Potente S, Kaiser C, Kettner M. Drying properties of bloodstains on com-mon indoor surfaces. Int J Legal Med 2012; 126: 739-46. [CrossRef] [PubMed]
10. Andrews C, Coquoz R. PCR DNA typing of washed stains. Advances in Forensic Haemogenetics: Series Haemogenetics 1994; 343-5.
11. Tobe SS, Watson N, Daéid NN. Evaluation of six presumptive tests for blood, their specificity, sensitivi-ty, and effect on high molecular-weight DNA. J Foren sic Sci 2007; 52(1): 102-9. [CrossRef] [PubMed]
12. Creamer JI, Quickenden TI, Crichton LB, Robertson P, Ruhayel RA. Attempted cleaning of bloodstains and its effect on the forensic luminol test. Luminescence 2005; 20(6): 411-3. [CrossRef] [PubMed]
13. Vennemann M, Scott G, Curran L, Bittner F, Tobe SS. Sensitivity and specificity of presumptive tests for blood, saliva and semen. Forensic Sci Med Pathol 2014; 10(1): 69-75. [CrossRef] [PubMed]
14. Adair TW, Shaw RL. Enhancement of Bloodstains on Washed Clothing Using Luminol and LCV Reagents. IABPA News 2005; 4-10.

DETEKCIJA KRVNIH MRLJA NA PAMUČNOJ TKANINI NAKON PRANJA

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Nakon krivičnog dela izvršilac može pokušati da opere tragove krvnih mrlja ili ručnim pranjem ili pranjem u veš mašini. Proces pranja može izmeniti prvobitni izgled krvnih mrlja na tkanini. Aktuelno istraživanje je bilo usredsređeno na utvrđivanje efekta protoka vremena od nanošenja krvne mrlje do pranja i efekta različitih modaliteta pranja (temperatura vode, upotreba deterdženta, mašinsko pranje i mašinski simulirano ručno pranje) na izgled krvnih mrlja na opranoj pamučnoj tkanini. Rezultat ovog eksperimenta ukazuje da obično ručno pranje u hladnoj vodi (30°C), nezavisno od protoka vremena od nanošenja krvi na tkaninu do pranja, ne bi bilo dovoljno za kompletno uklanjanje ili narušenje prvobitne konture krvne mrlje na pamučnoj tkanini. Svi uzorci prani na 60 i 95°C ukazuju da bi mašinsko pranje sa primenom deterdženta trebalo biti dovoljno za uklanjanje vidljivih krvnih mrlja sa pamučne tkanine, ali ne i onih nevidljivih. Savet tužilaštvima je da bi, u svim slučajevima izvršenja krivičnih dela povezanih sa krvoprolićem, čak i ako nema očiglednih tragova krvi, analitičar obrazaca krvnih mrlja trebalo da uvek izvrši pregled odeće osumnjičenog.

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Ključne reči: *krvna mrlja, analiza, prezumptivni test krvi*

IN VITRO COMPARISON OF THE ACCURACY OF TWO APEX LOCATORS OF DIFFERENT GENERATIONS

Tamara Karuntanović[1], Stefan Dačić[1,2], Nikola Miljković[1], Dragica Dačić-Simonović[1,2]

The accuracy of apex locators is very important for the correct working length determination of the root canal, and thus for successful endodontic treatment.

The aim of this study was to compare *in vitro* the accuracy of iPex II (the fourth generation) and Adaptive (the sixth generation) apex locators.

The material consisted of 28 root canals (16 premolars). The working length of all root canals was determined first by entering K-file #15 up to the apical foramen, what was checked by visual tracking of the top of the file. The fixed working length was then measured with a digital caliper and the obtained values were used to control measured canal lengths in two experimental groups. The teeth were immersed in the alginate before electronic measurements in order to simulate the clinical situation. In the first experimental group, the working length of the root canals was measured with iPex II, and in the second with Adaptive apex locator. All measurements were performed up to the apical foramen in the dry canal.

The results of One-way ANOVA showed that there was not statistically significant difference between examined experimental groups ($p > 0.05$). The biggest difference existed in comparison the values of Adaptive apex locator and the control group, and the lowest in comparison iPex II and Adaptive apex locators.

It can be concluded that both apex locators are accurate enough for clinical practice although they belong to different generations.
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Key words: *Adaptive, apex locator, iPex II, working length*

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Introduction

The accuracy of apex locators is very important for the correct working length determination of the root canal, and thus for successful endodontic treatment (1, 2). It is generally accepted that the endodontic treatment should be limited within the root canal (3, 4). The distance from a coronal reference point to the apical point at which canal preparation and obturation should terminate represents the working length of the root canal (5, 6).

There are six generations of apex locators and each generation has its working principle (7-9). The common characteristic of apex locators from the third to the sixth generation is a determination of the working length by using two or more frequencies. It enables the precise measurements in the presence of the electrolytes in the canal (10). The electric circuit must be established regardless of the principle used and it is achieved by the electrodes that are connected to the oral mucosa and to the endodontic instrument (8, 11).

iPex II apex locator (NSK Nakanishi inc., Tokyo, Japan) belongs to the fourth generation of apex locators, and Adaptive apex locator (Optica Laser, 405-10A, Sofia, Bulgaria) is a representative of the sixth generation. The difference between these two apex locators is that iPex II apex locator measures working length in the conditions of the dry canal (eventually in moist one), while Adaptive works in a wet canal as well as in the dry one (12-14). Both apex locators have been recently introduced, so there are not many data in the literature about them. This prompted us to investigate *in vitro* their accuracy in working length determination of the root canal, to see if they are reliable for clinical use.

The aim of this study was to compare *in vitro* the accuracy of iPex II (the fourth generation) and Adaptive (the sixth generation) apex locators. The working hypothesis was that there is a statistically

significant difference in the precision of these two apex locators since they belong to the different generations of apex locators.

Material and methods

The research was conducted at the Dental Clinic in Niš, at the Department of Restorative Dentistry and Endodontics. The material consisted of 28 root canals of 16 human premolars that were extracted for orthodontic reasons. The teeth had completed root growth and they were without visible fractures and resorptions. The width of the apical foramen was nevertheless sufficient to easily see the position of the top of the canal instrument at the visual working length determination. Teeth were stored in formalin from the extraction to the research start.

The access cavities were prepared on the occlusal tooth surface with a round diamond drill, in diameter 107-126 µm (Mesinger, Germany) using turbine with a water-air cooling. Trepanation of tooth and the removal of coronal pulp were done after that, by round steel drill (Mesinger, Germany). The root canals entrances were then found.

Pulp extirpation was done using the barbed broach #25 and #30, depending on the diameter of the canal. The root canals then were irrigated with 2 ml of 0.5% NaOCl and their patency was checked using a K-file #15. All teeth were used in research as there were no obstructions in the canals and were marked with numbers from one to 16. A total of 28 canals were included in the study. Out of 16 premolars, six were with one canal, eight with two and two with three root canals. The tops of the buccal and oral cusps were marked with felt pen and there the stopper of endodontic instrument leaned during the measurements of working length of the canal. The working length of the root canal was measured up to the apical foramen in the dry canal.

The research included three measurements of the working lengths of all 28 root canals. The first measurement was a control group, while the second and the third measurements represented experimental groups.

In the control group, the working length of the root canal was determined visually by using K-file #15. The file was placed in the canal up to the apical

foramen, and position of the top of the instrument was checked by observation under a magnifying glass with 5 × magnification (Hunan, China). The fixed working length was then measured with a digital caliper (Asimeto 307-06-1, Canada) with an accuracy of 0.01 mm and thus the control value was obtained.

Measurements in experimental groups were done after immersion of teeth in alginate since the alginate had the role to simulate the clinical situation. Alginate (Tropicalgin, Zhermack, Italy) was mixed according to the manufacturer's instruction and thus was inserted in a plastic bowl. The two-thirds of teeth roots were immersed in alginate, before its binding. All measurements were performed within two hours after mixing of alginate, while it still possessed humidity. The circuit was *in vitro* closed during the electronic measurements by labial and canal electrodes of corresponding apex locator which were connected with the alginate. The root canals were dried with paper points before measuring since the study was conducted under conditions of the dry canal.

In the first experimental group working lengths of the root canals up to the apical foramen were measured with iPex II apex locator (NSK Nakanishi inc., Tokyo, Japan), and in the second experimental group with Adaptive apex locator (Optica Laser, 405-10A, Sofia, Bulgaria). K-file #20 was used for measuring the working length of the root canal with apex locators. One value was measured for each sample and both devices, in a dry canal, up to the apical foramen. iPex II apex locator signaled the apical foramen with mark 0.0 on the screen, and Adaptive apex locator visually and by a beep. Measurements were first performed on all samples by one locator, and then with the other, so it was a total of 28 measurements for each group.

Statistical analysis was performed using the software package SPSS version 16.0 (SPSS Inc., Chicago, Illinois). Data were presented as the mean and standard deviation, and also as median, minimum and maximum value. Data normality was tested by Shapiro-Wilk test. Comparison of working lengths of the root canals of control and experimental groups was performed by One-way ANOVA (Analysis of variance).

Table 1. The working lengths values of the root canals of experimental and control groups

Groups	iPex II apex locator	Adaptive apex locator	Control group	F value	p value
	n = 28	n = 28	n = 28		
Working lengths (mm)	19.19 ± 1.88	18.94 ± 1.54	19.34 ± 2.12‡	0.32†	p > 0.05*
	18.89 (16.77-23.65)	19.41 (15.92-21.47)	19.06 (16.29-23.81)‖		

*no statistically significant difference
†One-way ANOVA, ‡mean ± standard deviation, ‖median (min-max value)

Results

Shapiro-Wilk test showed normal distribution of data in all examined groups. The values of the working lengths of the root canals of iPex II apex locator (19.19 ± 1.88 mm) were closer to the values of the control group (19.34 ± 2.12 mm) than the values of Adaptive apex locator (18.94 ± 1.54 mm).

The One-way ANOVA showed that there was not statistically significant difference in working lengths of the root canals between examined groups ($p > 0.05$)(Table 1).

Discussion

Precise determination of the working length of the root canal is a key factor that affects the outcome of endodontic treatment (2, 15, 16). Treatment of root canal up to the apical constriction represents a risk that part of the diseased pulp tissue might remain in the apical region and lead to treatment failure (17, 18). Therefore, treatment up to apical foramen is recommended (17).

The use of electronic devices for working length determination of the root canal has gained great popularity, especially in recent years with the introduction of the latest apex locators, which allow the measurements and in a humid environment (6, 17).

In our study, we applied alginate as a material to simulate the clinical situation because of its good electrical conductivity. The other good properties of alginate are that is inexpensive, easy to prepare, stable for hours and relative stiffness of alginate model that prevents fluid movement within the canal and premature readings (19, 20). It has been applied in a number of *in vitro* studies (6, 10, 17, 21). In the research of Lipski et al. alginate has proven to be a reliable medium for replacement of in vivo conditions during the electronic working length measurement. There was no statistically significant difference between the *in vitro* and *in vivo* measurements (21).

Previous *in vitro* studies examined the accuracy of apex locators in working length determination up to the apical foramen or apical constriction. However, apical foramen proved to be better for this type of research because it can locate consistently (22). We compared the accuracy of the apex locators up to the apical foramen for this reason. In the control group, apical foramen was visible and working length measurement was performed under the control of the eye. This indicates that apical foramen is a reliable point not only for examination of apex locator accuracy but also for comparison the apex locators measurements with the actual length of the root canal, which was visually determined.

The results of our research confirmed the null hypothesis as there was no statistically significant difference in working length determination of the root canal between the examined apex locators as well as between them and the control group. We compared the working length values of the three groups using One-way ANOVA, which is a better option than to apply Student's t-test three times because the possibility of errors is reduced. Some authors applied the same statistical test for comparing the values of more than two groups like us, while others applied the Student's t-test (6, 10, 12, 23).

The reason for the absence of statistically significant difference in the accuracy of iPex II and Adaptive apex locators can be the same physical principle of operation (two or more frequencies) (10). iPex II apex locator showed greater accuracy since its working lengths values of the root canals were closer to the values of the control group than the values of Adaptive apex locator. Higher precision of iPex II apex locator can be explained by the fact that the study was conducted in the dry canal because the fourth generation of apex locators to which it belongs is generally more accurate in the dry canal than in the moist one. On the other hand, Adaptive apex locator belongs to the sixth generation of apex locators that adapts to the conditions in the canal, so that the research was done and in the conditions of wet canal results would probably be different (14).

There are some studies that examined the accuracy of iPex II apex locator, while the precision of Adaptive apex locator was not examined. In the study of Kocak et al. iPex II apex locator showed accuracy in working length determination in a dry canal as well as in the presence of different irrigants (23). However, the aim of our study was different. The accuracy of iPex II apex locator was also examined in the study of Gurel et al. In that in vitro study iPex II apex locator showed accuracy in 50% of specimens and there was not a statistically significant difference between examined apex locators (Raypex 5, Raypex 6, iPex and iPex II) (12).

Conclusion

Our research showed that Ipex II apex locator is more precise since its values were closer to the control group compared to the Adaptive apex locator. However, it can be concluded that both devices are accurate enough due to the fact that this difference in precision was not statistically significant, so their use in clinical practice is recommended. It may also be concluded that representatives of the new generation of apex locators are not always more accurate in comparison to older generations, as it was demonstrated in this paper.

References

1. Bahrololoomi Z, Soleymani AA, Modaresi J, Imanian M, Lotfian M. Accuracy of an electronic apex locator for working length determination in primary anterior teeth. J Dent (Tehran) 2015; 12(4): 243-8. [PubMed]

2. Diwanji A, Rathore AS, Arora R, Dhar V, Madhusudan A, Doshi J. Working length determination of root canal of young permanent tooth: an in vitro study. Ann Med Health Sci Res 2014; 4(4): 554-8. [CrossRef] [PubMed]

3. Nekoofar MH, Ghandi MM, Hayes SJ, Dummer PM. The fundamental operating principles of electronic root canal length measurement devices. Int Endod J 2006; 39(8): 595-609. [CrossRef] [PubMed]

4. Yadav RK, Chand S, Verma P, Chandra A, Tikku AP, Wadhwani KK. Clinical negligence or endodontic mishaps: a surgeons dilemma. Natl J Maxillofac Surg 2012; 3(1): 87-90. [CrossRef] [PubMed]

5. Vieyra JP, Acosta J, Mondaca JM. Comparison of working length determination with radiographs and two electronic apex locators. Int Endod J 2010; 43(1): 16-20. [CrossRef] [PubMed]

6. Puri N, Chadha R, Kumar P, Puri K. An in vitro comparison of root canal length determination by DentaPort ZX and iPex apex locators. J Conserv Dent 2013; 16(6): 555-8. [CrossRef] [PubMed]

7. Gordon MP, Chandler NP. Electronic apex locators. Int Endod J 2004; 37(7): 425-37. [CrossRef] [PubMed]

8. Soi S, Mohan S, Vinayak V, Kaur P. Electronic apex locators. J Dent Sci Oral Rehab 2013: 24-7.

9. Kalhan A, Choudhary R, Chokshi S, Vaidya R. Evaluation of apex locators. JADCH 2010; 1(1): 4-7.

10. Kustarci A, Arslan D, Altunbas D. In vitro comparison of working length determination using three different electronic apex locators. Dent Res J (Isfahan) 2014; 11(5): 568-73. [CrossRef] [PubMed]

11. Ebrahim AK, Wadachi R, Suda H. Electronic apex locators - a review. J Med Dent Sci 2007; 54(3): 125-36.

12. Gurel MA, Helvacioglu-Kivanc B, Ekici A. A comparative assessment of the accuracies of Raypex 5, Raypex 6, iPex and iPex II electronic apex locators: an in vitro study. J Istanbul Univ Fac Dent 2017; 51(1): 28-33. [PubMed]

13. Srivastava V, Jain N, Bagchi S, Negi MPS. Evaluation of the use of sixth generation apex locators as a diagnostic tool to detect root perforations. Int J Dent Med Spec 2015; 2(4): 10-4. [CrossRef]

14. Dimitrov S, Roshkev D. Sixth generation adaptive apex locator. J IMAB 2009; 15(2): 75-8.

15. Bonilla M, Sayin TC, Schobert B, Hardigan P. Accuracy of a new apex locator in ex-vivo teeth using scanning electron microscopy. Endod Pract US 2014: 14-20. [CrossRef]

16. Pereira KF, Silva PG, Vicente FS, Arashiro FN, Coldebella CR, Ramos CA. An in vivo study of working length determination with a new apex locator. Braz Dent J 2014; 25(1): 17-21. [CrossRef] [PubMed]

17. Chopra V, Grover S, Prasad SD. In vitro evaluation of the accuracy of two electronic apex locators. J Conserv Dent 2008; 11(2): 82-5. [CrossRef] [PubMed]

18. Alothmani OS, Chandler NP, Friedlander LT. The anatomy of the root apex: a review and clinical considerations in endodontics. Saudi Endod J 2013; 3(1): 1-9. [CrossRef]

19. Kolanu SK, Bolla N, Varri S, Thummu J, Vemuri S, Mandava P. Evaluation of correlation between apical diameter and file size using propex pixi apex locator. J Clin Diagn Res 2014; 8(12): 18-20. [CrossRef] [PubMed]

20. Sadeghi S, Abolghasemi M. The effect of file size on the accuracy of the Raypex 5 apex locator: an in vitro study. J Dent Res Dent Clin Dent Prospects 2008; 2 (1): 24-7. [CrossRef] [PubMed]

21. Lipski M, Trabska-Swistelnicka M, Wozniak K, Dembowska E, Drozdik A. Evaluation of alginate as a substitute for root-surrounding tissues in electronic root canal measurements. Aust Endod J 2013; 39(3): 155-8. [CrossRef] [PubMed]

22. Altunbas D, Kustarci A, Arslan D, Er K, Kocak S. Comparison of various current electronic apex locators to determine the working length using the clearing technique. Niger J Clin Pract 2015; 18(3): 359-63. [CrossRef] [PubMed]

23. Kocak MM, Kocak S, Saglam BC, Turker SA. Influence of Qmix irrigant on the accuracy of four different electronic apex locators. Int J Exp Dent Sci 2016; 5(1): 23-7. [CrossRef]

IN VITRO POREĐENJE PRECIZNOSTI DVA APEKS LOKATORA RAZLIČITIH GENERACIJA

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Preciznost apeks lokatora je veoma važna za tačno određivanje radne dužine kanala korena, a time i za uspešan endodontski tretman.

Cilj ove studije bio je da se *in vitro* uporedi preciznost iPex II (četvrta generacija) i Adaptive (šesta generacija) apeks lokatora.

Materijal je činilo 28 kanala korena (16 premolara). Najpre je radna dužina svih kanala korena određivana unošenjem K-turpije #15 do apeksnog otvora, što je proveravano vizuelnim praćenjem vrha turpije. Fiksirana radna dužina je zatim merena digitalnim nonijusom, a dobijene vrednosti su služile za kontrolu merenih dužina kanala u dve eksperimentalne grupe. Zubi su uranjani u alginat pre elektronskih merenja, radi simulacije kliničke situacije. U prvoj eksperimentalnoj grupi radna dužina kanala korena je merena iPex II apeks lokatorom, a u drugoj, sa Adaptive apeks lokatorom. Sva merenja su izvršena do apeksnog otvora u suvom kanalu.

Rezultati jednostrane ANOVA su pokazali da nije bilo statistički značajne razlike između ispitivanih eksperimentalnih grupa (p > 0,05). Najveća razlika je postojala u poređenju vrednosti Adaptive apeks lokatora i kontrolne grupe, a najmanja u poređenju iPex II i Adaptive apeks lokatora.

Može se zaključiti da su oba apeks lokatora dovoljno precizna za kliničku praksu, iako pripadaju različitim generacijama.

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Ključne reči: *Adaptive, apeks lokator, iPex II, radna dužina*

DURATION OF THE MONITORING OF INTRACRANIAL PRESSURE CONCERNING THE PRESENCE OF INTRACRANIAL HYPERTENSION

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Duration of intracranial pressure (ICP) monitoring depends on the clinician`s necessity for the data that influence type and length of the management of intracranial hypertension (ICHT). On the other hand, it is also affected by the possibility of the development of the infection, which is very often fatal when it occurrs in the central nervous system.

A prospective study of the 32 patients with severe brain trauma (SBT) that had intracranial pressure (ICP) monitoring is presented in here. There were 22 patients with intracranial hypertension (ICHT) and 10 without it. In the ICHT group, the monitoring lasted 5.81 ± 2.70 and 4.45 ± 1.81 in the control group. We have not found a significant difference in the duration of the ICP monitoring between two groups (t = 1.71, p > 0.05). Patients with ICHT had significantly shorter survival than the control group (p = 0.04).

It seems that need for prolonged monitoring of the patients with ICHT is suppressed by their shorter survival, comparing to brain-injured patients with normal intracranial pressure.
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Key words: *monitoring, intracranial pressure, brain injuries*

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Introduction

The essential purpose of the ICP monitoring is the adequate therapy of the patients. Data obtained by the method are of some interest when deciding the type and length of the treatment. Today, clinicians are capable of performing specific or ultimate therapy options after obtaining the information from the ICP monitor. Specific therapy options include deep sedation, drainage of some amount of CSF from the ventricle, hyperventilation up to pCO_2 = 30-35 mmHg and intensifying antiedematous therapy. Ultimate therapies are high dose barbiturate treatment, hyperventilation up to pCO_2 = 25-30 mmHg, hypothermia, and decompressive treatment (1). Numerous treatment options suggest that neither of them is highly effective and that only their successive application or combination could be of some benefit for the patient with severe brain injury (SBI). The longer ICP moni-

toring usually means a bigger chance of successful treatment of the SBI patients.

The aim of this study was to show duration of ICP monitoring in both traumatized patients with ICHT and traumatized patients without it, and to find if there is a significant difference between them.

Patients and methods

In this prospective study, 32 patients underwent intracranial pressure monitoring after suffering SBI. It is defined as a brain injury resulting in an altered consciousness represented in the Glasgow Coma Scale from 3 to 8. Three stands for deep coma and 8 for state of motor, verbal and eye response when patients are aroused. Twenty -two of them had intracranial hypertension and 10 did not have it. They were divided into two groups: the group with intracranial hypertension (ICHT) and the control group. All of the patients had GCS ≤ 8 or abnormal CT scan of the brain in terms of present mass lesions. Mass effects that had been shown in the CT with midline shifting bigger than 5 mm were surgically explored (external or internal decompression). Patients with CT findings of no mass effect large enough and with ICHT were treated conservatively, in the Intensive care Unit.

ICHT was defined as a state of permanently elevated values of ICP, higher than 20 mmHg, for a period of time longer than 2 hours. We made continuous monitoring of the ICP by recording the data on an hourly basis and readings were performed from

the display device (Codman ICP Express). Seventeen patients had ICP monitor implanted subdural, 11 intraparenchymal and 4 intraventricular.

In all of the patients specific therapy options were administered in order to restore and\or preserve normal ICP, but in fifteen of them surgery had to be performed. Duration of the ICP monitoring lasted until its normalization, and then for 48 to 72 hours more, or, in some cases until the death occurs. In the patients where there was no intracranial hypertension initially, patients were monitored for another 48 to 72 hours, preemptively.

Besides general treatment measures, we provide specific and ultimate types of measures. The specific measures included: 1. deep sedation and/or relaxation (fentanyl, vecuronium), 2. Drainage 3 to 5 ml of CSF (in cases of intraventricular placed systems), 3. mannitol bolus at first and then application intravenously for 6 hours, 4. Hyperventilation to $pCO_2 = 30\text{-}35$ mmHg. Ultimate treatments that we have used were: 1. treatment with high doses of barbiturates (barbituric coma) 2. Hyperventilation to $pCO_2 = 25\text{-}30$ mmHg, 3. operation: internal or exterternal decompression. Appropriate nutritional support, glycemic control, and peptic ulcer prophylaxis were provided to all of the patients in the study. Internal decompression of traumatic mass lesions was done in 22 patients according to general neurosurgical indications.

For the quantitative statistical analysis Excel program from Microsoft Office, a software package was used for the tabular and graphical presentation of data. The calculations were performed using the SPSS program version 10.0. In all analysis the limits of statistical significance as the default error estimates of 0.05 or 5%. For a comparison of numerical values between the two groups, we used the Student t-test for independent samples. Comparison of representation of certain modes of attribute characteristics between the two groups was performed by Fisher test.

Results

There were 29 (90.62%) male patients and only 3 female (9.38%) in the study group. The average age of the patients was 40.97 ± 21.70. Average GCS in the study patients was 6.29 ± 2.11. We have determined ICHT in 22 (68.75%) of the monitored patients, 10 (31.25%) of them did not meet the criteria of intracranial hypertension, and that was the control group. In 13 (59.10%) patients ICHT was recorded in first 24 hours after trauma and in 9 (40.90%) it was recorded afterwards. The average period of time from injury until insertion of the ICP probe was 0.68 days.

In the ICHT group of the patients, ICP monitoring lasted 5.81 ± 2.70 days, minimal 2 days, and maximum 10 days (Graph 1). In the control group duration of the monitoring was 4.45 ± 1.81 days, minimum 3 days and maximal 9 days (Graph 2).
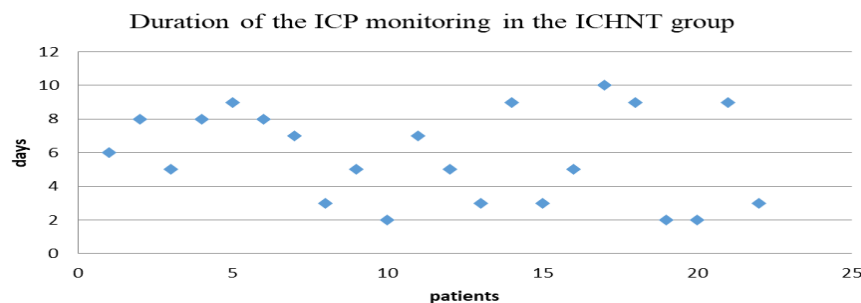
Graph 1. Distribution of duration (days) of the ICP monitoring in patients with ICHTN
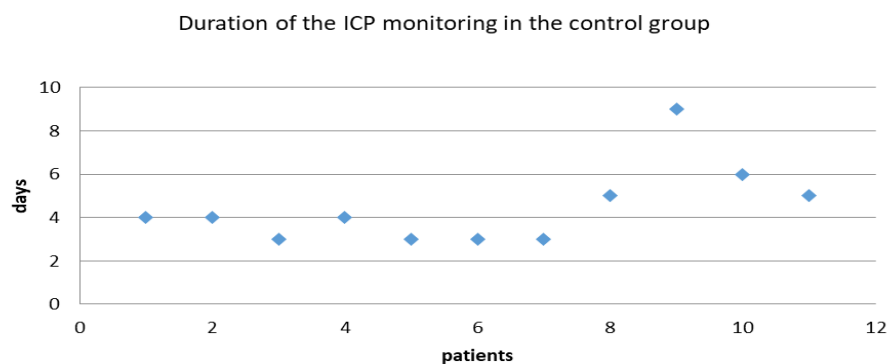
Graph 2 Distribution of the duration of the ICP monitoring in the control group

There was no significant difference in duration of the monitoring between ICHT and control group (t = 1.71 and p > 0.05) (Graph 3).

Percentage of the survived patients was significantly lower in patients with ICHT (Fisher test. p = 0,04; p < 0,05) (Table 1).

There were 20 registered complications during treatment of the study group. The most frequently pulmonary complications occurred, in 11 out of 32 patients (34.37%), while all others were very rare. In nine patients pneumonia occurred and in two mechanical pneumothorax occurred. No CNS infections were noted (Table 2).
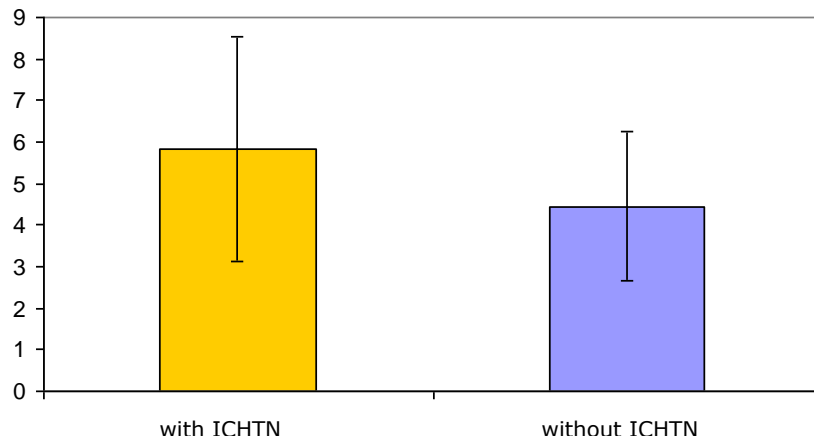
Graph 3. Comparison of duration of the ICP monitoring between ICHTN and control group (Xsr±SD)

Table 1. Deceased vs survived ratio in study group considering the ICHTN

Monitoring ICP	N	deceased	% deceased	survived	% survived
with ICHTN	22	13	59.10	9	40.90
without ICHTN	10	2	20.00	8	80.00
Σ	32	15		18	56.25

Table 2. General complication during treatment of SBI and ICP monitored patients

Complication	ICP monitored patients
Pneumonia	9
Mechanical pneumothorax	2
Thrombophlebitis	2
Osteomyelitis	1
CSF leaking	1
Infection of the digestive tract	4
Seizures	1
CNS infections	-
Σ	20

Discussion

A headache, nausea, and vomiting, symptoms of the elevated ICP, are impossible to elicit in coma-tose patients. Papilledema, a clinical sign of intracranial hypertension is uncommon after the head injury, even in patients with documented elevated ICP (2). Brain edema CT scan signs, such as midline shift and

compressed basal cisterns, are associated with increased ICP, nevertheless, intracranial hypertension can occur without those findings.

Several noninvasive ICP monitoring techniques have been proposed with the hope to replace the invasive techniques. Ocular sonography has been used to measure the changes in optic nerve sheath diameter to detect raised ICP, and it has been clinically shown that millimetric increases of the diameter correspond to increased ICP (3). Another method, pupillometry cannot be suggested for continuous ICP monitoring although it is a very useful method for screening patients with possibly increased ICP (4). Even today, invasive ICP monitoring is of the greatest significance in precise estimating both ICP and indirectly cerebral perfusion pressure (CPP). This gold standard, however, in most of the cases consists of the placement of an intraventricular catheter.

Duration of the ICP monitoring depends firstly on the severity of the injuries as they affect initial survival, then on the dynamics of normalization of the ICP, and at last but not the least, on the dedication of the clinician to struggle with the very frequent impregnable enemy (5). On the other hand, a patient's death or the occurrence of the complication of this intervention implies aborting the procedure.

The most common complication of ventriculostomy catheter placement is infection with an incidence of 5% to 14% (6). More than three decades ago, duration of ICP monitoring was shown to be associated with higher incidence of the infections, when Narayan found out that placement of an intraventricular catheter for a period of time longer than 5 days was accompanied with CNS infections in 85% (7).

The use of antibiotic-coated ventriculostomy catheters has been shown to reduce the risk of infection from 9.4% to 1.3% (8). In our study, we did not use catheters coated with antibiotics, but in the majority of the cases, we used subdural emplacement of the catheter, not intraventricular. Our patients had no case of infection. It seems that subdural location of the catheter`s tip is less associated with infection than intraventricular. However, reviewing the literature showed us no paper dealing with this issue. Another reason for the absence of the infection could be that we never prolonged ICP monitoring more than 10 days. In his study of 595 patients, Park et al. found out that relationship between duration of catheterization and infection is a fact. Nevertheless, in a group of 213 patients with prolonged ventricular drainage of 10 days or more, a non-linear increase in daily infection rate was observed over the initial 4 days but remained constant despite prolonged catheter use (9). Prophylactic antibiotic therapy seems to be of some benefit as some studies have shown (10) and we have implemented it in our therapy during all the time of the monitoring of the patients. Factors that are not associated with infection are the insertion of the catheter in the neurologic ICU, drainage of CSF, and the use of steroids. No reduction of the infection rate was achieved when prophylactic replacement of the catheters was practiced during ICP monitoring comparing to the group where they were in place all the time (11). Neither did we replace monitor catheters, during the period of ICP monitoring.

Secondly, the frequent complication of ventriculostomy catheters is hemorrhage in 1.4 % cases (12). However, such hemorrhages are unusually large, rarely the cause of neurological deterioration, and almost never require surgical removal. On the other hand, malfunction, obstruction, and malposition always require replacement or removal of the device`s catheter.

Other complications that occurred during the treatment of the ICP monitored patients were not implied in the process of the monitoring, as they had not been caused by this diagnostic procedure. The exception was if they directly influenced mortality of the patients. At the first place, pulmonary complications were noticed, pneumonia and pneumothorax, while other types of complications were rare. Prevalence of the pulmonary complications in critically ill neurological patients is well known (13). Besides polytraumas that often include direct chest trauma and consequently rib fractures, lung contusions, and pneumo or hemothorax, the most commonly noted respiratory complications are pneumonia, acute respiratory distress syndrome, pulmonary embolism, and mechanical pneumothorax. The main cause of these complications is the inability to protect the airway, low mobility of the patients, disruption of natural defense barriers in the patients with a depressed level of consciousness (13).

Our prime expectation was that patients with SBI that had ICHTN would have a significantly longer period of time ICP monitored, but results showed that duration was only 1.5 days longer (p > 0.05). This could be due to the fact that patients with ICHTN lived shorter than the control group (p = 0.04), despite all the efforts of the clinicians and staff at the ICU to prolong the survival of the patients. ICHTN is well known negative survival factor in SBI patients, no matter if they are in the deepest coma (14) or not (15). This fact also shows that the level of control of the ICP in SBI patients is not satisfactory despite all therapy options implemented during this study or elsewhere (15).

Conclusion

Invasive ICP monitoring remains the golden standard in the management of the severe brain injured patients despite attempts to be replaced by noninvasive monitoring methods. Longer monitoring, intraventricular placement of the catheter and omission of the antibiotic application are likely to enhance the chance for infection. It seems that need for prolonged monitoring of the patients with ICHT is suppressed by their shorter survival, comparing to brain-injured patients with normal intracranial pressure.

Conflicts of interest

The results presented in this paper have not been published previously in whole. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Oertel M, Kelly DF, Lee JH, McArthur DL, Glenn TC, Vespa P, et al. Efficacy of hyperventilation, blood pressure elevation, and metabolic suppression therapy in controlling intracranial pressure after head injury. J Neurosurg 2002; 97(5):1045-53. [CrossRef] [PubMed]
2. Selhorst JB, Gudeman SK, Butterworth JF, Harbison JW, Miller JD, Becker DP. Papilledema after acute head injury. Neurosurgery 1985; 16:357-63. [CrossRef] [PubMed]
3. Geeraerts T, Duranteau J, Benhamou D. Ocular sonography in patients with raised intracranial pressure: The papilloedema revisited. Crit Care 2008; 12:150. [CrossRef] [PubMed]
4. MN Khan, H Shallwani, MU Khan, MS Shamim. Non-invasive monitoring intracranial pressure – A review of available modalities. Surg-Neurol Int 2017; 8:51. [CrossRef] [PubMed]
5. Contant CF Jr, Narayan RK. Prognosis after head injury. In: Youmans JR, editor. Neurological surgery. 4th ed. Philadelphia: WB Saunders; 1996. P. 1792-812.
6. Mayhall CG, Archer NH, Lamb VA, Spadora AC, Baggett JW, Ward JD, et al. Ventriculostomy-related infections: a prospective epidemiologic study. N Engl J Med 1984; 310:553-9. [CrossRef] [PubMed]
7. Narayan RK, Kishore PRS, Becker DP, Ward JD, Enas GG, Greenberg RP, et al. Intracranial pressure: to monitor or not to monitor? A review of our experience with severe head injury. J Neurosurg 1982; 56(5): 650-9. [CrossRef] [PubMed]
8. Zabramski JM, Whiting D, Darouiche RO, Horner TG, Olson J, Robertson C, et al. Efficacy of antimicrobial-impregnated external ventricular drain catheters: a prospective, randomized, controlled trial. J Neurosurg 2003; 98:725-30. [CrossRef] [PubMed]
9. Park P, Garton HJL, Kocan MJ, Thompson BG. Risk of Infection with Prolonged Ventricular Catheterization. Neurosurgery 2004; 55:594-601. [CrossRef] [PubMed]
10. Sonabend AM, Korenfeld Y, Crisman C, Badjatia N, Mayer SA, Connolly ES Jr. Prevention of ventriculo-stomy-related infections with prophylactic antibiotics and antibiotic-coated external ventricular drains: a systematic review. Neurosurgery 2011; 68(4):996-1005. [CrossRef] [PubMed]
11. Holloway KL, Barnes T, Choi S, Bullock R, Marshall LF, Eisenberg HM, et al. Ventriculostomy infections: the effect of monitoring duration and catheter exchange in 584 patients. J Neurosurg 1996; 85:419-24. [CrossRef] [PubMed]
12. Ko JK, Cha SH, Choi BK, Lee JI, Yun EY, Choi CH. Hemorrhage Rates Associated with Two Methods of Ventriculostomy: External Ventricular Drainage vs. Ventriculoperitoneal Shunt Procedure 2014; 54(7): 545-51. [CrossRef] [PubMed]
13. Lee K, Rincon F. Pulmonary Complications in Patients with Severe Brain Injury. Critical Care Research and Practice 2012. doi:10.1155/2012/207247. [CrossRef]
14. Krbila S, Waczulikova I, Sobona V, Zahorec R. Impact of intracranial pressure measurement on survival in patients with severe traumatic brain injury. Bratisl Lek Listy 2013; 114(12):696-701. [CrossRef] [PubMed]
15. Miller JD, Becker D, Ward JD, Sillivan HG, Adams WE, Rosner MJ. Significance of intracranial hypertension in severe head injury. JNS 2010; 112(2):503-16. [PubMed]

TRAJANJE PRAĆENJA INTRAKRANIJALNOG PRITISKA UZIMAJUĆI U OBZIR PRISUTVO INTRAKRANIJALNE HIPERTENZIJE

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Trajanje praćenja intrakranijalnog pritiska (IKP) zavisi od potrebe kliničara da, potpomognut odgovarajućim podacima, modifikuje tip i dužinu terapije intrakranijalne hipertenzije. Sa druge strane, na monitoring IKP utiče mogućnost razvoja infekcije, koja je često fatalna kada zahvati centralni nervni sistem.

Prospektivna studija obuhvatila je 32 bolesnika sa teškom povredom mozga (TPM) kojima je praćen IKP. Bilo je 22 bolesnika sa intrakranijalnom hipertenzijom (IKHTN), a 10 bez. U grupi bolesnika sa IKHTN, monitoring je trajao 5,81 ± 2,70 dana, a 4,45 ± 1,81 u kontrolnoj grupi. Nismo našli značajnu razliku u trajanju praćenja IKP između ove dve grupe (t = 1,71, p > 0,05). Bolesnici sa IKHTN su značajo kraće preživljavali povrede mozga od kontrolne grupe (p = 0,04).

Čini se da je potreba dužeg praćenja bolesnika sa IKHTN kompromitovana njihovim kraćim preživljavanjem u odnosu na kontrolnu grupu.

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Ključne reči: *praćenje, intrakranijalni pritisak, teška povreda mozga*

ESTIMATION OF POSTOPERATIVE CARDIAC COMPLICATIONS WITH V-POSSUM MODEL IN PATIENTS PREPARED FOR MAJOR ELECTIVE VASCULAR SURGERY

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The Vascular Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity (V-POSSUM) is a vascular surgical modification of POSSUM. The aim of the study is to investigate role of V-POSSUM in estimation of major adverse cardiac events (MACE) in patents after major elective vascular surgery. We also wanted to examine relationship of various clinical and demographic data with postoperative cardiac complications. We prospectively enrolled all 122 patients prepared for major open elective vascular surgery (abdominal aortic aneurysm repair, inferior inguinal arterial reconstruction, or carotid endarterectomy). The analysis of the Kaplan-Meier curve showed that patients with a morbidity assessment of V-POSSUM score > 27 had a statistically significantly shorter time to develop cardiac complications in the first month compared to other patients (p = 0.026). Neither of clinical and demographic characteristics was not associated with postoperative cardiovascular events. V-POSSUM represents a way to improve the stratification for postoperative cardiac complications in patients prepared for major elective vascular surgery.

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Key words: *V-POSSUM, cardiac complications, vascular surgery*

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Introduction

The Vascular Physiological and Operative Severity Score for the enumeration of Mortality and Morbidity (V-POSSUM), is a vascular surgical modification of POSSUM in which the original developers examined 62 physiological parameters and used multi-variate analysis to identify the most powerful predictors of mortality. This eventually reduced the 62 to 12 physiological and 6 operative parameters. In the preoperative period only the 12 physiological parameters can be collected. The 12 physiological parameters required include age, evidence of cardiac failure (categorised based on current cardiac medication and on clinical evidence of heart failure), evidence of pulmonary disease (categorised based on severity of dyspnoea or evidence of consolidation), preoperative ECG changes (based on rate, ectopics, Q-waves and ST changes), systolic BP, resting pulse rate, Glasgow Coma Scale scoring, and serum levels of haemoglobin, white cell count, urea, sodium and potassium (1). These were placed into the online V-POSSUM calculator at www.riskprediction.org.uk to calculate individual scores. The aim of the study is to investigate role of V-POSSUM in estimation of major adverse cardiac events (MACE) in patents after major elective vascular surgery. We also wanted to examine relationship of various clinical and demographic data with postoperative cardiac complications.

Material and methods

The study was approved by the Ethics Committee of Medical Faculty University of Nis, Serbia. During 2017, we prospectively enrolled all 122 patients prepared for major open elective vascular surgery (abdominal aortic aneurysm repair, infrainguinal arterial reconstruction, or carotid endarterectomy) in Clinic for Cardiovascular and Transplantation Surgery, Clinical Center Niš, Niš, Serbia. Exclusion criteria were: 1) patients younger than 21 years, 2) unstable coronary disease and 3) decompensated

heart failure. All procedures were performed during general anesthesia. All patients initially underwent detail evaluation of medical history, physical examination, routine hematologic and biochemical blood analysis, 12-lead electrocardiogram, and chest radiography. We used online risk calculator software for V-POSSUM (http://www.riskprediction.org.uk/vasc-index.php). During the 30-days after the procedure, major adverse cardiac events such as: myocardial infarction, ventricular arrhythmias, decompensating heart failure, and new onset atrial fibrillation were recorded.

Statistical analysis

The obtained data are entered into the database, arranged by tables and shown graphically. As part of descriptive statistics, data are presented in the form of arithmetic mean and standard deviation, median and interquartile differences, minimum and maximum values, or in the form of absolute or relative numbers. Testing of the normality of data is carried out by Kolmogorov-Smirnov test. For the comparison of two groups of data, if a normal distribution was satisfied, a t-test was used, if the data distribution was not normal, Mann-Whitney's U test was used. For the comparison of three or more data sets, if the normal distribution was satisfied, ANOVA was used, and the Tukey test was used as a post hoc analysis. If the normal distribution was not satisfied when comparing three or more data sets, a Kruskal-Wallis test was used, in which case Mann-Whitney's U test was used as a post hoc analysis. To compare the attributes, a Hi-square test, or Fisher's Exact Probability Test, was used. Statistical data processing was carried out in the SPSS 16.0 program package (SPSS Inc., Chicago, Ill., USA). Statistical significance was determined for a p value of less than 0.05.

Results

The study included 122 patients (94 men – 77.0%, 28 women – 23.0%), average age 67.03 ± 4.50 years (Min 48, Max 84 years) (Table 1).

Table 1. Clinical characteristics

Characteristics	Number	%
Atrial fibrillation	6	4.9
Earlier stroke	32	26.2
Coronary disease	26	21.3
Cardiomyopathy	12	9.8
Prior PCI	7	5.7
Earlier myocardial infarction	21	17.2
Earlier CABG	2	1.6
Hypertension	104	85.2
DM	38	31.1
DMID	19	15.6
Hyperlipidemia	31	25.4
Smoking	49	40.2
Family history of cardiac diseases	49	40.2
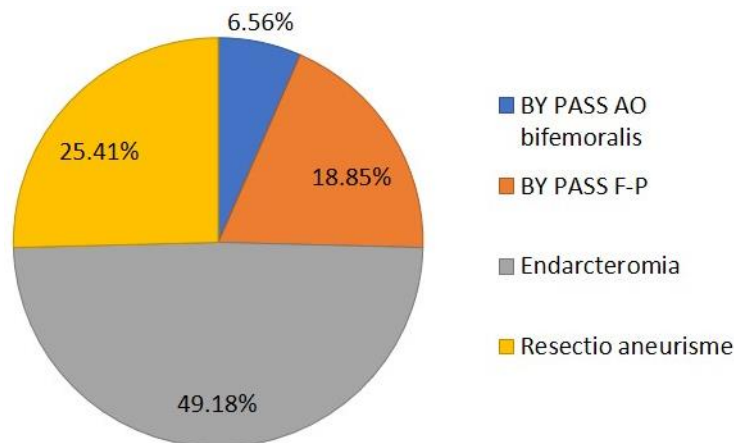
Graph 1. Representation of operative procedures

In the investigated population 8 patients underwent open aortic (by-pass aorto - bifemoralis (6.56%)), 23 patients underwent open reconstruction of inferior inguinal (BY-PASS F-P (18.85%)), 60 patients underwent open reconstruction of carotid artery (49.18%), and 31 patients underwent resection of infrarenal aortic resection (By pass aorto-biiliacalis (25.41%)) (Graph 1).

In the first 30 days after procedure 13 patients (10,7%) had 17 cardiovascular complications (Table 2). The most common complication was the new onset of atrial fibrilation (35,3%). Ten patients had one complication (76,9%), two patients had two complications (15,4%), and only one patient had three complications (7,7%). Only one patient died as a consequence of myocardial infarction. We did not have patients with pulmonary artery thromboembolism.

The occurrence of cardiac complications (Table 3) in the first month is equal regardless of the age (p = 0,182), gender (p = 0,736), atrial fibrillation (p = 1,000), stroke (p = 0,544), coronary artery disease (p = 0,601), cardiomyopathy (p = 0,229), prior PCI (p = 1,000), earlier myocardial infarction (p = 1,000), earlier CABG (p = 0,797), hypertension (p = 0,946), diabetes mellitus (p = 0,775), insulin dependent diabetes mellitus (p = 0,700), hyperlipidemia (p = 0,895), smoking (p = 0,868), family history of cardiac disease (p = 0,895).

Table 2. Cardiac complications during first month

Postoperative cardiac events	Number	%
Fatal myocardial infarction	1	5.9
Ventricular arrhytmias	4	23.5
CPR	1	5.9
Decompensation of heart failure	5	29.4
New onset of atrial fibrilation	6	35.3
Total	17	100.0

Table 3. Relation of cardiac and demographic characteristics with postoperative cardiac complications in first postoperative month

Parametar	Cardiac events		Others		p-value
	Number	%	Number	%	
Age	69.08 ± 5.47		66.79 ± 6.59		0.182
Gender					
Male	11	84.6	83	76.1	0.736
Female	2	15.4	26	23.9	
Atrial fibrillation	1	7.7	5	4.6	1.000
Stroke	2	15.4	30	27.5	0.544
Coronary disease	4	30.8	22	20.2	0.601
Cardiomyopathy	3	23.1	9	8.3	0.229
Prior PCI	1	7.7	6	5.5	1.000
Earlier MI	2	15.4	19	17.4	1.000
Earlier CABG	0	0	2	1.8	0.797
Hypertension	11	84.6	93	85.3	0.946
DM	5	38.5	33	30.3	0.775
DMID	3	23.1	16	14.7	0.700
Hyperlipidemia	4	30.8	27	24.8	0.895
Smoking	6	46.2	43	39.4	0.868
Family history of cardiac diseases	5	38.5	44	40.4	0.895

The distribution of various interventions is uniform in relation to the occurrence of cardiac complications (Table 4) in the first month (p = 0.607). In both examined groups, open surgical reconstructions of carotid artery (endarterectomy) (38.5% and 50.5%) were most performed.

The analysis of the Kaplan-Meier curve showed that patients with a morbidity assessment of V-POSSUM score > 27 had a statistically significantly shorter time to develop cardiac complications in the first month compared to other patients (p = 0.026) (Table 5).

Table 4. Impact of type of the surgery

Type of the surgery	Cardiac events		Others		p^1
	Number	%	Number	%	
By pass Ao- bifemoralis	2	15.4	6	5.5	0,607
By pass F-P	3	23.1	20	18.3	
Endarcteromia	5	38.5	55	50.5	
By pass Ao-biiliacalis	3	23.1	28	25.7	

Table 5. Kaplan-Meier's curve of survival after cardiac complications
in the first month compared to V-POSSUM values > 27

Characteristic	Average survival	SE	p-value†
V-POSSUM			
≤ 27	27.38	0.94	0.026
>27	24.32	2.03	

† - log rank test, SE – standard error

Discussion

V-POSSUM is a more reliable model then original POSSUM in the stratification of patients in vascular surgery (1). Its importance is known in the prediction of short-term mortality in elective and urgent major vascular surgery (2, 3). In this study, we wished to examine the role of V-POSSUM in the estimation of MACE. However, there is no unique definition of MACE. We define MACE as a large group of cardiovascular morbidities unlike in other definitions. Earlier our concern was to describe geographic variations in the accuracy of this model (4). We assumed that such good model characteristics in the prediction of mortality would have to be reflected in the assessment of postoperative cardiac complications. Neither of clinical and demographic characteristics was associated with postoperative cardiovascular events. A part of this group is independent predictors of major cardiac events, except renal failure, which is incorporated in Revised Cardiac Risk Index (RCRI). In this study, we could not determine the statistical significance of these factors. We consider it due to the fact that: 1) RCRI had a lower discriminatory potential for adverse cardiac events estimation; 2) narrow definition of MACE; 3) there was less than a quarter major vascular surgery patients (5). In this study, the patients with V-POSSUM > 27 had significantly shorter time to cardiovascular complications during the first month. A study that included a similar number of respondents as ours, in open elective abdominal aortic aneurysm repair, showed V-POSSUM as a valuable tool in stratification for MACE defined as a non-fatal myocardial infarction and cardiac death (6).

Conclusion

V-POSSUM represents a way to improve the stratification for postoperative cardiac complications in patients prepared for major elective vascular surgery.

References

1. Prytherch DR, Ridler BM, Beard JD, Earnshaw JJ. A model for national outcome audit in vascular surgery. Eur J Vasc Endovasc Surg 2001; 21(6):477-83. [CrossRef] [PubMed]
2. Harris JR, Forbes TL, Steiner SH, Lawlor DK, Derose G, Harris KA. Risk-adjusted analysis of early mortality after ruptured abdominal aortic aneurysm repair. J Vasc Surg 2005; 42(3):387-91. [CrossRef] [PubMed]
3. Tang TY, Walsh SR, Prytherch DR, Wijewardena C, Gaunt ME, Varty K, et al. POSSUM Models in Open Abdominal Aortic Aneurysm Surgery. Eur J Vasc Endovasc Surg 2007; 34(5):499-504. [CrossRef] [PubMed]
4. Walsh SR, Prytherch DR, Wijewardena C, Gaunt ME, Varty K, Boyle JR. POSSUM models in open abdominal aortic aneurysm surgery. Eur J Vasc Endovasc Surg 2007; 34(5):499-504. [CrossRef] [PubMed]

5. Lee TH, Marcantonio ER, Mangione CM, Thomas EJ, Polanczyk CA, Cook EF. Derivation and prospective validation of a simple index for prediction of cardiac risk of major noncardiac surgery. Circulation 1999; 100(10):1043-9. [CrossRef] [PubMed]

6. Bryce GJ, Payne CJ, Gibson SC, Kingsmore DB, Byrne DS, Delles C. Risk stratification scores in elective open abdominal aortic aneurysm repair: are they suitable for preoperative decision making? Eur J Vasc Endovasc Surg 2012; 44(1):55-61. [CrossRef] [PubMed]

PROCENA POSTOPERATIVNIH SRČANIH KOMPLIKACIJA POMOĆU V-POSSUM MODELA U PRIPREMI BOLESNIKA ZA VEĆE ELEKTIVNE VASKULARNE HIRURŠKE ZAHVATE

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Vaskularna fiziološka i operativna ocena težine za računanje mortaliteta i morbiditeta (V-POSSUM) je vaskularna hirurška modifikacija POSSUM-a. Cilj studije bio je istraživanje uloge V-POSSUM-a u proceni glavnih neželjenih srčanih manifestacija kod pacijenata nakon velikih elektivnih vaskularnih operacija. Takođe, želeli smo da ispitamo odnos različitih kliničkih i demografskih podataka sa postoperativnim srčanim komplikacijama. Prospektivno smo uključili 122 bolesnika, koji su pripremani za veliku vaskularnu hirurgiju (aneurizma abdominalne aorte, inferiorna ingvinalna arterijska rekonstrukcija ili karotidna endarterektomija). Analiza Kaplan-Majerove krivulje pokazala je da su bolesnici sa procenom morbiditeta skor V-POSSUM > 27, imali statistički značajno kraće vreme za razvoj srčanih komplikacija u prvom mesecu u poređenju sa drugim bolesnicima (p = 0,026). Nijedna klinička i demografska karakteristika nije bila povezana sa postoperativnim kardiovaskularnim događajima. V-POSSUM predstavlja način da se poboljša stratifikacija postoperativnih srčanih komplikacija kod bolesnika pripremljenih za veliku elektivnu vaskularnu operaciju.

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Ključne reči: *V-POSSUM, srčane komplikacije, vaskularna hirurgija*

A STRATEGY FOR THE TREATMENT OF PATIENTS WITH CORONARY ARTERY DISEASE AND THE PRESENCE OF SIGNIFICANT CAROTID ARTERY STENOSIS: ANALYSIS OF THE "STAGED" AND "CONCOMITANT" APPROACHES

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Today, there is no unequivocal overview of how to treat patients undergoing myocardial revascularization and have associated significant carotid artery stenosis. In this group of patients, there are several ways to treat, and our study analyzed patients who were exposed to a "Staged" and "Concomitant" approach.

During 2016 in three cardiac surgery centers of Serbia, the perioperative results of the patients subjected to a "Staged" and "Concomitant" approach were analyzed. Group 1 including patients with cardiac revascularization and endarterectomy was made at least 30 days earlier, and group 2 consisted of patients who underwent endarterectomy with cardiac revascularization simultaneously. In both groups, CVI appearance was observed 7 days after the revascularization of the heart. We followed the risk factors for the occurrence of CVI.

The conducted study included a total of 49 patients, divided into two groups. In 28 patients (group 1) Eversion Carotid endarterectomy was performed 30 days to 26 months prior to coronary revascularization. The average age of patients was 64 and 65 years, with the prevalence of male sex (86%: 14%). The only parameters that showed a significant difference between the groups were "left main syndrome" and unstable angina pectoris, both in Group 2. In the ensuing period, in both groups, there were no postoperative major neurological events (CVI). In one patient, clinical signs of neurological deficits occurred in the form of weakness of one side of the body, but CVI was not proven by CTom and MRA.

It can be concluded that the "Staged" and "Concomitant" approaches are safe and effective.

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Key words: *myocardial revascularization, carotid artery stenosis, Staged approach, Concomitant approach*

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Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in the world, responsible for 17.3 million deaths a year and is expected to rise to 23.6 million by 2030.y. About 80% of these deaths occur in low and medium-developed countries (1), which includes our country. Ischemic heart disease, caused predominantly by arteriosclerosis, is the most common form of heart disease. Arteriosclerosis is a diffuse process involving various vascular systems with significant overlap between coronary, cerebrovascular and peripheral arterial systems (2).

This condition is associated with similar predisposing risk factors and genetic predisposition. In the population of patients with coronary artery disease, the presence of 11.1-25.4% of cerebrovascular disease was observed (3, 4). One of the main risk factors for the development of arteriosclerotic disease, which equally affects the development of CAD and CVD, is age. All clinical studies show an increasing average age of patients undergoing CABG, so the risk of a significant CVD is increasing. The average age of 56 years old in 1980 jumped to 68 in 2001.y. In 1980, only 6% of patients were older than 70 years old, while according to data from 2001, even 45% were older than 70 and 13% older than 80

years (5). Stroke is a devastating complication that occurs in myocardial revascularization, not only because of the devastating consequences for the patient but also because of the increased costs of hospitalization and post-hospital care.

The rate of mortality among those who have experienced CVI is very high, close to 25%, with an average stay in hospital 28 days (6, 7). The risk of stroke in patients undergoing CABG is well defined. In 1986, Gardner et al. (8) found that the risk of stroke was in direct proportion to the age of the patient. Patients under 45 years of age have a statistically defined risk of 0.2%, and it rises to 3% for patients in sixties and 8% for patients over 79 years of age. Other risk factors associated with stroke in the perioperative period are previous neurological events (CVI, TIA), arteriosclerosis of the ascendant aorta, duration of cardiopulmonary bypass, and perioperative hypotension. The effectiveness of surgical treatment of significant carotid artery stenosis at the distant stroke is clear in all studies and has the highest possible level of recommendation today (9-15).

In many clinical studies, it has been shown that more than 50% of patients who come because of a significant carotid artery stenosis have coronary artery disease. Even 25% of them have a significant coronary disease, 7% have a severe inoperable coronary disease, and only about 7% have normal coronary blood vessels (16, 17).

The only question that becomes apparent when there is coronary artery disease with an indication for operative care and significant carotid stenosis is the choice of approach to the management of both diseases.

One approach is the performance of carotid endarterectomy and CABG as a stepwise procedure, carotid endarterectomy, and CABG ("staged" procedure), with endarterectomy being performed prior to the establishment of extracorporeal circulation (18-21). Most surgeons recommend this type of operative approach, especially for patients who have stable angina pectoris and who are hemodynamically stable (5). The introduction of everzione method and regional anesthesia (block) significantly improved the results in relation to the perioperative occurrence of stroke, death, and myocardial infarction.

The second approach is simultaneous, synchronous approach ("Concomitant"). Bernhard was the first one to report it in 1972. in 15 patients, in whom he performed endarterectomy of significant carotid artery stenosis and coronary revascularization in general anesthesia (5).

Daily in his analysis, he said that this approach does not lose efficiency and saves in the course of treatment (22).

Material and methods

The patients were treated with CABG in the Clinical Center of Serbia Clinic for cardiac surgery, KBC Dedinje and KC Niš Clinic for cardiovascular with transplant surgery, in the period from 1st January to 31st December 2016, with significant carotid artery stenosis. The significance of carotid artery stenosis is defined with ≥ 70% with the help of color duplex scans. Patients were divided into two groups:

Group 1. - Everzione endarterectomy of the carotid artery was performed at least 30 days prior to coronary revascularization

Group 2 - everzional endarterectomy of the carotid artery was performed synchronously with myocardial revascularization.

After myocardial revascularisation, CVI was confirmed by CT or MR in 7 days.

We analyzed the parameters that were emphasized in the literature as predictors of intra and perioperative CVI - perfusion pressure on EKK, length of occlusal clamp, duration of EKK, body temperature during EKK, representation of LM, perioperative arterial pressure.

The exclusion parameters were endovascular treatment of significant carotid artery stenosis, the presence of significant carotid artery stenosis on both sides, and the presence of atheromatous plaques on the ascending aorta.

Results

The conducted study included a total of 49 patients in 3 cardiovascular institutions (Clinic for Cardiac Surgery of the Clinical Center C of Serbia, IKVB Dedinje, Clinic for Cardiovascular with Transplantation Surgery KC Niš), divided into two groups.

In 28 patients (Group 1), endarterectomy of the carotid artery was performed for 30 days to 26 months, and on average 9.61 ± 6.97 months before coronary revascularization. In 21 patients (Group 2), endarterectomy of the carotid artery was performed synchronously with myocardial revascularization. In Group 1 age ranged from 54 to 73 years, and on average it was 63.86 ± 4.84 years. In Group 2, the average age was somewhat higher and amounted to 65.19 ± 3.98 years, and ranged from 58 to 72 years without significant difference compared to Group 1 (p = 0.310).

In Group 1 there were 25 (89.3%) men and 3 (10.7%) women. In Group 2 (80.9%) there were 17 men and 4 (19.0%) women.

There was no significant difference in the presence of significant localized carotid artery stenosis in relation to the side in both groups.

In the group of patients in whom the endarterectomy was performed prior to the CABG, the perfusion pressure value ranged from 55 to 70 mmHg, and on average it was 63.75 ± 2.93 mmHg.

In the group of patients in whom the endarterectomy was performed synchronously with CABG, the average perfusion pressure was slightly higher and amounted to 64.05 ± 2.56 mmHg. It ranged from 60 to 70 mmHg. There was no significant difference between the perfusion pressure value in the two groups of patients compared (p = 0.712).

In Group 1, the duration of the occlusion clamp ranged from 30 to 103 minutes, and on average it was 43.43 ± 13.42 minutes. In Group 2, the average duration of the occlusion clamp was somewhat shorter and amounted to 38.83 ± 12.12 minutes

and ranged from 24 to 64 minutes, without significant difference between groups (p = 0.246).

In the group of patients in whom endarterectomy was performed prior to CABG, the duration of EKK ranged from 55 to 158 minutes, and on average it was 79.57 ± 9.08 minutes. In the group of patients in whom the endarterectomy was performed synchronously with CABG, the average duration of EKK was slightly longer and amounted to 80.56 ± 19.97 minutes and ranged from 50 to 114 minutes. In the group of patients in whom the endarterectomy was performed prior to CABG the temperature was on average 33.04 ± 1.07 ºC, and in the group of patients in whom the endarterectomy was performed synchronously with CABG the average temperature was somewhat higher and amounted to 33.71 ± 2.00 ºC.

In the group of patients in whom the endarterectomy was performed before the revascularization of myocardial HTA was observed in 18 (64.3%) cases, and in the group of patients in whom the endarterectomy was performed synchronously with revascularization of myocardium HTA was recorded in a significantly higher percentage of HTA in two compared groups p = 0.014).

In the group of patients in whom endarterectomy was performed prior to myocardial revascularization, LM stenosis was observed in 2 (7.1%) cases, and in the group where the endarterectomy was performed synchronously with myocardial revascularization, LM was recorded in a significantly higher percentage, in 8 (38 , 1%) of cases (p = 0.012). (Table 1.)

Table 1. The presence of stenosis of the left main coronary artery

LM*	Group		In total (n = 49)
	1 (n = 28)	2 (n = 21)	
No	26 (92.9%)	13 (61.9%)	39 (79.6%)
Yes	2 (7.1%)	8 (38.1%)	10 (20.4%)

*Left main

Unstable angina pectoris was recorded in 8 (38.1%) patients in whom endarterectomy was performed synchronously with myocardial revascularization.

In a group of patients in whom endarterectomy was performed synchronously with myocardial revascularisation, there was one death outcome (4.8%).

Postoperative agitation was reported in one patient (4.8%) from the group of those in whom endarterectomy was performed synchronously with myocardial revascularization. Other postoperative complications have not been recorded. The patient who died during the operation had significant comorbidities left ventricular aneurysm and HOB. The cause of death was the inability to separate from cardiopulmonary by pass. The presence of HBI was recorded in one patient in the group of those in whom endarterectomy was performed synchronously with myocardial revascularization. Significant comorbidities were not observed in the group of patients in whom endarterectomy was performed prior to myocardial revascularisation. In the presence of comorbidity there were no significant differences between the groups compared (p = 0.179).

Multivariate logistic regression analysis was the only significant factor that separated subjects from the two comparing groups confirming the presence of LM stenosis (p = 0.016).

Discussion

Our study included a total of 49 patients divided into two groups. Group 1 (28 patients) who performed endarterectomy of the carotid artery at least a month before coronary surgery and Group 2 (21 patients) who had carotid endarterectomy were synchronized with coronary surgery.

In our study, the average age of patients was 64 and 65 years with the youngest patient aged 54 years and the oldest patient for 73 years. There was no significant difference in the age of patients in both groups.

The prevalence of male sex in our results is in line with world statistics (86%: 14%).

In the literature and in our results, no prevalence of involvement of some of the sides of the carotid artery stenosis was found.

The average value of the stenosis lesion in our study was 86%, linking to the average age and according to the literature data indicates a severe degree of stenosis and a high potential for neurological events.

The average perfusion pressure value during cardiopulmonary bypass was approximately 64 mmHg, not significantly higher in Group 2, and ranged between 55 and 70 mmHg. References cited as one of the main factors for the emergence of an intraoperative or perioperative stroke are just low perfusion pressure during cardiopulmonary bypass. Our data suggest that although patients were with

high-grade stenoses of the carotid arteries (86% on average), even with contralateral stenosis at the significance limit or occlusion of the contralateral carotid artery, this pressure represents the safety for performing surgery regardless of the approach.

The average occlusion of the aorta lasted for 42 minutes (30-103 minutes) and there was no significant difference in the duration of aortic occlusion in two groups.

Aorta also represents one of the main sources of embolism, but in our results, there were no significant neurological events, which may indicate careful handling of an ascendant aorta.

The average length of cardiopulmonary bypass was 80 minutes without significant differences in the average duration per group.

One of the parameters that contributed to such good operative results was moderate hypothermia in patients because the average temperature at EKK was 33 ° C. There was no significant difference in the groups.

There was no significant difference in the presence of risk factors for the development of arteriosclerotic disease in both groups. However, as the literature suggests, diabetes mellitus, hypertension, and hyperlipidemia have been seen as a risk factor for our patients as there was a significant presence in both groups.

Parameters that showed a significant difference between the groups were significant stenosis of the left coronary artery and unstable angina pectoris. However this discrepancy was a consequence of a different approach in patient care. Namely, surgeons more often opted for synchronous surgery in patients with unstable angina and high-risk patients due to the presence of stenosis of the left coronary artery, which is the recommendation of most surgeons in the world. In the literature, it is emphasized that in these patients the risk of myocardial infarction or CVI is increased in the "staged" procedure or "reverse staged" procedure, and the majority of authors recommend synchronous surgery.

There were no postoperative major neurological events (CVI) in the follow-up period, with one patient reporting clinical signs of neurological deficits in the form of weakness on one side of the body, but CT and MRA were not confirmed CVI.

One operation in group 2 was fatal due to haemodynamic instability and inability to separate from cardiopulmonary bypass, and the patient was with significant comorbidities (chronic obstructive pulmonary disease and left ventricular aneurysm with low ejection fraction).

Conclusion

Based on the conducted study, it can be concluded that the "Staged" procedure in the treatment of a group of patients with significant carotid artery stenosis and ischemic coronary disease represents a safe and effective procedure for patients who do not have unstable angina pectoris and who do not have a significant stenosis of the main tree of the left coronary artery.

The synchronous procedure is a safe and effective method for the group of patients who have a significant stenosis of the main tree of the left coronary artery with unstable angina pectoris with the presence of a significant carotid artery stenosis.

Perfusion pressure greater than 60 mmHg during cardiopulmonary bypass, during coronary surgery in patients in a "Synchronous" or "Staged" procedure, represents the prevention of neurological events.

Moderate hypothermia during cardiopulmonary bypass, in the case of coronary surgery in patients in a "Synchronous" or "Staged" procedure, prevents neurological events.

One of the conclusions that arise is the requirement to examine the carotid artery system and in neurologically asymptomatic patients who are preparing for coronary revascularization.

References

1. Mendis S, Puska P, Norrving B, editors. Global Atlas on Cardiovascular Disease Prevention and Control. Geneva: World Health Organization; 2011.
2. Criqui MH. Peripheral arterial disease – epidemiological aspects. Vascular Medicine 2001; 6 Suppl 1:3-7. [CrossRef] [PubMed]
3. Cheng SW, Wu LL, Lau H, Ting A, Wong J. Prevalence of significant carotid stenosis in Chinese patients with periferal and coronary artery disease. Aust NZJ Surg 1999; 69:44-7. [CrossRef] [PubMed]
4. Tanimoto S, Ikari Y, Tanabe K, Yachi S, Nakajima H, Nakayama T, et al. Prevalince of carotid artery stenosis in patients with coronary artery diseas in Japanese population. Stroke 2005; 36:2094-8. [CrossRef] [PubMed]
5. Cohn HL. Cardiac surgery in the adult. 3th ed. Boston (MA): The McGraw-Hill Companies; 2008.
6. Shroyer AL, Coombs LP, Peterson ED, Eiken MC, DeLong ER, Chen A, et al. The Society of Thoracic Surgeons: 30-day operative mortality and morbidity risk models. Ann Thorac Surg 2003; 75:1856-64. [CrossRef] [PubMed]
7. Hogue WC Jr, Murphy SF, Schechtman KB, Dávila-Román VG. Risk factors for early or delayed stroke after cardiac surgery. Circulation 1999; 100:642-7. [CrossRef] [PubMed]
8. Gardner TJ, Horneffer PJ, Manolio TA, Hoff SJ, Pearson TA. Major stroke after coronary artery bypass surgery: Changing magnitude of the problem. J Vasc Surg 1986; 3:684-7. [CrossRef] [PubMed]
9. Hertzer NR, Flanagan RA, O'Hara PJ, Beven EG. Surgical versus nonoperative treatment of symptomatic carotid stenosis. Ann Surg 1986; 204:154-62. [CrossRef] [PubMed]
10. North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. N Engl J Med 1991; 325:445-53. [CrossRef] [PubMed]
11. Barnett H, Taylor DW, Eliaszew M, Fox AJ, Ferguson GG, Haynes RB, et al. Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. N Engl J Med 1998; 339:1415-25. [CrossRef] [PubMed]
12. Mayberg MR, Wilson SE, Yatsu F, Weiss DG, Messina L, Hershey LA, et al. Carotid endarterectomy and prevention of cerebral ischemia in symptomatic carotid stenosis. JAMA 1991; 266:3289-94. [CrossRef] [PubMed]
13. European Carotid Surgery Trialist's Collaborative Group. MRC European Carotid Surgery Trial: Interim results for symptomatic patients with severe (70–99%) or with mild (0–29%) carotid stenosis. Lancet 1991; 337:1235-43. [CrossRef] [PubMed]
14. Gaparis AP, Ricotta L, Cuadra SA, Char DJ, Purtill WA, Van Bemmelen PS, et al. High-risk carotid endarterectomy: Fact or fiction? J Vasc Surg 2003; 37:40-6. [CrossRef] [PubMed]
15. Reed AB, Graccione P, Belkin M, Donaldson MC, Mannick JA, Whittemore AD, et al. Preoperative risk factors for carotid endarterectomy: Defining the patient at high risk. J Vasc Surg 2003; 37:1191-9. [CrossRef] [PubMed]
16. Hertzer NR, Lees CD. Fatal myocardial infarction following carotid endarterectomy. Ann Surg 1981; 194: 212-8. [CrossRef] [PubMed]
17. Mackey WC, O'Donnell TF, Callow AD. Cardiac risk in patients undergoing carotid endarterectomy: Impact on perioperative and long-term mortality. J Vasc Surg 1990; 11:226-33. [CrossRef] [PubMed]
18. Trachiotis GD, Pfister AJ. Management Strategy for Simultaneous Carotid Endarterectomy and Coronary Revascularization. Ann Thorac Surg 1997; 64:1013-8. [CrossRef] [PubMed]
19. Snider F, Rossi M, Manni R, Modugno P, Glieca F, Scapigliati A, et al. Combined surgery for Cardiac and Carotid disease: Management and Results of a rational Approach. Eur J Vasc Surg 2000; 20:523-7. [CrossRef] [PubMed]
20. Char D, Cuadra S, Ricotta J, Bilfinger T, Giron F, McLarty A, et al. Combined coronary artery bypass and carotid endarterectomy: long term results. Cardiovasc Surg 2002; 10(2):111-5. [CrossRef] [PubMed]
21. Dönmez AA, Adademir T, Sacli H, Koksal C, Alp M. Comparison of early outcomes with Tree Approaches for combined coronary revascularization and carotid endarterectomy. Braz J Cardiovasc Surg 2016; 31 (5): 365-70. [CrossRef] [PubMed]
22. Daily PO, Freeman RK, Dembitsky WP, Adamson RM, Moreno-Cabral RJ, Marcus S, et al. Cost reduction by combined carotid endarterectomy and coronary artery bypass grafting. J Thorac Cardiovasc Surg 1996; 111: 1185-92. [CrossRef] [PubMed]

STRATEGIJA ZBRINJAVANJA BOLESNIKA SA KORONARNOM BOLEŠĆU I PRISUTNOM SIGNIFIKANTNOM STENOZOM KAROTIDNE ARTERIJE: ANALIZA "STAGED" I "CONCOMITANT" PRISTUPA

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I danas ne postoji nedvosmislena, jasna prepopruka o za tretman bolesnika koji se podvrgavaju revaskularizaciji miokarda, u uslovima vantelesnog krvotoka, a imaju pridruženu signifikantnu stenozu karotidne arterije. Kod ove grupe bolesnika postoji nekoliko načina u tretmanu, a našom studijom analizirali smo one koji su bili podvrgnuti "Staged" i "Concomitant" pristupu.

Tokom 2016.godine u tri kardiohirurška centra Srbije, Klinika za kardiohirurgiju KCS, IKVB Dedinje i Klinika za kardiovaskularnu sa transplantacionom hirurgijom KC Niš, analizirani su perioperativni rezultati bolesnika, podeljenih u dve grupe: podvrgnuti "faznim" i "simultanim" pristupom. Signifikantnost stenoze karotidne arterije definisana je sa ≥ 70% uz pomoć kolor dupleks skena. Grupa 1- bolesnika kojima je rađena revaskularizacija miokarda, a everziona endarterektomija karotidne arterije urađena najmanje 30 dana ranije, i Grupa 2 – bolesnici kojima je istovremeno rađena everziona endarterektomija karotidne arterije sa revaskularizacijom miokarda. Nakon revaskularizacije miokarda praćena je pojava CVI verifikovanog CT-om ili MR-om u periodu od 7 dana. Praćeni su parametri koji su u ranijim kliničkim studijama isticani kao prediktori pojave intra i perioperativnog CVIa - perfuzioni pritisak na EKK, dužina trajanja okluzione kleme, vreme trajanja EKK, telesna temperatura tokom EKK, zastupljenost LM, perioperativni arterijski pritisak.

Sprovedeno ispitivanje obuhvatilo je ukupno 49 bolesnika koji su razvrstani u dve grupe. Kod 28 (Grupa 1) je everziona endarterektomija karotidne arterije urađena 30 dana do 26 meseci, a u proseku 9,61 ± 6,97 meseci pre koronarne revaskularizacije. Kod 21 bolesnika (Grupa 2), je everziona endarterektomija karotidne arterije urađena je sinhrono sa revaskularizacijom miokarda. Prosečna starost bolesnika bila je 64 i 65 godina, sa predominacijom muškog pola (86%:14%). Jedini parametri koji su pokazivali signifikantnu razliku među grupama bili su "left main syndrome" i nestabilna angina pektoris u Grupi 2. U praćenom periodu, u obe grupe, nije bilo postoperativnih velikih neuroloških događaja (CVI). Kod jednog bolesnika javili su se klinički znaci neurološkog deficita u vidu slabosti jedne strane tela, ali CTom i MRAom nije dijagnostifikovan CVI.

Na osnovu sprovedene analize, može se zaključiti da "Staged" procedura predstavlja sigurnu i efikasnu proceduru za bolesnike koji nemaju nestabilnu anginu pektoris i koji nemaju signifikantnu stenozu glavnog stabla leve koronarne arterije. Sinhrona procedura, za bolesnike koji imaju nestabilnu anginu pektoris i imaju stenozu glavnog stabla leve koronarne arteriije, predstavlja siguran i efikasan pristup. Perfuzioni pritisak ≥ 60 mmHg, kao i umerena hipotermija tokom kardiopulmonalnog by pass-a, u sinhronoj ili "Staged" proceduri, predstavlja prevenciju neuroloških događaja.

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Ključne reči: *revaskularizacija miokarda, stenoza karotidne arterije, "staged" procedura, "simultana" procedura*

POTENTIAL DUAL MECHANISM OF HYPOURICEMIC ACTIVITY OF DPP-4 INHIBITORS WITH PURINE-BASED SCAFFOLD

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Dipeptidyl peptidase-4 (DPP-4) binds to adenosine deaminase (ADA) and form a complex which catalyzes an irreversible deamination of extracellular adenosine to inosine, what leads to the generation of hypoxanthine, xanthine and finally uric acid by xanthine oxidase (XO) in purine catabolism with the production of reactive oxygen species. Xanthine-based DPP-4 inhibitor linagliptin showed inhibitory potential on XO. It exerts a hypouricemic effect by inhibiting DPP-4 activity and its binding to ADA, what causes the increase of adenosine and decrease of XO substrates levels, as well as by inhibiting XO activity. Based on the evidenced dual mechanism of hypouricemic activity of linagliptin, the possibility of other DPP-4 inhibitors with the purine-based scaffold to act in the same manner exists.
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Key words: *dipeptidyl peptidase-4, xanthine oxidase, adenosine deaminase*

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Introduction

Chronic hyperuricemia represents a risk factor for metabolic syndrome, cardiovascular and renal disorders (1-6). Although the elevation of uric acid levels acts as a risk factor and contributes to the development of mentioned disorders, it also represents a marker and consequence in cardiovascular and renal pathology (7). Hyperuricemia and oxidative stress generated via xanthine oxidase (XO) activity promote endothelial dysfunction. Uric acid stimulates proliferation of vascular smooth muscle cells, elevates levels of inflammatory markers including C-reactive protein and tumor necrosis factor-α, nuclear factor kappa B, monocyte chemoattractant protein-1, interleukin-1β and -6. Improvement might be associated with the administration of urate-lowering agents such as XO inhibitors (1, 3-6).

It has been shown in isolated postischemic rat heart that XO mediated generation of free radicals upon reperfusion is triggered by enhancement of formation of its substrates, hypoxanthine, and xanthine, during ischemia, while alterations in the enzyme activity are not sufficient as major limiting factor. During ischemia, the accumulation of hypoxanthine and xanthine occurs via degradation of adenosine triphosphate (ATP) to adenosine monophosphate (AMP), adenosine and inosine. Since substrates availability is significant in the production of free radicals and consequently in the determination of severity in postischemic injury, the decrease of their formation might offer novel therapeutic approach besides the inhibition of XO activity, with beneficial effect in terms of the increase of adenosine levels as well as the preservation of high energy phosphates (8). Moreover, it has been shown in postischemic rat heart that reduction of XO substrate generation by inhibition of adenosine deaminase (ADA) activity can prevent the production of free radicals and contractile dysfunction (9).

Dipeptidyl peptidase-4 (DPP-4) is ADA binding protein, with which in complex it contributes to T-cell stimulation and proliferation (10, 11). Bimolecular complex of DPP-4 and ADA catalyzes irreversible deamination of extracellular adenosine to inosine, than to hypoxanthine and xanthine finally oxidized to uric acid by xanthine oxidase (XO) in purine catabolic pathway, with generation of superoxide anions (Figure 1). If deamination is absent adenosine is rephosphorylated to 5'-AMP and ATP by adenosine kinase (12).

Therefore, compounds with XO and DPP-4 inhibitory potential will at the same time reduce XO activity, as well as its substrates generation by suppressing formation of the complex between DPP-4 and ADA.
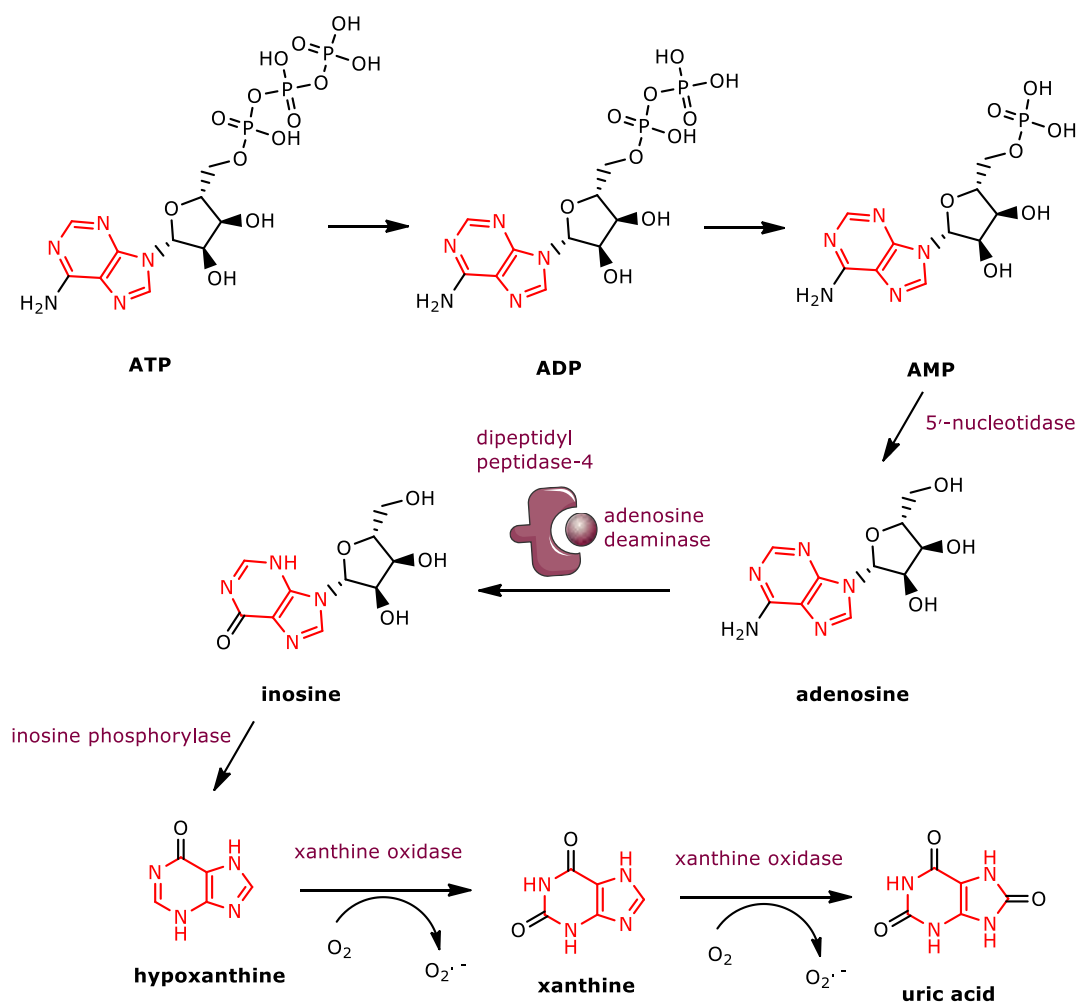
Figure 1. Schematic diagram of the irreversible deamination of adenosine to inosine, hypoxanthine and xanthine oxidized to uric acid (adapted from Lee et al. (12) and Xia et al. (9))
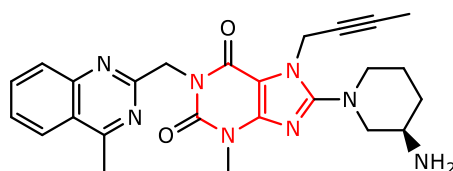
Figure 2. Chemical structure of linagliptin

Linagliptin, dipeptidyl peptidase-4 and xanthine oxidase inhibitor

It has been shown that linagliptin, DPP-4 inhibitor with xanthine-based structure (Figure 2) and reported IC_{50} value of $\sim$ 1 nM (13-16), at the oral dose of 5 mg/kg for 7 days normalized increased serum XO activity in septic rats (17). Additionally, linagliptin exerted an inhibitory effect on the activity of XO *in vitro* and in human serum derived from the healthy volunteer in a concentration-dependent manner up to 1 mM, as well as lowered uric acid levels in plasma of diabetic patients at the oral dose of 5 mg once daily for 24 weeks (18).
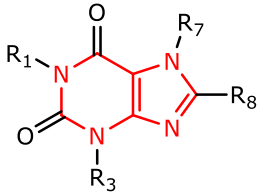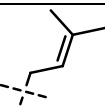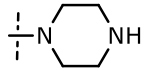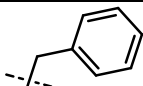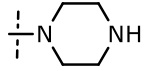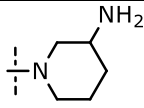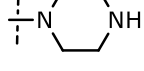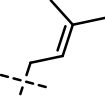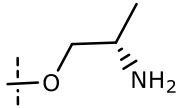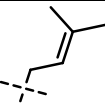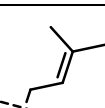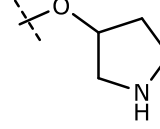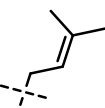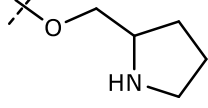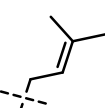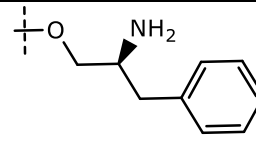
Generally, based on linagliptin as representative, DPP-4 inhibitors with purine-based scaffold may
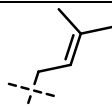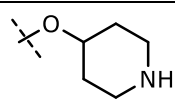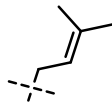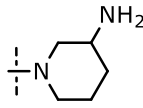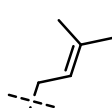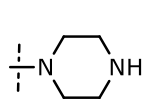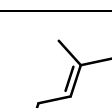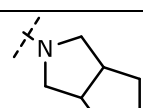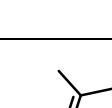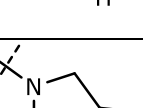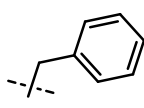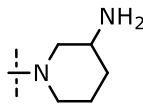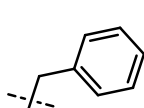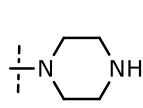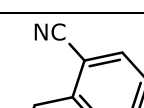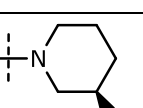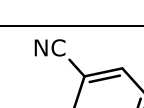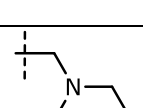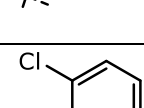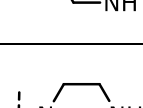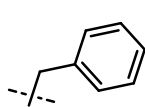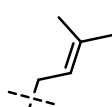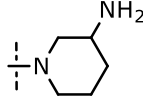
be hypothesized to exert hypouricemic effect via dual mechanism, inhibiting the activity of DPP-4 and its binding to ADA with subsequent increase of adenosine levels and decrease of availability of substrates for XO, as well as inhibiting XO activity, with finally reduced production of uric acid.
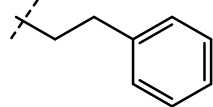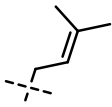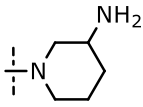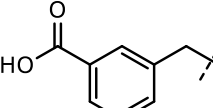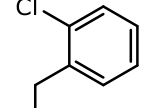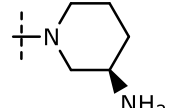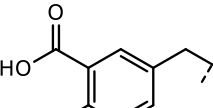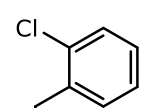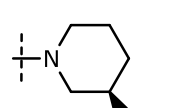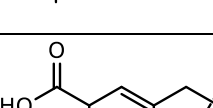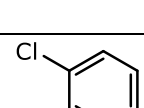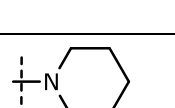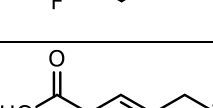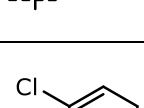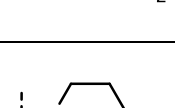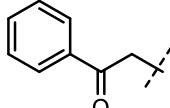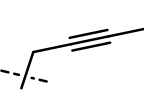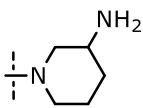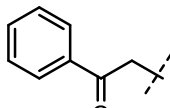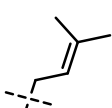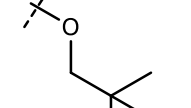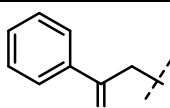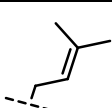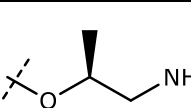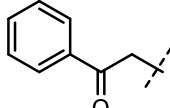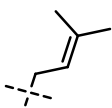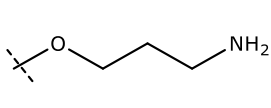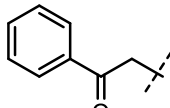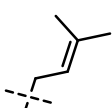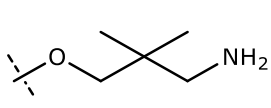
Overview of purine-based DPP-4 inhibitors
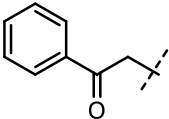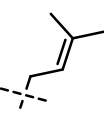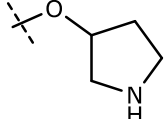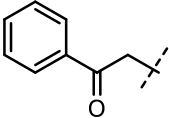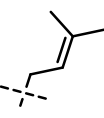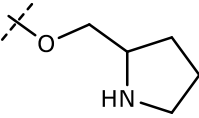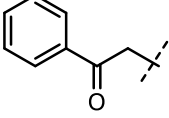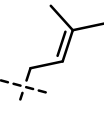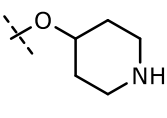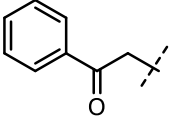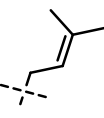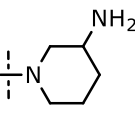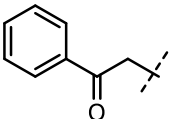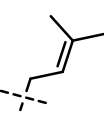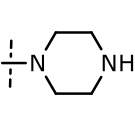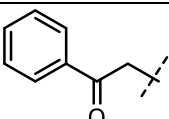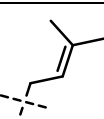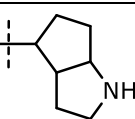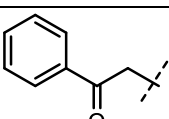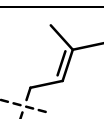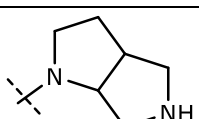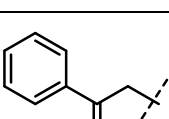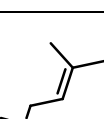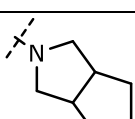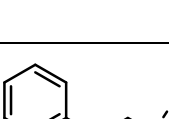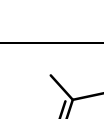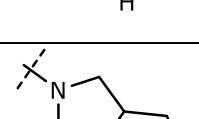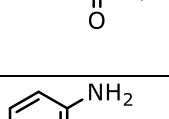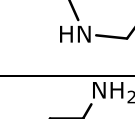
Some DPP-4 inhibitors (13-16, 19-28) have been derived from a xanthine scaffold, similar to the structure of linagliptin (Table 1, Figure 3).

Table 1. DPP-4 inhibitors derived from purine scaffold with determined IC_{50} values from 0.05 nM to > 24.50 µM (13-16, 19-23, 25-27)
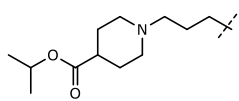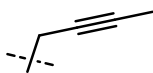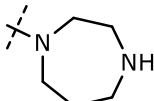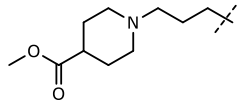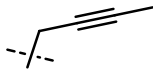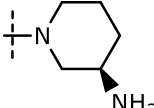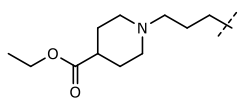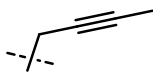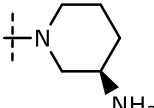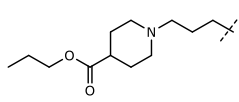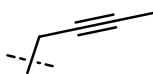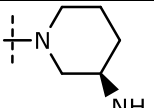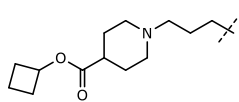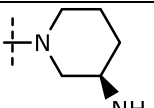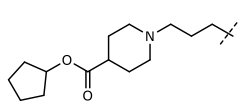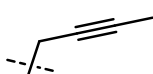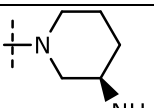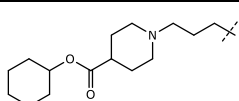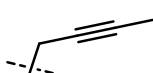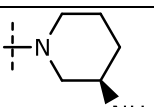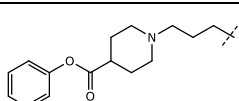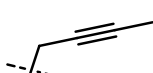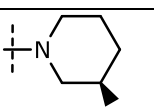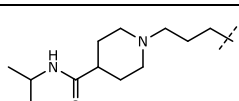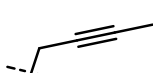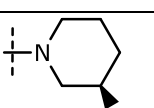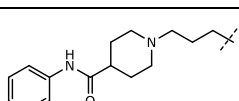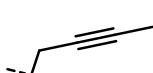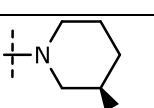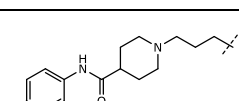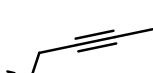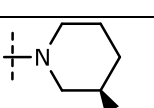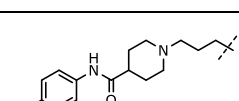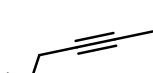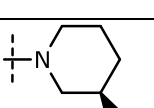
Compound	R_1	R_3	R_7	R_8
1	H	CH_3	3-methyl-2-butenyl	piperazin-1-yl (N–NH)
2	H	CH_3	benzyl	piperazin-1-yl (N–NH)
3	CH_3	CH_3	2-butynyl	3-amino-piperidin-1-yl (NH_2, N)
4	CH_3	CH_3	2-butynyl	piperazin-1-yl (N–NH)
5	CH_3	CH_3	3-methyl-2-butenyl	O–CH₂–CH(CH₃)–NH_2
6	CH_3	CH_3	3-methyl-2-butenyl	O–CH₂–C(CH₃)₂–CH₂–NH_2
7	CH_3	CH_3	3-methyl-2-butenyl	pyrrolidin-3-yloxy (N–H)
8	CH_3	CH_3	3-methyl-2-butenyl	(pyrrolidin-2-yl)methoxy (HN)
9	CH_3	CH_3	3-methyl-2-butenyl	O–CH₂–CH(NH_2)–CH₂–phenyl
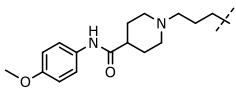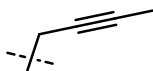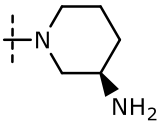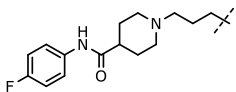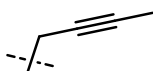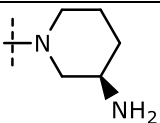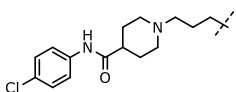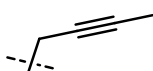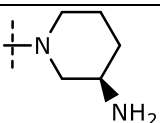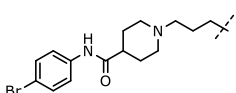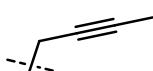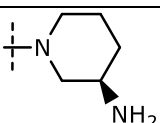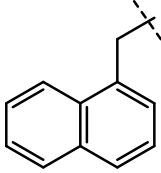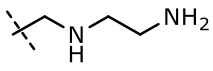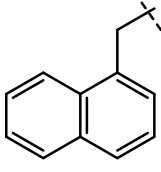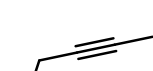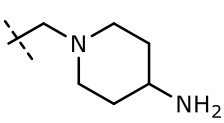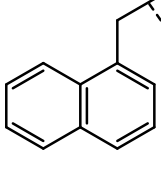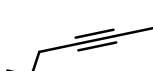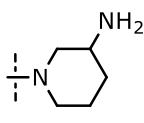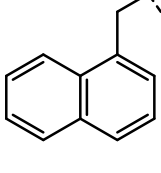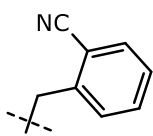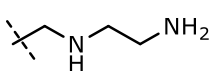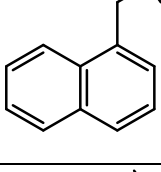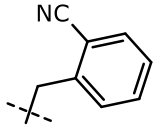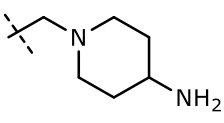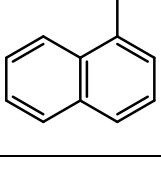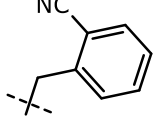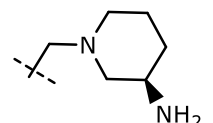
10	CH₃	CH₃		
11	CH₃	CH₃		
12	CH₃	CH₃		
13	CH₃	CH₃		
14	CH₃	CH₃		
15	CH₃	CH₃		
16	CH₃	CH₃		
17	CH₃	CH₃		
18	CH₃	CH₃		
19	CH₃	CH₃		
20		CH₃		

21		CH₃		
22		CH₃		
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49		CH₃		
50		CH₃		
51		CH₃		

52		CH$_3$		
53		CH$_3$		
54		CH$_3$		
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57		CH$_3$		
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59		CH$_3$		
60		CH$_3$		
61		CH$_3$		
62		CH$_3$		
63		CH$_3$		

64		CH₃		
65		CH₃		
66		CH₃		
67		CH₃		
68		CH₃		
69		CH₃		
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74		CH₃		
75		CH₃		
76		CH₃		
77		CH₃		
78		CH₃		
79		CH₃		
80		CH₃		
81		CH₃		
82		CH₃		

83	quinazoline structure	CH₃	pentynyl structure	piperazine (NH) structure
84	quinazoline structure	CH₃	pentynyl structure	1,4-diazepane (NH) structure
85	quinazoline structure	CH₃	2-cyanobenzyl structure	ethylenediamine structure
86	quinazoline structure	CH₃	2-cyanobenzyl structure	3-aminopiperidine structure
87	quinazoline structure	CH₃	2-cyanobenzyl structure	1,4-diazepane (NH) structure
88	benzothiazole structure	CH₃	pentynyl structure	3-aminopiperidine structure
89	fluoro-benzothiazole structure	CH₃	pentynyl structure	3-aminopiperidine structure
90	2-cyano-4-fluorobenzyl structure	2-cyano-4-fluorobenzyl structure	pentynyl structure	3-aminopiperidine structure
91	2-cyanopyridinyl structure	2-cyanopyridinyl structure	pentynyl structure	3-aminopiperidine structure
92	CH₂CH₂CH₃	CH₂CH₂CH₃	H	pyrazole-(4-methylphenyl)propynyl structure
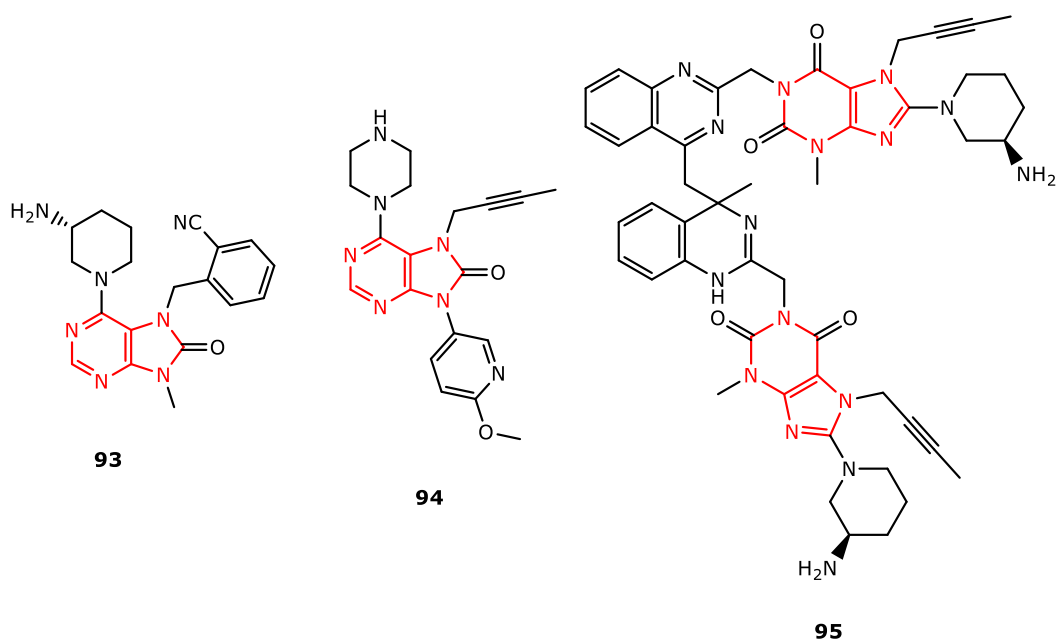
Figure 3. DPP-4 inhibitors with purine scaffold structurally little different from the above listed representatives with reported IC$_{50}$ values of 10 nM, 89 nM and 6 pM, respectively (21, 24, 28)

Conclusion

Besides XO activity, the availability of its substrates, affected by modulation of ADA activity, determine production of uric acid, and potential multitarget hypouricemic agents might offer a beneficial therapeutic approach. Based on evidenced linagliptin inhibitory potential on DPP-4 and XO, the possibility of DPP-4 inhibitors with the purine-based scaffold to exert hypouricemic effect via the same dual mechanism like linagliptin exists. Although structures possess the same scaffold, position and type of substituents affect and determine the structure-activity relationship, and inhibitory potential of other purine-based DPP-4 inhibitors on XO remains to be experimentally assayed.

References

1. Borghi C, Rosei EA, Bardin T, Dawson J, Dominiczak A, Kielstein JT, et al. Serum uric acid and the risk of cardiovascular and renal disease. J Hypertens 2015; 33(9):1729-41. [CrossRef] [PubMed]
2. Borghi C, Rodriguez-Artalejo F, De Backer G, Dallongeville J, Medina J, Nuevo J, et al. Serum uric acid levels are associated with cardiovascular risk score: A post hoc analysis of the EURIKA study. Int J Cardiol 2018; 253:167-73. [CrossRef] [PubMed]
3. Sharaf El Din UAA, Salem MM, Abdulazim DO. Uric acid in the pathogenesis of metabolic, renal, and cardiovascular diseases: A review. J Adv Res 2017; 8(5):537-48. [CrossRef] [PubMed]
4. Filiopoulos V, Hadjiyannakos D, Vlassopoulos D. New insights into uric acid effects on the progression and prognosis of chronic kidney disease. Ren Fail 2012; 34(4):510-20. [CrossRef] [PubMed]
5. Zoppini G, Targher G, Chonchol M, Ortalda V, Abaterusso C, Pichiri I, et al. Serum uric acid levels and

incident chronic kidney disease in patients with type 2 diabetes and preserved kidney function. Diabetes Care 2012; 35(1):99-104. [CrossRef] [PubMed]

6. Hahn K, Kanbay M, Lanaspa MA, Johnson RJ, Ejaz AA. Serum uric acid and acute kidney injury: a mini review. J Adv Res 2017; 8(5):529-36. [CrossRef] [PubMed]

7. Kutzing MK, Firestein BL. Altered uric acid levels and disease states. J Pharmacol Exp Ther 2008; 324(1):1-7. [CrossRef] [PubMed]

8. Xia Y, Zweier JL. Substrate control of free radical generation from xanthine oxidase in the postischemic heart. J Biol Chem 1995; 270(32):18797-803. [CrossRef] [PubMed]

9. Xia Y, Khatchikian G, Zweier JL. Adenosine deaminase inhibition prevents free radical-mediated injury in the postischemic heart. J Biol Chem 1996; 271 (17):1 0096-102. [CrossRef] [PubMed]

10. Abbott CA, McCaughan GW, Levy MT, Church WB, Gorrell MD. Binding to human dipeptidyl peptidase IV by adenosine deaminase and antibodies that inhibit ligand binding involves overlapping, discontinuous sites on a predicted β propeller domain. Eur J Biochem 1999; 266(3):798-810. [CrossRef] [PubMed]

11. Weihofen WA, Liu J, Reutter W, Saenger W, Fan H. Crystal structure of CD26/dipeptidyl-peptidase IV in complex with adenosine deaminase reveals a highly amphiphilic interface. J Biol Chem 2004; 279 (41): 43330-5. [CrossRef] [PubMed]

12. Lee TM, Chen WT, Yang CC, Lin SZ, Chang NC. Sitagliptin attenuates sympathetic innervation via modulating reactive oxygen species and interstitial adenosine in infarcted rat hearts. J Cell Mol Med 2015; 19 (2):418-29. [CrossRef] [PubMed]

13. Ikuma Y, Hochigai H, Kimura H, Nunami N, Kobayashi T, Uchiyama K, et al. Discovery of 3*H*-imidazo[4,5-c]quinolin-4(5*H*)-ones as potent and selective dipeptidyl peptidase IV (DPP-4) inhibitors. Bioorg Med Chem 2012; 20(19):5864-83. [CrossRef] [PubMed]

14. Eckhardt M, Langkopf E, Mark M, Tadayyon M, Thomas L, Nar H, et al. 8-(3-(*R*)-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione (BI 1356), a highly potent, selective, long-acting, and orally bio-available DPP-4 inhibitor for the treatment of type 2 diabetes. J Med Chem 2007; 50(26):6450-3. [CrossRef] [PubMed]

15. Lin K, Cai Z, Wang F, Zhang W, Zhou W. Synthesis and biological evaluation of xanthine derivatives on dipeptidyl peptidase 4. Chem Pharm Bull 2013; 61 (4):477-82. [CrossRef] [PubMed]

16. Lai ZW, Li C, Liu J, Kong L, Wen X, Sun H. Discovery of highly potent DPP-4 inhibitors by hybrid compound design based on linagliptin and alogliptin. Eur J Med Chem 2014; 83:547-60. [CrossRef] [PubMed]

17. Kröller-Schön S, Knorr M, Hausding M, Oelze M, Schuff A, Schell R, et al. Glucose-independent impro-

vement of vascular dysfunction in experimental sepsis by dipeptidyl-peptidase 4 inhibition. Cardiovasc Res 2012; 96(1):140-9. [CrossRef] [PubMed]

18. Yamagishi SI, Ishibashi Y, Ojima A, Sugiura T, Matsui T. Linagliptin, a xanthine-based dipeptidyl peptidase-4 inhibitor, decreases serum uric acid levels in type 2 diabetic patients partly by suppressing xanthine oxidase activity. Int J Cardiol 2014; 176(2):550-2. [CrossRef] [PubMed]

19. Czechtizky W, Dedio J, Desai B, Dixon K, Farrant E, Feng Q, et al. Integrated synthesis and testing of substituted xanthine based DPP4 inhibitors: application to drug discovery. ACS Med Chem Lett 2013; 4(8):768-72. [CrossRef] [PubMed]

20. Zhang Z, Wallace MB, Feng J, Stafford JA, Skene RJ, Shi L, et al. Design and synthesis of pyrimidinone and pyrimidinedione inhibitors of dipeptidyl peptidase IV. J Med Chem 2011; 54(2):510-24. [CrossRef] [PubMed]

21. Costante R, Stefanucci A, Carradori S, Novellino E, Mollica A. DPP-4 inhibitors: a patent review (2012–2014). Expert Opin Ther Pat 2015; 25(2):209-36. [CrossRef] [PubMed]

22. Mo DW, Dong S, Sun H, Chen JS, Pang JX, Xi BM, et al. Synthesis and potent inhibitory activities of carboxybenzyl-substituted 8-(3-(*R*)-aminopiperidin-1-yl)-7-(2-chloro/cyanobenzyl)-3-methyl-3,7-dihydro-purine-2,6-diones as dipeptidyl peptidase IV (DPP-IV) inhibitors. Bioorg Med Chem Lett 2015; 25(9):1872-5. [CrossRef] [PubMed]

23. Chyan YJ, Chuang LM. Dipeptidyl peptidase-IV inhibitors: an evolving treatment for type 2 diabetes from the incretin concept. Recent Pat Endocr Metab Immune Drug Discov 2007; 1(1):15-24. [CrossRef]

24. Gupta R, Walunj SS, Tokala RK, Parsa KVL, Singh SK, Pal M. Emerging drug candidates of dipeptidyl peptidase IV (DPP IV) inhibitor class for the treatment of type 2 diabetes. Curr Drug Targets 2009; 10(1):71-87. [CrossRef] [PubMed]

25. Augustyns K, Van der Veken P, Senten K, Haemers A. The therapeutic potential of inhibitors of dipeptidyl peptidase IV (DPP IV) and related proline-specific dipeptidyl aminopeptidases. Curr Med Chem 2005; 12 (8):971-98. [CrossRef] [PubMed]

26. Li G, Huan Y, Yuan B, Wang J, Jiang Q, Lin Z, et al. Discovery of novel xanthine compounds targeting DPP-IV and GPR119 as anti-diabetic agents. Eur J Med Chem 2016; 124:103-16. [CrossRef] [PubMed]

27. Eckhardt M, Hauel N, Himmelsbach F, Langkopf E, Nar H, Mark M, et al. 3,5-Dihydro-imidazo [4, 5-d] pyridazin-4-ones: a class of potent DPP-4 inhibitors. Bioorg Med Chem Lett 2008; 18(11):3158-62. [CrossRef] [PubMed]

28. Shu C, Ge H, Song M, Chen JH, Zhou H, Qi Q, et al. Discovery of imigliptin, a novel selective DPP-4 inhibitor for the treatment of type 2 diabetes. ACS Med Chem Lett 2014; 5(8):921-6. [CrossRef] [PubMed]

POTENCIJALNO DUALNI MEHANIZAM HIPOURIKEMIJSKE AKTIVNOSTI DPP-4 INHIBITORA PURINSKE STRUKTURE

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Dipeptidil peptidaza-4 vezuje adenozin deaminazu, pri čemu nastaje kompleks koji katalizuje ireverzibilnu deaminaciju ekstracelularnog adenozina u inozin, što vodi generisanju hipoksantina i ksantina do mokraćne kiseline ksantin-oksidazom u katabolizmu purina uz produkciju reaktivnih kiseoničnih vrsta. Inhibitor dipeptidil peptidaze-4 ksantinske strukturne osnove, linagliptin, pokazao je inhibitorni potencijal na ksantin-oksidazi. Linagliptin pokazuje hipourikemijski efekat inhibiranjem aktivnosti dipeptidil peptidaze-4 i formiranja kompleksa ove proteaze i adenozin deaminaze, što uzrokuje porast sadržaja adenozina i smanjenu raspoloživosti supstrata ksantin-oksidaze, kao i inhibiranjem aktivnosti ksantin-oksidaze. Usled dokaza o dualnom mehanizmu hipourikemijske aktivnosti linagliptina, postoji mogućnost da drugi inhibitori dipeptidil peptidaze-4 purinske strukture pokazuju istu aktivnost.

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Ključne reči: *dipeptidil peptidaza-4, ksantin-oksidaza, adenozin deaminaza*

DISORDERS OF COAGULATION STATUS AND HAEMOSTASIS AS PROGNOSTIC PARAMETERS OF IMMEDIATE AND EARLY RESULTS AFTER SURGICAL MYOCARDIAL REVASCULARISATION

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Surgical myocardial revascularization is one of the most commonly performed surgical procedures in the world. Over time, with the development of technology and modern diagnostic procedures, as well as the advancement of surgical techniques, the mortality rate for elective uncomplicated cases has fallen to below 2%. Nevertheless, despite the exceptional development of the surgical techiniques, the rate of postoperative complications, that can compromise the patients, is over 10%. The aim of this study was to define a group of patients with an increased risk of postoperative complications depending on the disorders of coagulation status and haemostasis.

Twenty eight patients who underwent surgical revascularization of the myocardium were included in this prospective, non-randomized study. The study was conducted at the Clinic for Cardiac Surgery, Clinical Center Nis, from January to April 2017. Preoperatively as well as 3 hours, 24 hours, 48 hours, 3 days, and 5 days postoperatively, the following parameters were determined: blood count, inflammation parameters (C reactive protein, presepsin); coagulation status (prothrombin time (PT), International Normalized Ratio (INR), activated partial thromboplastin time (APTT), fibrinogen, anti-thrombin III, D dimer).

The only preoperative independent prognostic parameter for increased postoperative drainage was INR. Activated clothing time (ACT) was an independent postoperative prognostic parameter of increased postoperative drainage, probably due to delayed or prolonged heparin activity. Inflammation parameters showed no association with the onset of postoperative complications. In relation to patients without bleeding, in patients with bleeding, significantly higher values of urea and the difference in APTT values, preoperatively and at the end of the monitoring period, were detected. Multivariate logistic regression analysis, confirmed the difference in APTT values preoperatively and at the end of the monitoring period, as the only factor, significantly associated with the risk of bleeding. Multivariate linear regression analysis, confirmed the value of the urea, as the only factor significantly associated with the change in total allogeneic transfusion value. Increase in urea levels is associated with an increase in the total amount of allogeneic transfusion. Correlation analysis showed that the increased number of days in the intensive care was significantly associated with female gender, number of grafts, prolonged ECC, clamping time, hematocrit (HCT), PT, INR preoperatively and at the end of the follow-up period.

Surgical myocardial revascularization is a safe method with a minimal morbidity rate. Using of modern methods for the preoperative monitoring of haemostasis may significantly reduce the risk of postoperative bleeding and the need for transfusion of red blood cells and other blood derivatives.

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Key words: *cardiosurgery, coagulation status, inflammation parameters, risk factors*

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Introduction

Cardiovascular (CV) diseases are leading cause of death in most developed countries and in many undeveloped countries. Epidemiological data indicate that CV diseases cause almost half of total mortality, while malignant tumors and lung diseases are represented by 22% and 10%, respectively. Every year, over 17 million people die from cardiovascular dise-

ases in the world, which is a third of the population dying (1, 2).

Cardiovascular diseases include: ischemic heart disease (stable angina pectoris, acute coronary syndrome, sudden cardiac death, cardiac insufficiency), acute stroke (ischemic stroke and haemorrhagic stroke), and peripheral arterial occlusive disease.

According to World Health Organization (WHO) estimation, the dying structure from cardiovascular diseases is as follows: ischemic heart disease is on average 41%, stroke with 32% and other heart disease with 27% (3). In our country the situation is significantly different: slightly more than half of those who died of CV disease had a diagnosis of one of the heart diseases, while the participation of the stroke was 29% (4).

Coronary artery disease (coronary or ischemic heart disease) is the most common cause of death, leading to death, disability and economic losses more than any other illness, despite a significant reduction in mortality over the past three decades. The frequency of may be been seen from the results of large studies, according to which, the risk of the onset of coronary artery disease during life, for a man at the age of 40, is almost 40% (3).

The role of the surgeon in the process of CV disease treatment, finds its significant place from the moment when it came to the knowledge that obstructive atherosclerotic lesions on the coronary arteries are directly responsible for the onset of coronary disease. Aortocoronary bypass is a procedure that is carried out at the heart in order to overcome narrowing of blood vessels that nourish the heart. The results of the conducted research show that in the United States alone, 1 500 000 patients suffer from coronary heart disease annually, while surgical myocardial revascularization is performed in almost every fourth patient. A similar trend was observed in the countries of Western Europe (1,000 revascularization per million inhabitants), while in our country this number is significantly lower (600 revascularization per million inhabitants) (5).

Although surgical revascularization of the myocardium is now the most commonly performed surgical procedure in general, with mortality of about 1% in elective cases, there is still a high risk of emergency surgery in patients with acute coronary syndrome (6). Previous studies suggest that surgical revascularization of the myocardium has been a method of choice for the treatment of most patients with coronary heart disease for more than 50 years. The results of surgical treatment are superior in comparison with empirical medication therapy, in almost all investigated subgroups of patients. After performing this surgical procedure, a longer survival, longer period without new myocardial infarction, and significant improvement in the quality of life is achieved, while the incidence of the new chest pain onset is lower and the working capacity of the majority of operatives is excellent. It is therefore important to note that there are many benefits of early surgical revascularization, including limiting of infarction expansion, avoiding left ventricular dysfunction and cardiac insufficiency. The underlying risk lies in the

ischemic-reperfusion myocardial damage, which can lead to the appearance of a hemorrhagic infarction, with all of its complications (5, 7, 8).

Material and methods

The aim of this study was to examine the correlation between mortality, bleeding and the need for transfusion, the length of hospitalization and the presence of postoperative complications (respiratory, renal and hepatic) in relation to parameters of coagulation and fibrinolysis activation, platelet function disorders and inflammatory parameters, in patients who underwent surgical myocardial revascularization.

According to the statistical tests and calculations in the G-Power software package, this study included 28 patients who underwent surgical myocardial revascularization at the Clinic for Cardiovascular with Transplantation Surgery KC Niš, in the period from January to April 2017, which is reduced the probability of α error to a level of significance r < 0.05 with a defined power of study of 80%.

Preoperatively as well as 3 hours, 24 hours, 48 hours, 3 days, and 5 days postoperatively, the following parameters were determined:

1. blood count (erythrocyte count, hemoglobin, hematocrit, leukocyte count, platelet count);

2. Inflammation parameters (C reactive protein, presepsin);

3. coagulation status (prothrombin time, International Normalized Ratio, activated partial thromboplastin time, fibrinogen, anti-thrombin III, D dimer);

4. parameters of thrombocyte function (platelet activation adenosine di-phosphate (ADP HS), platelet activation with arachidonic acid (ASPI));

5. Rotational thrombelastometry parameters (direct activation of the thrombin peptide receptor (TRAP), internal and external coagulation pathway (clotting time, maximal clotting firmness, clot amplitude after 10 minutes, alpha angle, maximum lysis, functional fibrinogen).

The correlation of the above parameters with comparison to the duration of the extracorporeal circulation and the duration of the aortic cross clamp is determined.

Statistical analysis

Quantitative statistical analysis was carried out on a computer. Excel program from the Microsoft Office 2010 software package was used for typing, ranking, grouping, table and graphical data presentation. The calculations were made using the SPSS program in version 18.0.

The following statistical parameters were displayed: arithmetic mean (AS), standard deviation (SD) and structure index (%).

Comparison of mean values of numerical features between groups of patients with and without bleeding was performed by Student's T test or Man-Whitney U test, in cases where the distribution of values did not meet the requirements of normal

schedule. Comparison of mean values of numerical features in the same patients between two measurements was done by the Paired-samples t test.

A comparison of the frequency of attributes between groups was performed by Mantel-Hensel's Quadratic Test (Mantel-Haenszel Chi square test) or Fisher exact test of the exact probability of a zero hypothesis (Fisher exact test) when one of the expected frequency of the mark was less than five. Changes in the value of the mark during the follow-up period were examined by Repeated measures ANOVA.

The study of the relationship between the amount of allogeneic transfusion, the number of days of total hospitalization, the number of days of hospitalization in intensive care, total drainage and all other investigated features was carried out by Spearman's rank correlation.

Determination of significant bleeding predictors was performed by multivariate logistic regression analysis. The odds ratio (OR) and the 95% confidence interval (CI) were calculated. By using the Wald method step-by-step (Backward: Wald) from a mul-

tivariate model, all factors, the statistical significance of which, was not confirmed were excluded.

Multivariate linear regression analysis was used to determine factors significantly related to the values of allogeneic transfusion, the number of days of hospitalization overall, the number of days in intensive care and total drainage. The values of regression coefficients (B) and the limits of their 95% confidence interval were calculated. Using stepwise methods in final models, only those factors that are significantly related to dependent variables are retained. As a threshold of statistical significance in the conclusion, the estimation error was less than 5% ($p < 0.05$). The statistical analysis results were showed as tables and graphically.

Results

The study included 28 patients, 22 men (79%) and 6 women (21%), the average age of 64 years. The basic characteristics of the respondents are shown in Table 1.

Table 1. The basic characteristics of the respondents

Characteristic	Value
Age	64.14 ± 6.85
Gender	
Male	22 (78.6)
Female	6 (21.4)
Smoking	6 (21.4)
Diabetes	13 (46.4)
Regulation of diabetes	
Without regulation	1 (3.6)
Oral	6 (21.4)
Insulin	6 (21.4)
BMI	29.94 ± 4.4
Triglycerides	1.86 ± 0.85
Cholesterol	4.36 ± 2.12
Urea	6.38 ± 1.46
Kreatinin	95.67 ± 18.34
EF percentage	53.86 ± 10.7
Surgery type (number of grafts)	2.57 ± 0.79
Duration extra corporal ECC	102.36 ± 22.22
Time of clamping	45.79 ± 11.63
The amount of given	1158.93 ± 278.57
Surgery type (number of grafts)	
CABG I	1 (3.6)
CABG II	13 (46.4)
CABG III	12 (42.9)
CABG IV	1 (3.6)
CABG V	1 (3.6)
Defibrilation	26 (92.9)
Diuresis	1346.43 ± 569.26
ACT at admittance	138.96 ± 13.33
ACT afterAH	584.46 ± 104.5
ACT after AP	125.79 ± 12.19
Autotransfusion	569.11 ± 124.27
Hospitalization in intensive care unit	4.54 ± 1.29
Hospitalization in semi-intensive care unit	2.43 ± 1.4
Total hospitalization	6.96 ± 0.51
Allogenic transfusion total	583.33 ± 224.11
Drainage total	975.75 ± 387.97
Diuresis total	11577.14 ± 3638.94

NOTE: values are displayed as arithmetic mean ± SD or as number (percentage)

Table 2. Application of allogeneic transfusion, blood derivatives, voluven and crystalloids,
drainage and diuresis per follow-up days

	I day	II day	III day	IV day	V day	VI day	VII day	Total
Allogenic Transfusion	3 (933 ± 728.6)	2 (350.0 ± 0.0)	1 (700.0 ± 0.0)	4 (350.0 ± 0.0)	-	2 (350.0 ± 0.0)	2 (350.0 ± 0.0)	2 (583 ± 224)
Application of plasma	-	-	-	-	-	-	-	-
Application of cryo	-	-	-	-	-	-	-	-
Application of thrombocytes	4 (6.25 ± 2.87)	-	-	-	-	-	-	4 (6.25 ± 2.87)
Crystaloids	-	-	-	-	-	-	-	-
Drainage	28 (414.3 ± 207)	28 (416.9 ± 141.9)	18 (190.8 ± 140.6)	3 (203.3 ± 131.3)	-	-	-	28 (976 ± 388)
Diuresis	28 (2779 ± 764)	28 (3004 ± 1406)	28 (2795 ± 1263)	17 (2852 ± 1205)	9 (3105 ± 686)	2 (2850 ± 0.0)	1 (1800 ± 0.0)	28 (11577 ± 3639)

NOTE: values are displayed as number of patients (arithmetic mean ± SD)

Allogeneic transfusion was administered in 3 patients on the first postoperative day, and the average blood amount was 933.3 ml (Table 2). On the second day it was applied in two patients, 350 ml each, the third day in one patient, 700 ml, the fourth day in 4 patients, 350 ml each.

On the fifth postoperative day allogeneic transfusion was not applied to any patient. On the sixth and seventh day, allogeneic transfusion was administered in 2 patients, in 350 ml each. In total, allogeneic transfusion was received by 12 patients, and the approximate amount of blood was 583 ml.

The drainage was measured for the first two days in all 28 patients, and the average value was 414.3 ml during the first day and 416.9 ml during the second day. On the third day, the drainage was measured in 18 patients, on average 190.8 ml, and on the fourth day in 3 patients on average 203.3 ml. After the fourth day, the drainage was not measured in any patient.

The diuresis was measured for the first three days in all 28 patients, and the average value was 2779 ml, 3004 ml and 2795 ml, by day. On the fourth day, the diuresis was measured in 17 patients, on average 2852 ml, on the fifth day in 9 patients on average 3105 ml, the six in 2 patients at 2850 ml, and on the seventh day in one patient, 1800 ml.

Platelets were administered only on the first day, in 4 patients with an average of 6.25 units, plasma, cryoprecipitate, volume and crystalloids were not applied.

The analysis of variance for repeated measurements showed that changes in the values of most of the features during monitoring were statistically significant (Table 3).

In almost all features, difference between preoperative values, values 2 hours after surgery and at the end of the follow-up period, was significant. In the period between 2 hours after the operation and at the end of the follow up period, the values of WBC, RBC, HGB and ACT decreased significantly, while the HCT and PLT values increased significantly. Compared to preoperative values, the values of MPV and INTEM CT were significantly lower than the preoperative values, until the values of CRP, P SEP, INR, fibrinogen, D dimer, ADP HS, ASPI, TRAP, EXTEM CT, EXTEM MCF , EXTEM A10, EXTEM ALPHA ANGLE, INTEM MCF, INTEM A10, INTEM ALPHA ANGLE, FIBTEM CT, FIBTEM MCF, FIBTEM A10, FIBTEM ALPHA ANGLE and troponin, were singnificantly higher.

Comparison of values in patients with and without bleeding, within the examined groups is shown in Table 4.

Diabetes was significantly more commonly reported in patients without bleeding (Table 4). In patients without bleeding., significantly higher BMI, triglycerides, cholesterol, ACT before surgery, PLT count preoperatively and INTEM ALPHA ANGLE were seen preoperativly, compare to patients with bleeding. In patients with bleeding, significantly higher values of the urea and difference in APTT preoperatively and at the end of the follow-up period, were detected compared to patients without bleeding. The values of all other features did not differ significantly in patients with and without bleeding.

Table 3. Values of the blood count, inflammation parameters, coagulation status, platlets, rotational thrombelastometry, internal and external coagulation pathway

Parameter	Preoperatively	2 hours after Surgery	24 hours after surgery	48 hours after surgery	72 hours after surgery	5 days after surgery	7 days after surgery	Effect time
WBC	7.2 ± 1.6	9.6 ± 4.1	9.2 ± 3.0	8.8 ± 2.6	7.6 ± 1.9	6.5 ± 2.9	7.1 ± 2.7	**<0.001**
RBC	4.6 ± 0.4	4.2 ± 0.5	4.1 ± 0.5	3.6 ± 0.4	3.6 ± 0.4	3.7 ± 0.4	3.8 ± 0.6	**<0.001**
HGB	140.3 ± 10.1	121.0 ± 10.7	115.7 ± 10.0	103.5 ± 10.5	103.9 ± 10.6	105.1 ± 11.0	103.5 ± 22.4	**<0.001**
HCT	39.4 ± 8.2	34.7 ± 6.9	34.1 ± 3.6	30.6 ± 3.5	30.9 ± 3.1	41.0 ± 50.5	35.3 ± 14.0	0.272
PLT	241.6 ± 70.2	175.0 ± 50.0	191.9 ± 42.1	160.7 ± 50.5	172.2 ± 45.6	236.6 ± 59.1	271.1 ± 97.1	**<0.001**
MPV	8.7 ± 0.8	7.8 ± 0.7	8.2 ± 0.6	11.2 ± 15.1	8.3 ± 0.7	7.7 ± 0.7	7.7 ± 0.7	0.209
CRP	3.7 ± 3.9	4.6 ± 4.2	79.6 ± 39.8	188.9 ± 61.9	192.9 ± 79.3	125.6 ± 73.4	106.1 ± 134.2	**<0.001**
P SEP	177.6 ± 59	413.4 ± 196.2	461.6 ± 207.6	443.9 ± 254.7	496.1 ± 353.3	469.4 ± 284.2	504.1 ± 296.9	**<0.001**
PT	11.4 ± 1.3	18.8 ± 30.8	12.6 ± 1.6	12.2 ± 1.4	11.3 ± 1.5	16.3 ± 23.3	12.8 ± 4.1	0.444
INR	1.1 ± 0.1	1.8 ± 2.4	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.4	0.228
APTT	26.4 ± 3.3	30.1 ± 3.3	38.1 ± 52.0	30.4 ± 3.2	30.9 ± 5.9	27.2 ± 3.9	27.5 ± 4.0	0.364
Fibrinogen	4.1 ± 2.0	3.7 ± 1.5	5.1 ± 0.9	7.7 ± 1.8	18.9 ± 24.0	11.2 ± 10.8	9.2 ± 2.2	**0.005**
AT III	100.6 ± 14.1	77.4 ± 14.2	75.0± 13.6	75.5 ± 7.4	77.5 ± 11.6	89.3 ± 15.7	96.2 ± 17.0	**<0.001**
D dimer	282 ± 166.9	431.2 ± 852.6	279.9 ± 241.6	426.5 ± 856.7	447.3 ± 676.3	788.8 ± 982.3	1450.9 ± 1376.9	**<0.001**
ADP HS	435.4 ± 179.5	424.0 ± 199.0	629.3 ± 148.4	582.7 ± 125.2	605.1 ± 168.6	645.4 ± 169.3	804.1 ± 308.2	**<0.001**
ASPI	579.5 ± 238.6	958.4 ± 351.0	1055.9 ± 334.3	793.8 ± 209.2	775.1 ± 239.2	667.8 ± 287.1	918.2 ± 383.3	**<0.001**
TRAP	1021.7 ± 211.9	1142.8 ± 201.7	1123.6 ± 192.5	1033.1 ± 201.4	1074.6 ± 254.2	1131.3 ± 288.5	1230.5 ± 358.9	**0.022**
EXTEM CT	62.4 ± 6.1	70.5 ± 15.2	61.1 ± 18.7	61.4 ± 7.0	67.2 ± 16.0	66.6 ± 9.6	65.6 ± 11.1	0.074
EXTEM MCF	63.7 ± 4.5	57.2 ± 5.2	60.3 ± 5.8	65.3 ± 4.3	66.5 ± 3.7	70.5 ± 4.3	70.6 ± 3.5	**<0.001**
EXTEM A10	60.0 ± 4.6	51.4 ± 5.1	58.9 ± 4.8	60.7 ± 4.8	61.8 ± 3.7	66.4 ± 4.1	67.1 ± 4.2	**<0.001**
EXTEM ALPHA ANGLE	77.7 ± 2.2	72.3 ± 3.7	76.8 ± 2.3	78.3 ± 2.1	73.0 ± 18.5	79.0 ± 4.4	80.1 ± 2.3	**0.048**
INTEM CT	200.3 ± 44.7	216.1 ± 57.1	172.7 ± 38.0	191.7 ± 43.9	197.4 ± 68.1	176.6 ± 33.4	162.3 ± 33.6	**0.001**
INTEM MCF	59.0 ± 4.5	54.4 ± 5.4	58.9 ± 4.3	61.3 ± 5.2	63.2 ± 5.7	68.2 ± 4.5	68.9 ± 4.2	**<0.001**
INTEM A10	57.8 ± 4.6	50.5 ± 5.2	57.9 ± 3.8	59.5 ± 4.2	59.5 ± 10.2	65.8 ± 4.0	66.4 ± 4.7	**<0.001**
INTEM ALPHA ANGLE	74.5 ± 5.2	71.4 ±5.1	76.0 ± 4.5	76.8 ± 3.5	76.6 ± 5.3	79.3 ± 1.8	79.5 ± 3.1	**<0.001**
FIBTEM CT	54.3 ± 12.2	60.0 ± 6.0	53.6 ± 6.0	55.8 ± 18.7	57.8 ± 7.1	71.0 ± 38.2	61.7 ± 9.4	**0.049**
FIBTEM MCF	21.0 ± 5.1	14.6 ± 3.8	22.2 ± 4.4	29.4 ± 4.5	30.1 ± 3.7	31.3 ± 6.8	32.5 ± 6.1	**<0.001**
FIBTEM A10	20.8 ± 5.0	14.0 ± 3.8	21.6 ± 4.3	28.8 ± 4.6	28.9 ± 3.4	30.3 ± 6.4	31.4 ± 5.9	**<0.001**
FIBTEM ALPHA ANGLE	76.0 ± 5.6	69.8 ± 11.9	77.2 ± 3.4	76.9 ± 5.1	78.6 ± 3.4	79.6 ± 2.9	80.4 ± 2.0	**<0.001**
Troponini	0.1 ± 0.2	3.4 ± 2.4	5.8 ± 6.1	3.9 ± 4.8	2.3 ± 2.8	1.2 ± 1.7	0.7 ± 1.0	**<0.001**
CK MB	21.7 ± 9.4	54.0 ± 19.9	54.5 ± 37.8	43.4 ± 40.1	34.4 ± 28.6	26.2 ± 27.9	22.7 ± 24.6	**<0.001**
ACT	-	130.0 ± 15.0	128.0 ±9.2	127.7 ± 7.3	127.1 ± 8.1	125.2 ± 8.3	125.9 ± 7.8	**0.044**

NOTE: values are displayed as arithmetic mean ± SD

Table 4. Comparison of values in patients with and without bleeding

Parameter	Without bleeding (n=16)	With bleeding (n=12)	p
Age	63.25 ± 6.71	65.33 ± 7.14	0.441
Gender			
Male	13 (81.3%)	9 (75%)	0.690
Female	3 (18.8%)	3 (25%)	
Smoking	3 (18.8%)	3 (25%)	0.690
Diabetes	10 (62.5%)	3 (25%)	**0.049**
Regulation of DM			
Without regulation	0 (0%)	1 (8.3%)	0.053
Oral	5 (31.3%)	1 (8.3%)	
Insulin	5 (31.3%)	1 (8.3%)	
BMI	31.43 ± 4.44	27.97 ± 3.64	**0.032**
Trigliceridi	2.18 ± 0.89	1.44 ± 0.57	**0.012**
Cholesterol	5.16 ± 1.91	3.29 ± 1.96	**0.019**
Urea	5.75 ± 1.14	7.21 ± 1.45	**0.009**
Creatinine	91.85 ± 15.64	100.76 ± 21.05	0.232
EF percents	54.56 ± 12.91	52.92 ± 7.22	0.672
Type of surgery (number of grafts)	2.56 ± 0.51	2.58 ± 1.08	0.952
Duration of EKK	100.75 ± 15.62	104.5 ± 29.51	0.694
Clamping time	43.56 ± 8.66	48.75 ± 14.59	0.289
The amount of given cardioplegia	1196.88 ± 315.95	1108.33 ± 222.42	0.392
Defibrilation	14 (87.5%)	12 (100%)	0.204
Diuresis	1400 ± 476.1	1275 ±690.36	0.597
ACT at admittance	144.44 ± 11.84	131.67 ± 11.97	**0.010**

ACT after AH	599.56 ± 95.68	564.33 ± 116.39	0.403
ACT after AP	125.63 ± 14.8	126 ± 8.14	0.933
Autotransfusion	578.44 ± 121.2	556.67 ± 132.59	0.660
Hospitalization in intensive unit	4.5 ± 1.32	4.58 ± 1.31	0.869
Hospitalization in semi- intensive unit	2.44 ± 1.46	2.42 ± 1.38	0.970
Hospitalization total	6.94 ± 0.57	7 ± 0.43	0.744
WBC preoperatively	7.3 ± 1.39	7.11 ± 1.94	0.775
RBC preoperatively	4.58 ± 0.39	4.61 ± 0.36	0.841
HGB preoperatively	141.75 ± 8.92	138.42 ± 11.62	0.418
HCT preoperatively	38.59 ± 10.49	40.48 ± 3.62	0.512
PLT preoperatively	269.31 ± 74.43	204.67 ± 43.9	**0.008**
MPV preoperatively	8.61 ± 0.71	8.89 ± 0.82	0.357
CRP preoperatively	4.38 ± 3.51	2.87 ± 4.34	0.334
P SEP preoperatively	172.07 ± 65.86	184.92 ± 50.38	0.564
PT preoperatively	11.46 ± 1.22	11.38 ± 1.49	0.892
INR preoperatively	1.07 ± 0.1	1.07 ± 0.13	0.903
APTT preoperatively	27.23 ± 3.06	25.25 ± 3.31	0.120
Fibrinogen preoperatively	3.99 ± 2.35	4.23 ± 1.32	0.739
AT III preoperatively	99.18 ± 11.49	102.41 ± 17.36	0.583
D dimer preoperatively	244.38 ± 122.48	332.17 ± 207.52	0.210
ADP HS preoperatively	449.75 ± 199.9	416.33 ± 154.6	0.622
ASPI preoperatively	587.19 ± 220.69	569.25 ± 270.47	0.853
TRAP preoperatively	962.38 ± 216.98	1100.83 ± 184.52	0.080
EXTEM CT preoperatively	62.81 ± 5.55	61.83 ± 6.95	0.692
EXTEM MCF preoperatively	64.25 ± 5.27	63 ± 3.22	0.446
EXTEM A10 preoperatively	60.94 ± 5.35	58.83 ± 3.16	0.205
EXTEM ALPHA ANGLE preoperatively	78.25 ± 2.02	76.92 ± 2.28	0.121
INTEM CT preoperatively	187.25 ± 39.77	217.75 ± 46.45	0.081
INTEM MCF preoperatively	59.88 ± 4.52	57.75 ± 4.31	0.218
INTEM A10 preoperatively	58.75 ± 4.7	56.42 ± 4.19	0.178
INTEM ALPHA ANGLE preoperatively	76.44 ± 2.78	71.92 ± 6.59	**0.042**
FIBTEM CT preoperatively	57.56 ± 5.53	50 ± 16.94	0.161
FIBTEM MCF preoperatively	22.38 ± 5.57	19.25 ± 3.77	0.089
FIBTEM A10 preoperatively	21.81 ± 5.62	19.33 ± 3.7	0.172
FIBTEM ALPHA ANGLE preoperatively	77.31 ± 5.4	74.33 ± 5.69	0.175
Troponines preoperatively	0.01 ± 0.03	0.11 ± 0.33	0.350
CK MB preoperatively	20.06 ± 5.23	23.87 ± 13.14	0.359
Drainage total	875.06 ± 313.4	1110 ± 448.46	0.137
Diuresis total	11718.13 ± 4002.87	11389.17 ± 3253.59	0.812
WBC diffrence preoperatively and 2 h after surgery	2.07 ± 3.31	2.9 ± 4.36	0.587
RBC difference preoperatively and 2 h after surgery	-0.35 ± 0.47	-0.39 ± 0.39	0.819
HGB difference preoperatively and 2 h after surgery	-19.69 ± 13.39	-18.75 ± 10.64	0.838
HCT difference preoperatively and 2h after surgery	-2.41 ± 10.84	-7.65 ± 8.99	0.175
PLT difference preoperatively and 2h after surgery	-75.25 ± 44.28	-55.08 ± 42.29	0.233
WBC difference 2h after surgery and at the end of follow-up	-2.93 ± 3.84	-2.02 ± 5.36	0.624
RBC difference 2h after surgery and at the end of follow-up	-0.33 ± 0.92	-0.58 ± 0.52	0.371
HGB difference 2h after surgery and at the end of a follow-up	-13.44 ± 16.65	-14.67 ± 15.16	0.840

HCT difference 2h after surgery and at the end of follow-up	2.14 ± 18.35	-1.47 ± 10.49	0.518
PLT difference 2h after surgery and at the end of follow-up	105.26 ± 119.96	83.83 ± 32.21	0.504
MPV difference preoperatively and at the end of follow-up	-1.14 ± 0.71	-0.93 ± 0.95	0.511
CRP difference preoperatively and at the end of follow up	113.78 ± 168.61	87.08 ± 68.8	0.573
P SEP difference preoperatively and at the end of follow-up	299.62 ± 289.07	362.42 ± 307.98	0.589
PT difference preoperatively and at the end of follow-up	0.51 ± 1.87	2.54 ± 5.31	0.226
INR difference preoperatively and at the end of follow-up	0.1 ±0 .14	0.26 ± 0.51	0.321
APTT difference preoperatively and at the end of follow-up	-0.56 ± 3.22	3.3 ±4.35	**0.018**
Fibrinogen difference preoperatively and at the end of follow-up	4.94 ± 3.26	5.27 ± 2.57	0.762
AT III difference preoperatively and at the end of follow-up	-1.01 ± 23.4	-8.83 ± 22.11	0.375
D dimer difference preoperatively and at the end of follow-up	843.38 ± 1076.16	1602.92 ± 1658.62	0.184
ADP HS difference preoperatively and at the end of follow-up	403.88 ± 349.86	321.67 ± 356.48	0.549
ASPI difference preoperatively and at the end of follow-up	378.94 ± 394.24	285 ± 496.68	0.595
TRAP difference preoperatively and at the end of follow-up	256.31 ± 497.59	145.42 ± 338.83	0.490
EXTEM CT difference preoperatively and at the end of follow-up	1.63 ± 12.96	5.33 ± 15.65	0.512
EXTEM MCF difference preoperatively and at the end of follow-up	6.5 ± 4.82	7.5 ± 5.16	0.607
EXTEM A10 difference preoperatively and at the end of follow-up	6.31 ± 5.1	8.08 ± 5.65	0.401
EXTEM ALPHA ANGLE difference preoperatively and at the end of follow-up	2.31 ± 2.75	2.67 ± 3.7	0.783
INTEM CT difference preoperativelyand at the end of follow-up	-20.69 ± 46.07	-61.08 ± 63.53	0.078
INTEM MCF difference preoperatively and at the end of follow-up	8.75 ± 4.93	11.42 ± 7.37	0.292
INTEM A10 difference preoperatively and at the end of follow-up	7.38 ± 5.2	10.25 ± 7.47	0.268
INTEM ALPHA ANGLE difference preoperatively and at the end of follow-up	3.06 ± 2.59	7.67 ± 8.42	0.091
FIBTEM CT difference preoperatively and at the end of follow-up	3.19 ± 9.93	12.92 ± 24.93	0.223
FIBTEM MCF difference preoperatively and at the end of follow-up	9.63 ± 6.63	13.83 ± 6.9	0.118
FIBTEM A10 difference preoperatively and at the end of follow up	9.19 ± 6.12	12.67 ± 7.02	0.185
FIBTEM ALPHA ANGLE difference preoperatively and at the end of follow-up	3.19 ± 5.96	6 ± 5.56	0.211
Troponines- difference preoperatively and at the end of follow-up	0.64 ± 0.87	0.68 ± 1.2	0.910
CK MB difference preoperatively and at the end of follow-up	-4.38 ± 12.89	8.06 ± 22.02	0.100
ACT difference 2h after surgery and at the end of follow up	-6 ± 12.12	-1.67 ± 4.7	0.206

Multivariate logistic regression analysis, confirmed that the only factor significantly associated with the risk of bleeding, is a difference in APTT values preoperatively and at the end of the follow-up period (Table 5). An increase in this difference is associated with an increased risk of bleeding.

Variance analysis for repeated measurements showed that significant differences between the group of patients with and without bleeding existed at the PLT and ACT values over the entire period of follow-up (Table 6, Graph 1).

Table 5. Association of the investigated parametres and risk for bleeding, results of multivariate logistic regression analysis

Parameter	OR	95% confidence interval		P
		Lower	Upper	
APTT difference preoperatively and at he end of follow-up	2.87	1.141	7.22	0.025
Constant	1421.1			0.023

Table 6. Evaluation of the effects of the group (bleeding) and interaction between time and group on the values of individual parameters during the entire monitoring period,
results of variance analysis for repeated measurements (RM ANOVA)

Parameter	Bleeding	Time*bleeding
WBC	0.345	0.225
RBC	0.381	0.540
HGB	0.299	0.771
HCT	0.746	0.263
PLT	**0.001**	0.780
MPV	0.117	0.270
CRP	0.435	0.705
PSEP	0.332	0.227
PT	0.341	0.552
INR	0.514	0.404
APTT	0.479	0.439
Fibrinogen	0.210	0.051
ATIII	0.603	0.616
Ddimer	0.118	0.288
ADPHS	0.898	0.301
ASPI	0.152	0.112
TRAP	0.293	0.478
EXTEMCT	0.240	0.181
EXTEMMCF	0.170	0.160
EXTEMA10	0.080	0.682
EXTEMALPHAANGLE	0.567	0.234
INTEMCT	0.439	0.218
INTEMMCF	0.120	0.299
INTEMA10	0.118	0.153
INTEMALPHAANGLE	0.143	0.212
FIBTEMCT	0.269	0.519
FIBTEMMCF	0.834	0.227
FIBTEMA10	0.838	0.315
FIBTEMALPHAANGLE	0.975	0.540
Troponines	0.774	0.652
CKMB	0.136	0.139
ACT	**<0.001**	0.296
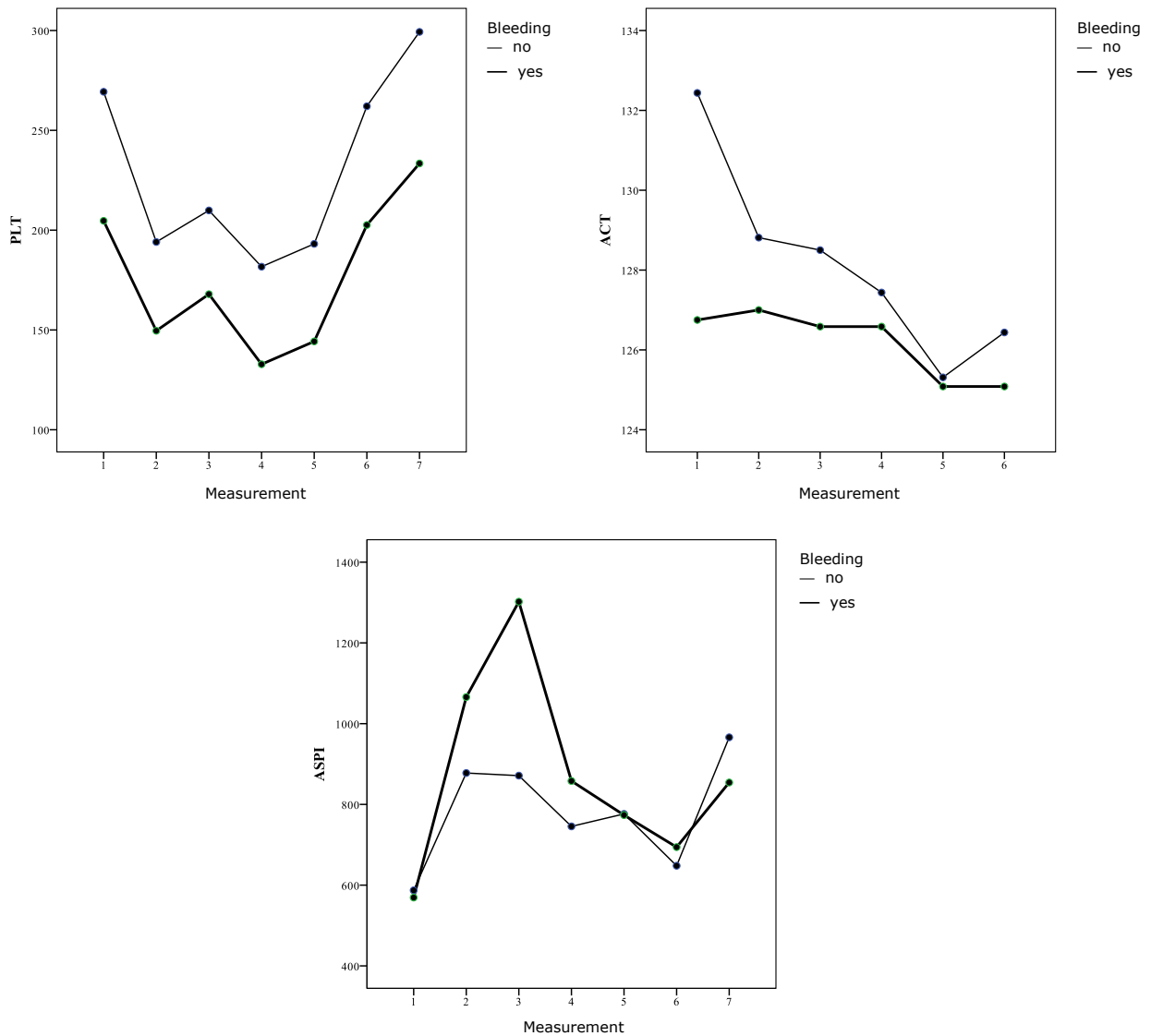
Graph 1. PLT, ACT, and ASPI values in patients with and without bleeding

Changes in ASPI values occurred in a significantly different way throughout the entire monitoring period in patients with and without bleeding.

There were no significant effects of the group (bleeding) and the interaction between time and group in the overall monitoring period regarding the value of other parameters.

Multivariate linear regression analysis showed that the only factor significantly associated with the change in the total allogeneic transfusion value was the value of urea (Table 7). Increase in urea levels is associated with an increase in total allogeneic blood transfusion values (Graph 2).

Table 7. Connection betweenl total allogeneic transfusion and values of other investigated features, results of multivariate linear regression analysis

Parameter	B	95% confidence interval		
		Lower	Upper	P
(Constant)	-887.191	-1541.135	-233.246	0.010
Urea	178.383	78.292	278.474	0.001
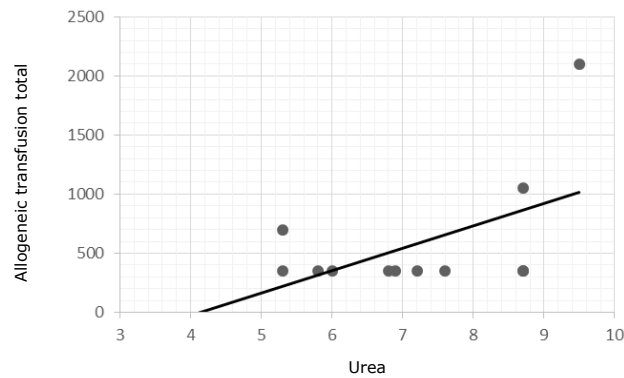
Graph 2. The relationship between the value of total allogeneic transfusion amount and urea values

Multivariate linear regression analysis confirmed: the type of surgery (number of grafts), diuresis, ACT after AP, and an increase in HCT from preoperative period to period 2h after surgery, as factors significantly associated with changes in the number of days in intensive care (Table 8).

An increase in the number of grafts and diuresis is associated with an increase in the number of days in intensive care, while the rise in ACT after AP and HCT difference preoperatively and 2h after surgery, are associated with a decrease in the number of days in intensive care (Graph 3).

Multivariate linear regression analysis, confirmed the amount of cardioplegia, the difference in RBC values in the period of 2 hours after the operation to the end of the monitoring period, and the difference in values of PT and EXTEM CT in the period before surgery to the end monitoring period as factors sig-

nificantly associated with changes in the number of days of total hospitalization (Table 9).

An increase in the amount of cardioplegia has been associated with a reduction in the number of days of total hospitalization, while an increase in RBC values in the period from 2 hours after operation to the end of the follow-up period, as well as an increase in the values of PT and EXTEM CT from the period before surgery to the end of the monitoring period, has been associated to increased number of days of total hospitalization.

Multivariate linear regression analysis, confirmed the preoperative value of INR as the only factor significantly associated with changes in total drainage value (Table 10). The increase in preoperative INR value is associated with an increase in the total drainage value (Graph 4).

Table 8. The relationship between the number of days in intensive care and the value of all other investigated features, the results of the multivariate linear regression analysis

Parameters	B	95% confidence interval		
		Lower	Upper	p
(Constant)	6.722	3.410	10.034	<0.001
Type of surgery (number of grafts)	0.674	0.310	1.038	0.001
ACT after AP	-0.042	-0.065	-0.019	0.001
HCT difference preoperatively and 2h after surgery	-0.059	-0.087	-0.031	<0.001
Diuresis	0.001	<0.001	0.001	0.004
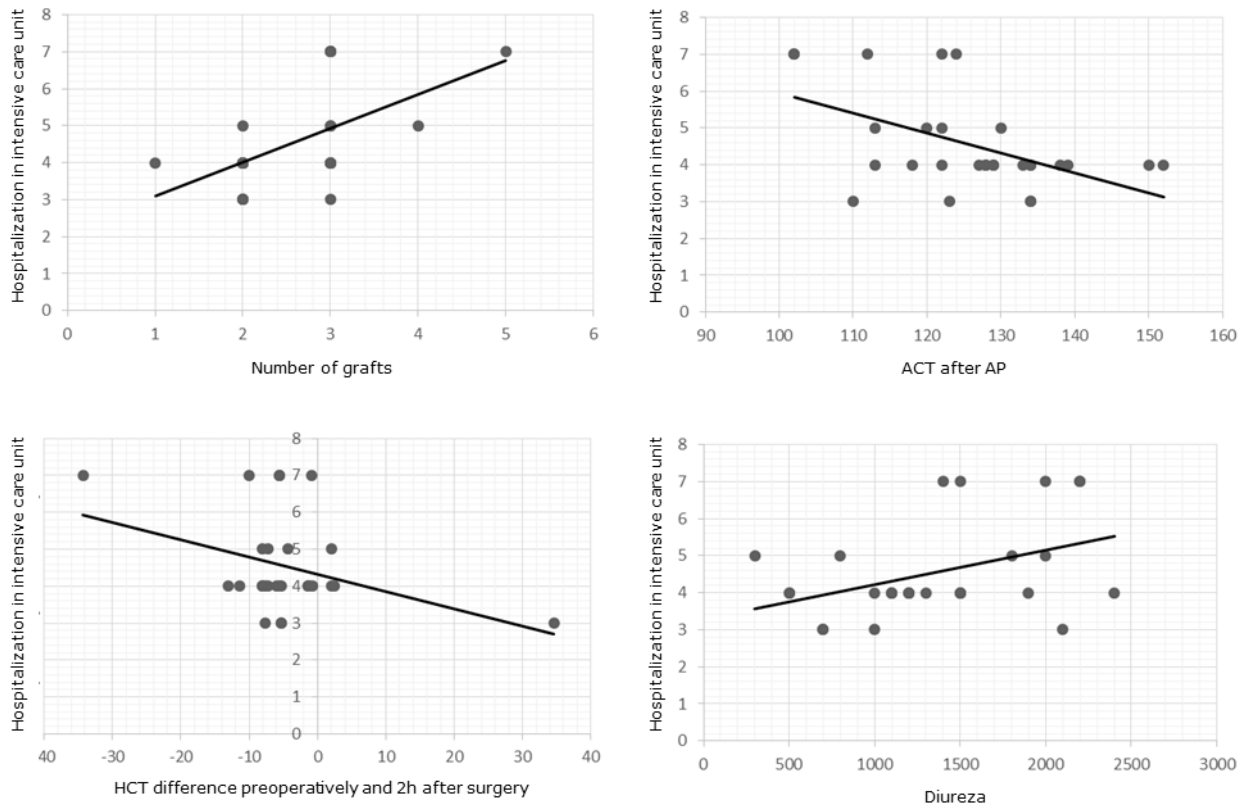
Graph 3. The relationship between the number of days in intensive care and the number of grafts, ACT after AP, HCT difference preoperatively and 2h after surgery and diuresis

Table 9. The relationship between the number of days of total hospitalization and the value of all other investigated features, the results of multivariate linear regression analysis

Parameter	B	95% confidence interval		
		Lower	Upper	p
(Constant)	7.786	7.302	8.270	<0.001
The amount of given cardioplegia	-0.001	-0.0012	-0.0008	0.002
RBC diffrence 2h after surgery and at the end of follow-up	0.260	0.106	0.413	0.002
EXTEM CT difference preoperatively and at the end of follow-up	0.016	0.008	0.024	0.001
PT difference preoperatively and at the end of follow-up	0.035	0.004	0.067	0.030

Table 10. The relationship between the total drainage value and the value of all other investigated features, the results of the multivariate linear regression analysis

Parameter	B	95% confidence interval		
		Lower	Upper	p
(Constant)	3052.12	1817.42	4286.82	<0.001
INR preoperatively	-1942.87	-3092.06	-793.67	0.002
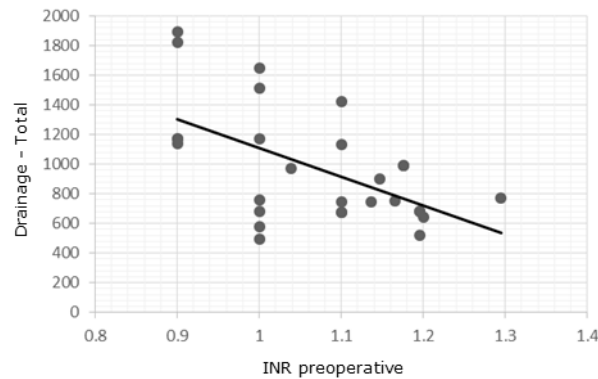
Graph 4. The relationship between total drainage and preoperative value of INR

Discussion

Cardiosurgical interventions carry a certain risk with them. This risk depends on age, gender, heart function, condition of the whole organism, urgency of the intervention, etc. Statistical analysis of a large number of cardio-surgical patients has determined the factors that influence the operational risk.

According to the National Blood Collection & Utilization Survey (NBCUS), blood transfusions and blood derivatives, with the exception of platelet transfusions, declined between 2008. and 2011. in the United States (9). In this period, a reduction rate of 8.2% of red blood cell transfusions (RBC) was recorded, as well as a reduction in plasma transfusion by 13.4%. In the United States, contrary to the general trend, there has been an increase in blood transfusions and blood derivatives during cardiovascular surgery during 2010, when a total of 34% of patients undergoing transfusion received red blood cells or other blood derivatives (10). Moreover, cardiac surgery was a branch of surgery that consumed the largest amounts of blood, just before orthopedic surgery (11, 12). Transfusion is often necessary during cardiovascular surgery to cure coagulopathy, blood loss, and hemodilution due to priming (13). Very often, patients who undergo cardiac surgery have numerous comorbidities such as anemia or myocardial infarction, which increase the risk of complications, and therefore the need for blood transfusion is higher (10, 11, 14).

This study included a total of 28 patients who underwent surgical revascularization of the myocardium at the Clinic for Cardiovascular with Transplantation Surgery, Clinical Centre of Niš, 22 men (79%) and 6 women (21%), with average age of 64. The most common risk factor for these patients was diabetes mellitus, which was present in 46.4% of patients. In our study, there was 21.4% smokers fewer than in other published studies (13-14). This study also showed that BMI was a significant indicator of the incidence of coronary disease, so the average BMI for examiners in our study was 29.94.

The most commonly performed procedure for these patients was double and triple aorto-coronary bypass, with 46.4% of patients having a double aorto-coronary bypass, and 42.9% of patients with triple aorto-coronary bypass. The average number of grafts that patients received was 2.57.

The average ECC time was 102 minutes and the time for the aortic cross clamp was 45 minutes, on average, 1158 ml of cardioplegia was given.

Allogeneic transfusion was administered in 3 patients on the first postoperative day, and the average blood amount was 933.3 ml. On the second day it was applied in two patients, 350 ml each, the third day in one patient, 700 ml, the fourth day in 4 patients, 350 ml each.

On the fifth postoperative day allogeneic transfusion was not applied to all patients. On the sixth and seventh day, allogeneic transfusion was administered in 2 patients, in 350 ml each. In total, allogeneic transfusion was received by 12 patients, and the approximate amount of blood delivered to them was 583 ml.

The diuresis was measured for the first three days in all 28 patients, and the average value was 2779 ml, 3004 ml and 2795 ml, by day. On the fourth day the diuresis was measured in 17 patients, on average 2852 ml, on the fifth day in 9 patients on average 3105 ml, the six in 2 patients at 2850 ml, and on the seventh day in one patient 1800 ml.

Platelets were administered only on the first day, in 4 patients with an average of 6.25 units, plasma, cryoprecipitate, volume, and crystalloids were not applied.

Several studies published the results and effects of blood transfusion in anemic and non-anemic, haemodynamically stable patients. (15-26). A retrospective study carried out in Cleveland Clinic showed that patients undergoing cardio-pulmonary bypass and who did not have anemia during the ECC procedure (hematocrit > 25%), but who received an intraoperative blood, had a need for longer use of mechanical ventilation and they had reduced long-term survival compared to a non-anemic group of patients or with patients who had anemia but did not receive blood (27). Blood transfusions in patients un-

dergoing surgical myocardial revascularization, who were preoperatively classified in a group with a low or moderate risk according to EuroSCORE (< 8) and who had a postoperative hemoglobin greater than 10 g / dL and a minimum postoperative blood loss and without postoperative complications in the first 24 hours of surgery, caused an increased risk of postoperative events and infections in relation to those patients who did not receive blood replacement (17, 21-23, 28).

Shaw et al. compared the effects of blood transfusion in a group of patients who were stratified by the value of hematocrit. Preoperative hematocrit values were compared in patients undergoing cardiac surgery depending on whether they received blood transfusion or not (29). This study showed a statistically significantly higher rate of mortality after 30 days, for that group of patients who received blood transfusion compared to a group of patients who did not receive blood. These results are in agreement with previously published studies that confirm that there is a higher mortality rate in patients receiving blood transfusion (28, 30–33).

A retrospective study published by Schwann et al. investigated the correlation between blood transfusion and mortality in 6,947 patients who underwent surgical myocardial revascularization (34). The overall rate of the red blood cells transfusions was 33.9%. Postoperative complications were present in 35.2% of patients, and this was statistically significantly higher in comparison to a group of patients who did not receive blood transfusion. The most common complications of this type were present in older women with comorbidities. Early mortality (30 days after surgery) and five-year survival were higher among the group of patients receiving blood transfusion, compared to those who did not receive red blood cells. The authors concluded that red blood cell transfusion increased the risk of cardiac and non-cardiac mortality in patients who underwent surgical myocardial revascularization (34-36).

The initial decline after the operation, with a subsequent increase at the end of the monitoring period, was recorded for values of RBC, HGB, PLT, AT III, EXTEM MCF, EXTEM A10, EXTEM ALPHA ANGLE, INTEM MCF, INTEM A10, INTEM ALPHA ANGLE, FIBTEM MCF, FIBTEM A10 , FIBTEM ALPHA ANGLE and ACT.

The initial increase after surgery, with subsequent decline at the end of the monitoring period, was recorded for the values of WBC, CRP, fibrinogen, INTEM CT, troponins and CK MB.

The increasing trend during the entire monitoring period was recorded for values of P SEP, D dimer, ADP HS, ASPI, TRAP and FIBTEM CT.

In relation to preoperative values, the number of WBC significantly increased 2 hours after surgery, while the values of RBC, HGB, HCT, and PLT significantly dropped in the same period.

Diabetes was significantly more common in patients without bleeding. In patients without bleeding, significantly more BMI, triglyceride, cholesterol, preoperative ACT, PLT and INTEM ALPHA ANGLE were preoperatively detected, compare to patients with bleeding.

In patients with bleeding, significantly higher values of the urea and APTT difference preoperatively and at the end of the follow-up period were detected compared to patients without bleeding. The values of all other parameters did not differ significantly in patients with and without bleeding.

The multivariate logistic regression analysis, confirmed that the only factor significantly associated with the risk of bleeding, is a difference in APTT values preoperatively and at the end of the follow-up period. An increase in this difference is associated with an increased risk of bleeding.

The variance analysis for repeated measurements showed that significant differences between the group of patients with and without bleeding existed at the PLT and ACT values.

The changes in ASPI values occurred in a significantly different way throughout the entire monitoring period in patients with and without bleeding.

There were no significant effects of the group (bleeding) and the interaction between time and group in the overall monitoring period, in the value of other features.

The correlation analysis showed that the increased values of total allogeneic transfusion were significantly associated with elevated urea values and increased differences in APTT and CK MB values preoperatively and at the end of the follow-up period.

The multivariate linear regression analysis confirmed the value of urea as the only factor significantly associated with the change in the total allogeneic transfusion value. The increase in urea levels is associated with an increase in the total amount of allogeneic transfusion.

The correlation analysis showed that the increased number of days in intensive care was significantly associated with female gender, higher number of grafts, prolonged ECC time, aortic cross clamp time, diuresis, AT III preoperative, EXTEM CT preoperatively, increased differences in HCT, PT, INR preoperatively and at the end of the monitoring period.

An increased number of total hospitalization days was significantly associated with lowered values of given cardioplegia and PLT preoperative values with declines in WBC and HGB values in the period before surgery to 2 hours after surgery, as well as with the decline in troponine levels in the period before surgery to the end of the follow-up period.

The multivariate linear regression analysis confirmed the amount of cardioplegia, the difference in RBC values in the period of 2 hours after the operation to the end of the monitoring period, and the difference in values of PT and EXTEM CT in the period before surgery to the end of monitoring period as factors significantly associated with changes in the number of days of total hospitalization.

An increase in the amount of cardioplegia has been associated with a reduction in the number of days of the hospitalization, while an increase in RBC values in the period of 2 hours after operation to the end of the follow-up period, as well as an increase in the values of PT and EXTEM CT in the period before surgery to the end of the monitoring period, has been associated with increased number of hospitalization days overall.

The correlation analysis showed that an increase in the total drainage value was significantly associated with an increase in triglyceride and preoperative HCT values, while the increase in total drainage value was significantly associated with a fall in preoperative INR. The multivariate linear regression analysis confirmed the preoperative value of INR as the only factor significantly associated with changes in total drainage value. The increase in preoperative INR value is associated with an increase in the total drainage value. A major dilemma in bleeding patients is whether coagulopathy or mechanical bleeding is the cause of the increase drainage and whether it is necessary to continue with the administration of hemostasic therapy and blood products or perform revision in the operating room. In less than ten minutes, ROTEM test guide doctors in which direction they should work.

The number of transfused allogeneic blood products is declining year after year, which points to the importance of modern monitoring in the indication of blood transfusions by the doctors. Transfusion of fresh plasma is also reduced. Transfusion of concentrated platelets remained at the same level, but percentage of cryoprecipitate transfusions increased year after year (37).

Despite improvements made with existing new techniques, most surgeons tend to accept a significant amount of blood loss as a feature of cardiac surgery (38). It is important to ensure adequate drainage and removal of blood from pericardium and pleura (it has high fibrinolytic activity and tissue factor of coagulation). Removing this blood and clot probably not only reduces the chance of excessive blood loss by preventing systemic coagulopathy, but it is likely to have beneficial effects on several other factors associated with surgery such as, for example, inflammation, atrial fibrillation, pericardial effusion (tamponade), and development of the adhesions (39). After careful evaluation, hemodilution appears to be the most pronounced factor associated with the development of coagulopathy after cardiac surgery, and probably plays an important role in the occurrence of blood loss after heart surgery (40). Fibrinogen is one of the most important factors in coagulation and it is possible that the clotting process falls below the critical level during hemodilution and therefore care should be taken and, if necessary administration of fibrinogen concentrate initiated (41).

Fibrinogen is an acute phase protein the level of which gradually increases during and after surgery in response to a surgical trauma and the use of ECC. The increased concentration of D-dimer and prothrombin fragment 1 + 2 together with increased thrombin production indicate that the hypercoagulable state develops up to 5 days after cardiac surgery. The combination of these two factors (hypercoagulable state and the use of fibrinogen concentrates) can increase the risk of thromboembolic complications in the postoperative period. Therefore, adequate anticoagulant and (or) antiaggregation therapy to prevent the occurrence of thromboembolic complications in the postoperative course is necessary, especially in patients who do not receive vitamin K antagonists, even in patients without prior

bleeding. A delicate balance between bleeding and hypercoagulable states must be maintained. The use of POC evaluation can provide a quicker and more complete insight into this delicate balance, creating more individualized treatment oriented to each patient in particular. A large variation in the patient's sensitivity to the use of clopidogrel often results in very different individual results before surgery, which requires further use and determination of the POC before, during and after cardiac surgery. An individual approach oriented to each patient can contribute to the reduction of perioperative and postoperative blood loss and minimizes the need for transfusion to a minimum (42).

Transfusion of red blood cells is common in cardiac surgery. The percentage of patients receiving blood transfusion during the perioperative period varies in literature: from 95%, up to 10 years ago, to 49% of CABG patients over the past few years. Blood transfusion can improve systemic transport and distribution of oxygen, relieve regulation of vasomotor response, improve delivery of oxygen to the myocardium. On the other hand, there are data in the literature that indicate that transfusion damage is probably more serious than it has been valued so far and that blood transfusions are used more often than necessary (43). Even transfusion of one blood unit is associated with a significant risk of serious postoperative morbidity, the immediate goal should be to avoid transfusion whenever possible and not to apply it just to treat low hemoglobin levels, which is a common practice in over 50% of all patients receiving transfusion. Traditional concern over transfusion of blood and blood derivatives is reduced to the possibility of transmitting viral and bacterial infections or the occurrence of haemolytic reactions, which is rarely occurring. However, immunosuppression, lung damage, or dysfunction of the organs can occur with each recipient. Recently, Cleveland Clinic investigators have found that the administration of erythrocytes that have been stored for a longer period (> 14 days) is independently associated with an increased risk of complications and increases the estimated risk of death (30). This happens because the erythrocytes over time develop lesions and release cytokines, cell membranes fragment and release hemoglobin and free oxygen radicals. Obviously, there is a challenge to determine the circumstances in which transfusion is used. Unfortunately, the existing evidence is scarce, and the existing guidelines and recommendations are based on a low level of evidence. Assuring doctors to change their practice is not an easy task and an appropriate clinical assessment is used as a justification for transfusion.

Numerous strategies exist to minimize the need for a transfusion of blood and blood products in the perioperative period.

Some of the existing guidelines recommend: discontinuation of preoperative application of antithrombotic drugs, applying a restrictive attitude about the level of hemoglobin requiring red blood cell transfusion, the application of intraoperative blood savage techniques, and offpump CABG as one of the of surgical techniques that can reduce bleeding in the postoperative course. The study we conducted showed that using the POC and Rotem methods in the

preoperative and postoperative period, can reduce postoperative bleeding to an acceptable level that in most patients does not require the use of blood and blood derivative transfusion. Antiagregation therapy was stopped before surgery and was re-administered after taking drains out. Reinfusion of the remaining blood at the end of the CPB and the use of the Cell Saver system were applied to all patients involved in our study. But perhaps most importantly, doctors should be encouraged to use a restrictive hemoglobin compensation model, which is also recommended in special guidelines: patients should be given blood transfusions when hemoglobin is less than 7 g / dL, where transfusion is not indicated for improving oxygen transport and when the hemoglobin concentration was higher than 10 g / dL. About 20% of all CABG operations are in off-pump technique. The use of CPB during CABG is associated with more harmful effects, including hemodilution, activation of coagulation factors, and a decrease in the number and function of platelets, leading to coagulopathy that can lead to extensive bleeding and the need for massive blood transfusions. Because of this, off-pump procedures would be expected to lead to a reduction in the incidence of postoperative complications, and are also recommended to reduce the need for transfusion. However, in our study, we did not notice the significant differences between individual patient groups in whom increased bleeding could be expected in the intra and postoperative period. The number of platelets and their function was tracked at 8 different time points. Our study did not show that there was statistically significant correlation between individual groups of patients compared to basic biochemical and inflammatory parameters with the number and function of platelets. There has been a decrease in platelet counts intraoperatively, and a gradual increase in their number in the immediate postoperative period. Increased perioperative bleeding was associated only with a reduced number and platelet function as well as elevated ACT rates.

The only parameter in our study that was associated with increased postoperative drainage was increased preoperative value of INR. In our study, only 4 patients (14.28%) received platelets while 12 patients received a blood supply (42%), an average of 583 ml. We didn't have any reintervention because of bleeding. Our study could not show independent prognostic factors of greater postoperative drainage compared to the classic operational parameters that are being monitored (aortic cross-clamp and ECC time...), nor in relation to the parameters of inflammation.

Conclusion

Based on the conducted research and the obtained results, it can be concluded:

- Surgical myocardial revascularization is a safe technique with a minimum morbidity rate.
- By using of modern methods for preoperative hemostasis monitoring (Multiplate, Rotem), the risk of postoperative bleeding can be significantly reduced, as well as the need for transfusion of red blood cells and other blood derivatives.
- The only preoperative independent prognostic parameter for increased postoperative drainage was INR.
- ACT was an independent postoperative prognostic parameter of increased postoperative drainage, probably due to delayed or prolonged heparin activity
- Inflammation parameters did not show association with the occurrence of postoperative complications.
- Diabetes was significantly more common in patients without bleeding. The same conclusion was for patients with higher BMI.
- In patients with bleeding significantly higher values of urea and the difference in APTT preoperatively and at the end of the follow-up period were detected compared to patients without bleeding.
- Multivariate logistic regression analysis confirmed the difference in APTT values preoperatively and at the end of the monitoring period, as the only factor significantly associated with the risk of bleeding.
- Correlation analysis showed that the increased values of total allogeneic transfusion were significantly associated with the elevated values of urea and increased differences in APTT and CK MB values preoperatively and at the end of the follow-up period.
- Multivariate linear regression analysis confirmed the urea value as the only factor significantly associated with the change in the total transfusion value. The increase in urea levels is associated with the increase in the total amount of allogeneic transfusion.
- The correlation analysis showed that the increased number of days in the intensive care was significantly associated with female gender, high number of grafts and prolonged ECC and aortic cross-clamp time, HCT, PT, and INR values preoperatively and at the end of the follow-up period.
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References

1. De Backer G. Epidemiology and prevention of cardio-vascular disease: Quo vadis? Eur J Prev Cardiol 2017; 24(7): 768-72. [CrossRef][PubMed]
2. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J 2014; 35(42): 2929. [CrossRef][PubMed]
3. Fuster V, Kelly BB, editors. Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health. Washington (DC): National Academies Press (US); 2010.
4. Incidencija i mortalitet od akutnog koronarnog sindroma u 2006, 2007, 2008, 2009, Srbija. Institut za javno zdravlje republike Srbije „Dr Milan Jovanović Batut"; Available from: http://www.batut.org.rs.
5. Wijns W, Kolh P, Danchin N, Di Mario C, Falk V, Folliguet T, et al. Guidelines on myocardialrevascularization: The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J 2010; 31(20): 2501-55. [CrossRef][PubMed]
6. Herzog CA, Ma JZ, Collins AJ. Comparative survival of dialysis patients in the United Statesafter coronary angioplasty, coronary artery stenting, and coronary artery bypass surgery andimpact of diabetes. Circulation 2002; 106(17): 2207-11. [CrossRef][PubMed]
7. Fox K, Garcia MA, Ardissino D, Buszman P, Camici PG, Crea F, et al. Guidelines on themanagement of stable angina pectoris: executive summary: The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Eur Heart J 2006; 27(11): 1341-81. [CrossRef][PubMed]
8. Trullàs JC, González-Franco Á. Major developments in the 2016 european guidelines for heart failure. Rev Clin Esp 2017; 217(7): 405-9. [CrossRef][PubMed]
9. US Department of Health and Human Services: The 2011 national blood collection and utilization survey report. Washington, DC: US Department of Health and Human Services, Office of the Assistant Secretary for Health. 2011; 15 Available from: https://www.aabb.org/research/hemovigilance/bloodsurvey/Documents/11-nbcus-report.pdf
10. Robich MP, Koch CG, Johnston DR, Schiltz N, Chandran Pillai A, Hussain ST, et al. Trends in blood utilization in United States cardiac surgical patients. Transfusion 2015; 55(4): 805-14. [CrossRef][PubMed]
11. Geissler RG, Rotering H, Buddendick H, Franz D, Bunzemeier H, Roeder N, et al. Utilisation of blood components in cardiac surgery: a single-centre retrospective analysis with regard to diagnosis-related procedures. Transfus Med Hemother 2015; 42(2): 75-82. [CrossRef][PubMed]
12. Stoicea N, Bergese SD, Ackermann W, Moran KR, Hamilton C, Joseph N, et al. Current status of blood transfusion and antifibrinolytic therapy in orthopedic surgeries. Front Surg 2015; 2:3. [CrossRef][PubMed]
13. Koch CG. Tolerating anemia: taking aim at the right target before pulling the transfusion trigger. Transfusion 2014;54(10 Pt 2):2595-7. [CrossRef][PubMed]
14. Ad N, Massimiano PS, Burton NA, Halpin L, Pritchard G, Shuman DJ, et al. Effect of patient age on blood product transfusion after cardiac surgery. J Thorac Cardiovasc Surg 2015; 150(1): 209-14. [CrossRef][PubMed]
15. Vincent JL, Baron JF, Reinhart K, Gattinoni L, Thijs L, Webb A, et al. Anemia and blood transfusion in critically ill patients. JAMA 2002; 288(12): 1499-507. [CrossRef][PubMed]
16. Anía BJ, Suman VJ, Fairbanks VF, Rademacher DM, Melton LJ 3rd.. Incidence of anemia in older people: an epidemiologic study in a well defined population. J Am Geriatr Soc 1997; 45(7): 825-31. [CrossRef][PubMed]
17. Dejam A, Malley BE, Feng M, Cismondi F, Park S, Samani S, et al. The effect of age and clinical circumstances on the outcome of red blood cell transfusion in critically ill patients. Crit Care 2014; 18(4): 487. [CrossRef][PubMed]
18. Rodriguez RM, Corwin HL, Gettinger A, Corwin MJ, Gubler D, Pearl RG. Nutritional deficiencies and blunted erythropoietin response as causes of the anemia of critical illness. J Crit Care 2001; 16(1): 36-41. [CrossRef][PubMed]
19. Corwin HL, Parsonnet KC, Gettinger A. RBC transfusion in the ICU. Is there a reason? Chest 1995; 108(3): 767-71. [CrossRef][PubMed]
20. Littenberg B, Corwin H, Gettinger A, Leichter J, Aubuchon J. A practice guideline and decision aid for blood transfusion.Immunohematology 1995; 11(3): 88-94. [PubMed]
21. Lako A, Bilali S, Memishaj S, Daka A, Dedej T, Nurka T, et al. The impact of blood use on patients undergoing coronary artery bypass surgery: a prospective study. G Chir 2014; 35(1-2): 20-6. [CrossRef][PubMed]
22. Westenbrink BD, Kleijn L, de Boer RA, Tijssen JG, Warnica WJ, Baillot R, et al. Sustained postoperative anaemia is associated with an impaired outcome after coronary artery bypass graft surgery: insights from the IMAGINE trial. Heart 2011; 97(19): 1590-6. [CrossRef][PubMed]
23. Patel NN, Avlonitis VS, Jones HE, Reeves BC, Sterne JA, Murphy GJ. Indications for red blood cell transfusion in cardiac surgery: a systematic review and meta-analysis. Lancet Haematol 2015; 2(12): e543-53. [CrossRef][PubMed]
24. Murphy GJ, Reeves BC, Rogers CA, Rizvi SI, Culliford L, Angelini GD. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. Circulation 2007; 116 (22): 2544-52. [CrossRef][PubMed]
25. Litmathe J, Boeken U, Feindt P, Gams E. Predictors of homologous blood transfusion for patients undergoing open heart surgery. Thorac Cardiovasc Surg 2003; 51(1):17-21. [CrossRef][PubMed]
26. Pattakos G, Koch CG, Brizzio ME, Batizy LH, Sabik JF 3rd, Blackstone EH, et al. Outcome of patients who refuse transfusion after cardiac surgery: a natural experiment with severe blood conservation. Arch Intern Med 2012; 172(15): 1154-60. [CrossRef][PubMed]
27. Loor G, Li L, Sabik JF, 3rd, Rajeswaran J, Blackstone EH, Koch CG. Nadir hematocrit during cardiopulmonary bypass: end-organ dysfunction and mortality. J Thorac Cardiovasc Surg 2012; 144(3): 654-662. [CrossRef]
28. Paone G, Herbert MA, Theurer PF, Bell GF, Williams JK, Shannon FL, et al. Red blood cells and mortality after coronary artery bypass graft surgery: an analysis

of 672 operative deaths. Ann Thorac Surg 2015; 99(5): 1583-9. [CrossRef][PubMed]

29. Shaw RE, Johnson CK, Ferrari G, Zapolanski A, Brizzio M, Rioux N, et al. Balancing the benefits and risks of blood transfusions in patients undergoing cardiac surgery: a propensity-matched analysis. Interact Cardiovasc Thorac Surg 2013; 17(1):96-102. [CrossRef][PubMed]

30. Kuduvalli M, Oo AY, Newall N, Grayson AD, Jackson M, Desmond MJ, et al. Effect of peri-operative red blood cell transfusion on 30-day and 1-year mortality following coronary artery bypass surgery. Eur J Cardiothorac Surg 2005; 27(4): 592-8. [CrossRef][PubMed]

31. Engoren MC, Habib RH, Zacharias A, Schwann TA, Riordan CJ, Durham SJ. Effect of blood transfusion on long-term survival after cardiac operation. Ann Thorac Surg 2002; 74(4): 1180-6. [CrossRef][PubMed]

32. Möhnle P, Snyder-Ramos SA, Miao Y, Kulier A, Böttiger BW, Levin J, et al. Postoperative red blood cell transfusion and morbid outcome in uncomplicated cardiac surgery patients. Intensive Care Med 2011; 37 (1): 97-109. [CrossRef][PubMed]

33. Loor G, Rajeswaran J, Li L, Sabik JF 3rd, Blackstone EH, McCrae KR, et al. The least of 3 evils: exposure to red blood cell transfusion, anemia, or both? J Thorac Cardiovasc Surg 2013; 146(6): 1480-1487. [CrossRef][PubMed]

34. Schwann TA, Habib JR, Khalifeh JM, Nauffal V, Bonnell M, Clancy C, et al. Effects of Blood Transfusion on Cause-Specific Late Mortality After Coronary Artery Bypass Grafting-Less Is More. Ann Thorac Surg 2016; 102(2): 465-73. [CrossRef][PubMed]

35. Du Pont-Thibodeau G, Harrington K, Lacroix J. Anemia and red blood cell transfusion in critically ill cardiac patients. Ann Intensive Care 2014; 4: 16. [CrossRef][PubMed]

36. Azarfarin R, Ashouri N, Totonchi Z, Bakhshandeh H, Yaghoubi A. Factors influencing prolonged ICU stay after open heart surgery. Res Cardiovasc Med 2014; 3(4): e20159. [PubMed]

37. Mehta RH, Grab JD, O'Brien SM, Glower DD, Haan CK, Gammie JS, et al. Clinical characteristics and in-hospital outcomes of patients with cardiogenic shock undergoing coronary artery bypass surgery: insights from the Society of Thoracic Surgeons National CardiacDatabase. Circulation 2008; 117(7): 876-85. [CrossRef][PubMed]

38. Jubelirer SJ, Mousa L, Reddy U, Welch CA. Coronary artery bypass grafting (CABG) in patients with immune thrombocytopenia (ITP): a community hospital experience and review of the literature. W V Med J 2011; 107(6): 10-4. [PubMed]

39. Engoren M, Arslanian-Engoren C. Long-term survival in the intensive care unit after erythrocyte blood transfusion. Am J Crit Care 2009; 18(2): 124-31. [CrossRef][PubMed]

40. Vamvakas EC, Carven JH. RBC transfusion and post-operative length of stay in the hospital or the intensive care unit among patients undergoing coronary artery bypass graft surgery: the effects of confounding factors. Transfusion 2000; 40(38): 832-9. [CrossRef][PubMed]

41. Koster A, Zittermann A, Borgermann J, Knabbe C, Diekmann J, Schirmer U, et al. Transfusion of 1 and 2 units of red blood cells does not increase mortality and organ failure in patients undergoing isolated coronary artery bypass grafting. Eur J Cardiothorac Surg 2016; 49(3): 931-6. [CrossRef][PubMed]

42. Haanschoten MC, van Straten AH, Verstappen F, van de Kerkhof D, van Zundert AA, Soliman Hamad MA. Reducing the immediate availability of red blood cells in cardiac surgery, a single-centre experience. Neth Heart J 2015; 23(1): 28-32. [CrossRef][PubMed]

43. Ferraris VA. Blood transfusion in cardiac surgery: who should get transfused? Lancet Haematol 2015; 2(12). [CrossRef][PubMed]

Originalni rad

POREMEĆAJI KOAGULACIONOG STATUSA I HEMOSTAZE KAO PROGNOSTIČKI PARAMETRI NEPOSREDNIH I RANIH REZULTATA NAKON HIRURŠKE REVASKULARIZACIJE MIOKARDA

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Hirurška revaskularizacija miokarda predstavlja jednu od najčešće izvođenih hirurških procedura u svetu. Vremenom, razvojem tehnologije i savremenih dijagnostičkih postupaka kao i unapređenjem hirurške tehnike, stopa smrtnosti kod elektivnih nekomplikovanih slučajeva pala je na ispod 2%. Ipak, i pored izuzetnog razvoja hirurške metode, stopa postoperativnih komplikacija koje mogu ugroziti bolesnike kreće se i preko 10%. Cilj ovog istraživanja bio je da se definiše grupa bolesnika sa povećanim rizikom od postoperativnih komplikacija u zavisnosti od poremećaja koagulacionog statusa i hemostaze.

Sprovedeno je prospektivno, nerandomizovano istraživanje koje je obuhvatilo 28 bolesnika koji su podvrgnuti hirurškoj revaskularizaciji miokarda u Klinici za kardiohirurgiju KC Niš od januara do aprila meseca 2017. godine. Preoperativno, kao i tri sata, 24 sata, 48 sati, tri dana i pet dana postoperativno, određivani su sledeći parametri: krvna slika, parametri inflamacije (C reaktivni protein, presepsin); koagulacioni status (protrombinsko vreme, International Normalized Ratio, aktivisano parcijalno tromboplastinsko vreme, fibrinogen, anti-trombin III, D dimer).

Jedini preoperativni nezavisni prognostički parametar povećane postoperativne drenaže bio je INR. ACT je bio nezavisni postoperativni prognostički parametar povećane postoperativne drenaže verovatno zbog odloženog ili protrahovanog dejstva heparina. Parametri inflamacije nisu pokazali povezanost sa nastankom postoperativnih komplikacija. U odnosu na bolesnike bez krvarenja, kod onih sa krvarenjem evidentirane su značajno više vrednosti uree i razlike vrednosti APTT preoperativno i na kraju perioda praćenja. Multivarijantna logistička regresiona analiza je kao jedini faktor značajno povezan sa rizikom za nastanak krvarenja potvrdila razliku vrednosti APTT preoperativno i na kraju perioda praćenja. Multi-varijantna linearna regresiona analiza je kao jedini faktor značajno povezan sa promenom vrednosti ukupno date alogene transfuzije potvrdila vrednost uree. Povećanje nivoa uree povezano je sa porastom vrednosti ukupno date alogene transfuzije. Korelaciona analiza je pokazala da je povećan broj dana boravka u intenzivnoj nezi bio značajno povezan sa ženskim polom, povećanjem broja graftova i povišenim vrednostima trajanja EKK, vremena klemovanja, vrednosti HCT, PT, INR preoperativno i na kraju perioda praćenja.

Hirurška revaskularizacija miokarda je bezbedna i sigurna metoda sa minimalnom stopom morbiditeta. Primenom savremenih metoda za preoperativni monitoring hemostaze može se značajno smanjiti rizik postoperativnog krvarenja i smanjiti potreba za transfuzijom crvenih krvnih zrnaca i drugih derivata krvi.

Ključne reči: *kardiohirurgija, koagulacioni status, parametri inflamacije, faktori rizika*

SEVERE KETOACIDOSIS IN A NEWLY DIAGNOSED PATIENT WITH TYPE 2 DIABETES AND METABOLIC SYNDROME

Vojislav Ćirić[1,2]

Diabetic ketoacidosis (DKA) is well-known complication of type 1 diabetes, however, its presence is increasingly recognized in type 2 diabetes patients, even as initial presentation. Case report Male patient, 54 years old, was hospitalized due to newly diagnosed type 2 diabetes (BG 21,3 mmol/l, HbA1c 10.5%) accompanied with severe diabetic ketoacidosis (pH 7.00, base excess -24,6, serum bicarbonate 6,7 mEg/l). The patient was obese (BMI 35), hypertensive (160/90 mmHg), with extreme dyslipidaemia (TC 25,22 mmol/l, HDL 2,45 mmol/l, TG 31,21 mmol/l). During hospitalization, the patient was diagnosed with acute pancreatitis, cholelithiasis, GERD, and hepatic steatosis. The patient was treated with rehydration, intravenous insulin infusion, antibiotic therapy, proton pump inhibitor, antihypertensive therapy (ACE inhibitor and beta blocker), and dietary restriction. The patient was discharged with NPH insulin once daily, metformin, PPI, ACEi, BB and statin. Six months later BMI was 30,2, FBG 6,2 mmol/l, HbA1c 5.6%, TC 3,91 mmol/l, HDL 1,19 mmol/l, LDL 2.27 mmol/l, TG 0.99 mmol/l, amylase 78, CRP 6,9 mg/l, BP 130/80 mmHG. Seven months later laparoscopic cholecystectomy was done, and nine months later insulin therapy was discontinued. The weight, glycaemic control, lipid status and blood pressure remained stable during follow up of 24 months. The patient continues with metformin, statin, ACEi, and BB. Conclusion In newly diagnosed type 2 diabetes DKA could be from constant hyperglycaemia (glucose toxicity) and the presence of stressors that cause increase lipolysis due to counterregulatory hormones. Majority of patients are able to discontinue insulin after the resolution of DKA.
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Key words: *Type 2 diabetes, diabetic ketoacidosis, metabolic syndrome*

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Introduction

Diabetic ketoacidosis (DKA) is a well known complication of type 1 diabetes mellitus, however, its presence is increasingly recognized in type 2 diabetes patients, even as the initial presentation of type 2 diabetes. DKA is characterized by hyperglycaemia, ketosis and metabolic acidosis (increased anion gap) along with a number of secondary metabolic derangements (1). DKA results from absolute or relative insulin deficiency which is inadequate to prevent ketosis combined with counteregulatory hormone excess (2). Hyperglycaemia results from three processes: increased glyconeogenesis, accelerated glycogenoliysis and impared glucose utilization in peripheral tissues (3). This is magnified by transient insulin resistance due to hormone imbalance itself as well as the elevated free fatty acids (4). The combination of insulin deficiency and increased counterregulatory hormones in DKA leads to release of free fatty acids from adipose tissue (lipolysis) and to unrestrained hepatic fatty acid oxidation in the liver to ketone bodies (5). The most common precipitating factors are infections, inadequate insulin therapy, pancreatitis, myocardial infarction and illicit drug use (6).

The rate of hospitalization due to DKA among adults in USA in 2014 is 7,7 per 1000 persons with diabetes (168,000 discharges) (7). DKA is the most common cause of death in children and adolescents with type 1 diabetes (8). The mortality rate in adults is < 1%, however, a mortality rate >5% is reported in elderly and in patients with concomitant life-threatening diseases (9).

Diagnostic criteria for DKA are shown in Table 1.

Table 1. Diagnostic criteria for DKA (2)

	Mild DKA (plasma glucose > 13,9 mmol/l)	Moderate DKA (plasma glucose > 13,9 mmol/l)	Severe DKA (plasma glucose > 13,9 mmol/l)
Arterial pH	7.25-7.30	7.00 to <7.24	< 7.00
Serum bicarbonate (mEq/l)	15-18	10 to < 15	< 10
Urine ketone	Positive	Positive	Ppositive
Serum ketone	Positive	Positive	Positive
Serum osmolality	Variable	Variable	Variable
Anion gap	> 10	> 12	> 12
Mental status	Alert	Alert/drowsy	Stupor/coma

Treatment of DKA includes replacement of fluids, administration of short-acting insulin IV, replacement of K+, bicarbonate or phosphate supplementation. Frequent assessment of the serum electrolytes (K, Na, Mg, Cl, bicarbonates, phosphates), acid-base status, renal function is necessary, as well as monitoring of blood pressure, pulse, respirations, mental status, fluid intake and output (2).

Case report

In January 2016, male patient, aged 54, was admitted to the Clinic for Endocrinology, Diabetes and Metabolic Diseases of Clinical Center Niš, due to newly diagnosed type 2 diabetes mellitus accompanied with severe diabetic ketoacidosis. The patient complains of symptoms that last for about ten days, including thirst, dry mouth, frequent urination, fatigue, nausea, vomitus, loss of appetite. The patient reported that he was being treated for hypertension for 25 years (ACE inhibitor, beta blocker), also reported that he was overweight for about 15 years. There was a positive family history of diabetes (the mother suffered from type 2 diabetes). The patient is a former smoker (he had smoked 30 years ago) and uses no alcohol.

Physical examination showed signs of dehydration, tachycardia (122/min), hypertension (160/90 mmHg), dyspnea, diffusely painfully sensitive abdomen, obesity (weight 124kg, height 188cm, BMI 35.1).

Laboratory report revealed diabetes mellitus with highly elevated glycaemic parameters (blood glycose 21.3 mmol/l, HbA1c 10.5%) and metabolic acidosis (pH 7,00, serum bicarbonates 6.7 mEq/l, anion gap -24.6). Other clinically significant results included extreme dyslipidaemia (total cholesterol 25.22 mmol/l, HDL cholesterol 2.45 mmol/l, triglycerides 31.21 mmol/l), elevated amylases (459 IU/l), elevated lypases (903 IU/l), elevated inflammation parameters (CRP 106.7 mg/l, WBC 14,0). Electrolytic status was normal.

Additional diagnostic methods have been applied. Esophagogastroduodenoscopy showed esophaphagitis grade B of the distal part of esophagus. Abdominal ultrasound showed hepatic steatosis, cholelythiasis (gallblader calculi up to 13 mm) and increased echogenicity of the pancreatic head.

The patient was treated with rehydration, intravenous insulin infusion, antibiotic therapy, proton pump inhibitor and antihypertensive therapy (ACE inhibitor and beta blocker) and dietary restriction. After the resolution of DKA, NPH insulin was introduced.

Table 2. Regular control six monts later

Parameters	Six months later	Baseline
BMI (kg/m^2)	30.2 (↓)	35.1
FBG (mmol/l)	6.2 (↓)	21.3
HbA1c (%)	5.6 (↓)	10.5
Total cholesterol (mmol/l)	3.91 (↓)	25.22
LDL cholesterol (mmol/l)	2.27	-
HDL cholesterol (mmol/l)	1.19 (↓)	2.45
Triglycerides (mmol/l)	0.99 (↓)	31.21
Amilases (IU/l)	78 (↓)	459
CRP (mg/l)	6.9 (↓)	106.9
BP (mmHg)	130/80 (↓)	160/90

Table 3. Regular controls up to 24 months

Parameters	12 months	18 months	24 months
BMI (kg/m^2)	29.1	28.5	29.5
FBG (mmol/l)	5.5	5.9	5.9
HbA1c (%)	5.2	5.6	5.8
Total cholesterol (mmol/l)	3.56	3.95	3.93
LDL cholesterol (mmol/l)	1.58	1.87	1.89
HDL cholesterol (mmol/l)	1.37	1.32	1.35
Triglycerides (mmol/l)	1.35	1.68	1.52
BP (mmHg)	120/80	130/70	120/70

The patient was discharged 11 days later with significant improvement in glycaemic control and without any subjective symptoms. The patient was discharged with NPH insulin once daily, metformin, PPI (pantoprazole), ACEi (ramipril), BB (bisoprolol) and statin (atorvastatin).

The patient came to regular check-ups and was compliant with dietary requirements and performed the moderate-intensity physical activity. The patient performed regular SMBG (self -monitoring of blood glucose). At control after 6 months a weight loss was obvious, parameters of glycaemic control were much better, hyperlipidaemia and hypertension were satisfactorily controlled, there were no elevated inflammation markers. Relevant parameters are presented in Table 2.

Seven months later laparoscopic cholecystectomy was done, and nine months later insulin therapy was discontinued. The patient continued with metformin, atorvastatin, ACEi and BB. The weight, glycemic control, lipid status, and blood pressure remained stable up to 24 months, as shown in Table 3.

Discussion

Insulin is known to inhibit glyconeogenesis and glycogenolysis and to enhance glucose uptake in peripheral tissues. However, in insulin-resistant states glucose output from the liver is increased (10). In insulin-resistant states the body still remains sensitive to antilypolytic effect of insulin. There are data suggesting that the amount of insulin required to prevent lipolysis is one-tenth of that required for glucose utilization (11). This is the reason why it had been thought that patients with type 2 diabetes did not develop ketoacidosis (type 2 diabetes is a predominantly a disease of increased insulin resistance, so residual beta cell function in these patients could produce enough insulin to prevent ketogenesis but not to satisfy glucose metabolism requirements).

The occurrence of DKA in type 2 diabetes is thought to be due to coexisting stressors, predominantly infections. Other reported causes include myocardial infarction, cerebrovascular accidents, antipsychotic usage, malignancy, poor compliance with medication etc (2, 12). Sometimes no stressors can be found and it can be the initial presentation of type 2 diabetes (13).

The occurrence of DKA in type 1 diabetes is due to the presence of insulinopaenia. A similar mechanism can occur in long-standing type 2 diabetes due to complete loss of beta cell function. However, this is not always the case as same patients present within a few years from diagnosis or at the time of diagnosis, when complete beta cell dysfunction is unlikely (12). The cause could be relative insulin deficiency that comes from constant hyperglycaemia as the result of poor control and the presence of stressors that cause increased lypolysis due to counter regulatory hormones (glucagon, cortisol, growth hormone) (14). Hyperglycaemia itself reduces insulin secretion and glucose removal by down-regulating glycose transporter systems and even reducing insulin gene transcription (mechanisms known as glucose toxicity) (15).

DKA in type 2 diabetes tends to be less severe and potassium level is more likely to be normal (12). Type 2 diabetes patients with DKA tend to have typical insulin resistance features (large body habitus, acantosis nigricans), positive family history, no autoimmune markers, and may require larger insulin doses to correct hyperglycaemia (15). The majority of patients with DKA and newly diagnosed type 2 diabetes are able to discontinue insulin after the acute episode and to continue with oral antidiabetic therapy (up to 66% in some studies) (16). This may be related with beta cell recovery after the resolution of the acute hyperglycemic episode (17). The importance of recognizing DKA as the feature of type 2 diabetes lies in this finding, ensuring that patients are not unnecessarily continued with insulin and providing significant cost, economic and emotional benefit to patients (17).

Some aspects of our case are in accordance with literature data. The patient had features of insulin resistance (obesity, metabolic syndrome, hepatic steatosis). Hyperglycemia was long and uncontrolled (HbA1c 10.5%), most likely leading to further beta cell dysfunction (glucose toxicity). Coexisting stressor was identified (cholecystitis, pancreatitis). After the resolution of DKA, the patient experienced satisfactory glycaemic control with NPH insulin and metformin, being compliant with the dietary regimen and performing regular physical activity. The insulin could be discontinued earlier, but this therapeutic approach was continued probably due to expected surgical intervention. After the surgery insulin was discontinued and the patient remained off insulin for more than a year without any worsening of glycemic control, enabling him to lose some more weight.

References

1. Powers AC. Diabetes Mellitus: Complications. In: Jameson JL, editors. Harrison's Endocrinology. 4th ed. Boston: McGraw-Hill Education; 2017. p. 309.
2. Kitabchi AE, Umpierezz GE, Miles JM, Fisher JN. Hyperglycemic crises in patients with diabetes mellitus. Diabetes Care 2009; 32:1335. [CrossRef] [PubMed]
3. DeFronzo RA, Matzuda M, Barret E. Diabetic ketoacidosis: a combined metabolic nephrologic approach to therapy. Diaebetes Rev 1994; 2:209-38.
4. Barrett EJ, DeFronzo RA, Bevilacqua S, Ferrannini E. Insulin resistence in diabetic ketoacidosis. Diabetes 1982; 31:923-8. [CrossRef] [PubMed]
5. Miles JM, Haymond MW, Nissen S, Gerich JE. Effects of free fatty acids aviability, glucagoe excess and insulin deficiency on ketone body production in postapsorptive man. J Clin Invest 1983; 71:1554-61. [CrossRef] [PubMed]
6. Ennis ED, Stahl EJVB, Kreisberg RA. The hyperosmolar hyperglycemic syndrome. Diabetes Rev 1994; 2:115-26.
7. National Diabetes Statistics Report. Available from: URL: https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf
8. Wolfsdorf J, Glaser N, Sperling MA. Diabetic ketoacidosis in infants, children, and adolescents: a consensus statement from the American Diabetes Association. Diabetes Care 2006; 29:1150-2259. [CrossRef] [PubMed]
9. Malone ML, Gennis V, Goodwin JS. Characteristics of diabetic ketoacisosis in older versus younger adults. J Am Geriatr Soc, 1992; 40:1100-41. [CrossRef] [PubMed]
10. Barthel A, Schmoll D. Novel concepts in insulin regulation of hepatic gluconeogenesis. Am J Physiol. Endocrinol Metab 2003; 285:685-92. [CrossRef] [PubMed]
11. Zirler KL, Rabinowitz D. Effect of very small concentrations of insulin on forearm metabolism. Persistence of its action on potassium and free fatty acids without its effect on glucose. J Clin Invest 1964; 43:950-62. [CrossRef] [PubMed]
12. Newton Ca, Raskin P. Diabetic ketoacidosis in type 1 and type 2 diabetes mellitus: clinical and biochemical differences. Arch Inter med 2004; 164:1925-31. [CrossRef] [PubMed]
13. Rao VD, Pradhan B, Mallikarjuna Y, Reddy R. Clinical profile of diabetic ketoacidosis in adults. Health Renaissance 2012; 10(2):80-6. [CrossRef]
14. Lin MV, Bishop G, Benito-Herrero M. Diabetic ketoacidosis in type 2 diabetics: A novel presentation of pancreatic adenocarcinoma. J Gen Intern Med 2010; 25:369-73. [CrossRef] [PubMed]
15. Robertson PR, Olson LK, Zhang HJ. Differentiating glucose toxicity from glucose desensitization: a new message from insulin gene. Diabetes 1994; 43:1085-9. [CrossRef] [PubMed]
16. Jabbar A, Farooqui K, Habib A, Islam N, Haque N, Akhter J. Clinical characteristics and outcomes of diabetic ketoacidosis in Pakistani aduts with type 2 diabetes mellitus. Diabet Med 2004; 21:920-3. [CrossRef] [PubMed]
17. Puttana A, Padinjakara RNK. Diabetic ketoacidosis in type 2 diabetes. Practical Diabetes 2014; 31(4):155-8. [CrossRef]

Prikaz bolesnika

TEŠKA DIJABETIČKA KETOACIDOZA KOD BOLESNIKA SA NOVOOTKRIVENIM DIJABETESOM TIPA 2 I METABOLIČKIM SINDROMOM

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Dijabetička ketoacidoza (DKA) je dobro poznata komplikacija dijabetesa tipa 1, ali njeno postojanje se sve više prepoznaje kod bolesnika sa dijabetesom tipa 2, čak i kao inicijalna prezentacija. Bolesnik muškog pola, star 54 godine, hospitalizovan je zbog novootkrivenog dijabetesa tipa 2 (glikemija 21,3 mmol l, HbA1c 10,5%) praćenog teškom dijabetičkom ketoacidozom (pH 7,00, bazni eksces -24,6, serumski bikarbonat 6,7 mEg/l). Bolesnik je bio gojazan (BMI 35), hipertenzivan (160/90 mmHg), sa ekstremnom dislipidemijom (TC 25,22 mmol/l, HDL 2,45 mmol/l, TG 31,21 mmol/l). Tokom hospitalizacije mu je dijagnostikovan akutni pankreatitis, holelitijaza, GERB i hepatična steatoza. Lečen je rehidratacijom, intravenskom infuzijom insulina, antibiotskom terapijom, inhibitorom protonske pumpe, antihipertenzivnom terapijom (ACE inhibitor i beta blokator), kao i restriktivnom dijetom. Bolesnik je otpušten sa propisanom terapijom: NPH insulin jednom dnevno, metformin, PPI, ACEi, BB i statin. Šest meseci kasnije, BMI je bio 30,2, glikemija našte 6,2 mmol/l, HbA1c 5,6%, TC 3,91 mmol/l, HDL 1,19 mmol/l, LDL 2,27 mmol/l, TG 0,99 mmol/l, amilaza 78, CRP 6,9 mg/l, TA 130/80 mmHg. Sedam meseci kasnije obavljena je laparoskopska holecistektomija, a devet meseci kasnije prekinuta insulinska terapija. Glikoregulacija, telesna težina, krvni pritisak i lipidni status ostali su stabilni tokom praćenja od 24 meseca. Bolesnik nastavlja sa metforminom, atorvastatinom, ACEi i BB. Zaključak je da novodijagnostikovani dijabetes tipa 2 DKA može nastati usled konstantne hiperglikemije (glukotoksičnost) i prisustva stresora koji uzrokuju ubrzanu lipolizu usled povećanja kontraregulatornih hormona. Većina bolesnika mogu da prekinu insulinsku terapiju nakon korekcije DKA.

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Ključne reči: *dijabetes tipa 2, dijabetička ketoacidoza, metabolički sindrom*

COMPARATIVE RESONANCE FREQUENCY ANALYSIS OF THE PRIMARY STABILITY AT DIFFERENT DENTAL IMPLANT DESIGNS

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Primary implant stability appears to be a prerequisite for successful osseointegration of dental implants. Different factors may contribute to initial implant stability, and these include implant design, surgical technique and bone quality.

The aim of this study was to determine the effect of different macro design on primary stability, and the evaluation of primary stability relative to the percentage contact surface of the implant and bone.

The research was conducted *in vitro*, with pig ribs as analogue of human bone (cortical thickness 2 mm, non-self taping implants Nobel Biocare Replace 3.5x10 mm and self-tapping implants Bredent 3.5x10 mm. The primary stability was measured with Osstell mentor instrument and Student's t-test was used for statistical data processing.

The average value of primary stability after three measurements with 5mm contact of bone and non-self tapping for Nobel Biocare is 30 ISQ. In self-tapping Bredent implants, the ISQ values were 42 ISQ. When the contact with the bone was on 10 mm, the following average values of primary stability were recorded: Nobel Biocare 70 ISQ, and Bredent 72 ISQ. Chi-square test (p < 0.05) showed that there is a statistically significant difference in the values of primary stability in implants with different designs.

Implant design plays an important role in achieving adequate primary stability. In this study, there were statistically significant higher values of primary stability recorded in self-tapping compared to non-self-tapping implants at the 5mm depth, thus recommending it for immediate placement.

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Key words: *primary stability, resonance frequency analysis, implants*

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Introduction

Osseointegration is a prerequisite for achieving success in implant therapy, while the primary stability of the implant is marked as a precondition for achieving osseointegration (1). Also, some authors state that the primary stability can be used to evaluate and predict the osseointegration success (2, 3). Numerous factors affect the primary stability of the implant, but the following three are the most important: implant design, surgical implantation technique, and the quality and quantity of bone (4). Macro design of implants plays an essential role in achieving adequate primary stability (5). Macro design entails the implant shape and thread design, as well as the depth, width, density, angle and shape of the thread. The difference between macro-design of self-tapping and non-self-tapping implants is particularly noteworthy. Some studies show that in lower bone density, type 3 and type 4 of bone types, according to the classification of Lekholma and Zarb (6), use of an example of self-tapping implants in combination with different preparation of site preparation can achieve better primary stability compared to standard surgical technique with non-taping implants (7). Under certain conditions, implants placed by immediate method can be regarded as an attractive substitute compared to classic single-phase or two-phase delayed application techniques (8), and achieving adequate primary stability is stated as a basic parameter for evaluation of success (9).

Aim

The aim of the study was to compare resonance frequency analysis differences in the primary stability of different implant designs with type 3 and type 4 bone, as well as to assess the difference in

primary stability in comparison to the percentage contact between the implant and bone.

Materials and methods

Bone

The research was conducted under in vitro conditions, pig ribs with equal cortical thickness of 2 mm (10, 11) were used as a skeletal model of the human upper jaw (bone density types 3 and 4 according to the classification of Lekholma and Zarb).

All samples were obtained from experimental animals - males (due to higher bone density analogue to human density), six month old and weighing 120 kg. The samples were taken from a local slaughterhouse. In order to preserve and cause minimal changes in the physical properties of bones, samples were prepared according to the protocol published by Sedliņe and Hirsch, which means that bone was kept moist, whilst being kept in the saline solution frozen at -10°C, and it was used in the period of 3 to 4 weeks (12, 13).

Twenty samples were used for the purpose of this study.

Implants

In this research, we used 10 cylindrical non-self-tapping Nobel Biocare Replace implants with dimensions 3.5x10 mm, and 10 self-tapping implants Bredent diameter 3.5x10 mm.

Measurement of primary stability

Primary implant stability is measured by resonance frequency Osstell mentor instrument.

The method of resonant frequency analysis (RFA - Resonance Frequency Analysis) represents a non-invasive diagnostic method that enables the measurement of clinical implant stability and monitoring biological response of tissues, as well as osseointegration in the function of time. The method that analyses the resonance frequency uses sophisticated technology with computer-based measurement of the resonant frequency, which is determined by two parameters: the degree of bone density at the intermediate implant – bone, and the level of marginal alveolar bone around the transducer (14).

The measured amplitude of the resonance frequency is displayed numerically and graphically on the analyser, and its maximum value represents the stability of the implant quantified through ISQ units (implant stability quotient units) which is the stability coefficient of the implant. The resulting value in the ISQ units reflects the rigidity of the system transducer - implant - bone and calibration parameters of the transducer. Its measures scale from 0 ISQ (3500 Hz) to 100 ISQ units (8500 Hz), where the higher value of ISQ indicates stronger implant stability. The method is non-invasive and comfortable for patients since it does not cause painful sensations and takes 1 to 2 seconds (13, 14).

Osstell is representative of the RFA technique, which was first tested in 1997. Instruments include an Osstell transducer and Osstell analyzer connected to a PC, or standalone. The transducer is either S-shaped or L shaped screw (SmartPeg) and screwing firmly positioned on the implant or its superstructure (a force from 4 to 5 N/cm^2), and it is composed of two piezo ceramic transducers. High energy pulse like sinusoidal pulse oscillation continuously excites the implant to record the mechanical vibrations of the intermediate zone of the implant and bone (13, 14).

The research was conducted in two phases

Phase I

The first phase included the preparation of implants sites in bone with standard surgical technique using the drill as recommended by the manufacturer's protocol. After that, the implants are embodied in the bones at the 5mm depth, which provides conditional percentage contact of implants and bone of 50% (Figure 1). All implants are set to prepared bearing mechanic by using the force of 35 N/cm^2 fixed on phisiodispenser Bien Air Chiropo 980.

SmartPegs are placed on the implants and, according to the recommendation from the manufacturer, primary stability is measured from four different directions with the help of an Osstel mentor instruments, with mean value being the reference value. (Figure 2)

In this way, we simulated immediate placing of implants in cases when surface of implants has no full contact with bone wall side of post-extraction alveoli.

Phase II

The second phase of the study included the preparation of the implant site and their placing at its full length of 10 mm, which provides conditional contact between the implant and the bone of 100%.

SmartPeg is placed on the implants and according to the recommendation from the manufacturer, primary stability is measured from four different directions with the help of an Osstel mentor instruments, with mean value as the reference value. (Figure 2)

SmartPegs are placed on the implants and primary stability is measured from four different directions with the help of an Osstell mentor instruments, with mean value as the reference value.

Statistical analysis

The Student's t-test was used for statistical data analysis and comparative analysis of the average value of the primary stability between self-tapping and non self-tapping implants.
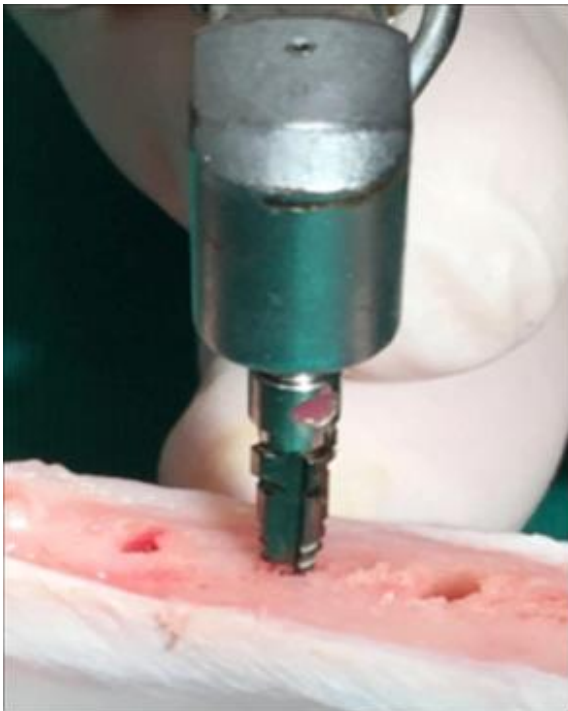
Figure 1.

Figure 2.

Results

In the first phase of the study, the average value of primary stability in non-self-tapping implants was 30 ISQ (Graph 1). On the other hand, the average value obtained in the self-tapping implants varied significantly compared to non-self-tapping implants, and its value was 42 ISQ. Comparative analysis by Student's t-test showed a statistically significant difference in stability between primary stability between self-tapping and non-self-tapping implants.

In the second phase of the study, approximately the same values have been recorded: i.e. 70 ISQ, on average. The comparative analysis made by Student's t-test showed that there was no statistically significant difference in primary stability between different implantation systems and macro design of implants (Graph 2).

	ISQ Value
■ Nobel biocare	30
■ Bredent Blue Sky	42
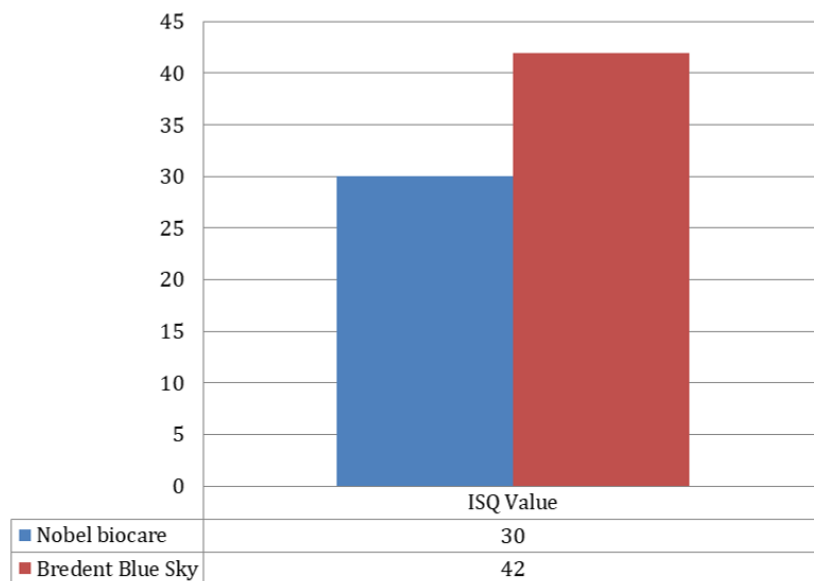
Graph 1. Values of primary stability at a depth of 5 mm

	ISQ Value
■ Nobel biocare	70
■ Bredent Blue Sky	72
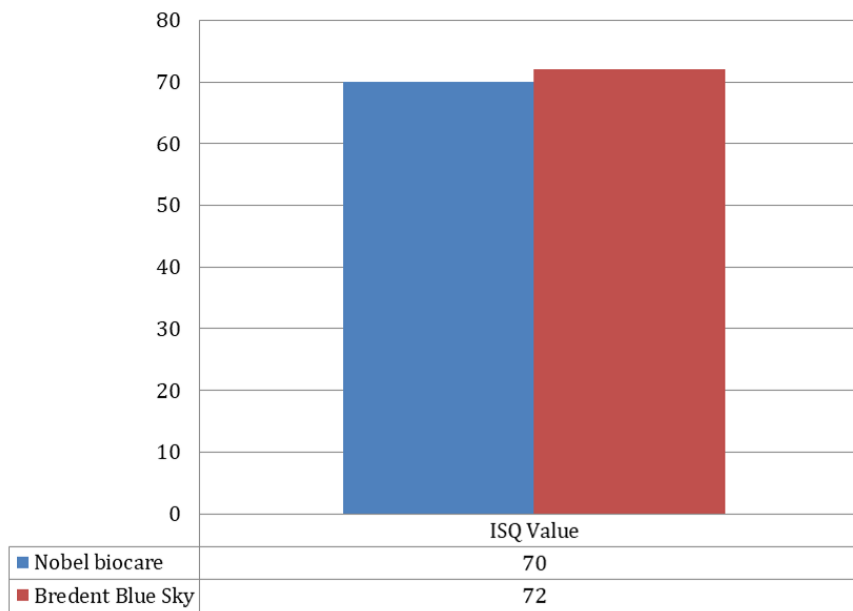
Graph 2. Values of primary stability at a depth of 10 mm

Discussion

Numerous studies have shown that the design of the implant plays an important role in achieving good primary stability. Also, the primary stability depends on the quantity and quality of available bone tissue, depending on the localization and individual dispositions (11, 13, 15, 16). Studies on human cadavers showed that the value of the primary stability of 12 mm length implant into the region of the extracted lower premolars and immediate implants amounted to an average of 69 ISQ (ranging between 64 and 73) (15).

However, a particular problem was to achieve adequate primary stability in the region of the upper lateral sector where is the bone type 3 and type 4 according to the classification of Leksholma and Zarb, bone dominated by spongiosa with a thin cortical layer of compact (11). The researches show that better primary stability can be achieved with the use of self-tapping implants in combination with lateral and apical bone condensation in low bone quality cases (6). In our study, the values of primary stability do not significantly differ compared to macro design of implants when the percentage contact of the implant and the bone was 100%, and when implants were built to their full 10 mm length using standard surgical installation techniques.

Regarding the immediate implant placement, the problem is to achieve adequate primary stability due to the difference in the shape of implants and alveolus of the extracted tooth during, where it is impossible to achieve full contact between the implant and the bone. In self-tapping implants, there are vertical cuts in the apical third that enable the implant without taps. Also, bone splinters, occurring during twisting, were used for greater implant-bone contact (8, 9).

In our study, due to percentage contact between the bone and the implant of 50%, when the implants were built to a 5 mm depth, the obtained values of primary stability are statistically significantly different in self-tapping compared to non self-tapping implants.

Conclusion

Macro design plays an important role in achieving adequate primary stability. This study recorded statistically significant higher values of primary stability in self-tapping compared to non-self-tapping implants in the contact between the implant with the bone of 50% in percentage terms, which in turn recommends this macro design during instalment of immediate implants when the contact of the implant with bone tissue is not complete.

References

1. Branemark PI, Hansson BO, Adell R, Breine U, Lindstrom J, Hallen O, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg 1977; 16:1-132. [PubMed]
2. Nedir R, Bischof M, Szmukler-Moncler S, Bernard JP, Samson J. Predicting osseointegration by means of implant primary stability. A resonance-frequency analysis study with delayed and immediately loaded ITI SLA implants. Clin Oral Impl Res 2004; 15:520-8. [CrossRef] [PubMed]
3. Vlahović Z, Mihailović B, Lazić Z, Golubović M. Comparative radiographic and resonance frequency analyses of the peri-implant tissue after dental implants placement using flap and flapless techniques: an experimental study on domestic pigs. Vojnosanit Pregl 2013; 70:586-94. [CrossRef] [PubMed]
4. Meredith N. Assessment of implant stability as a prognostic determinant. Int J Prosthodont 1998; 11:491-501. [PubMed]
5. Chong L, Khocht A, Suzuki JB. Effect of implant design on initial stability of tapered implants. J Oral Implantol 2009; 35:130-5. [CrossRef] [PubMed]
6. Lekholm U, Zarb GA. Patient selection and preparation. In: Branemark P-I, Zarb GA, Albrektsson T, editors. Tissue-Integrated Prostheses: Osseointegration In Clinical dentistry. Chicago: Quintessence; 1985. p. 199-209. [PubMed]
7. Markovic A, Calvo-Guirado JL, Lazić Z, Gomes-Moreno G, Ćalasan D, Guardia J, et al. Evaluation of primary stability of self-tapping and non-self-tapping dental implants. A 12-week clinical study. Clin Implant Dent Relat Res 2013; 15:341-9. [CrossRef] [PubMed]
8. Esposito M, Grusovin MG, Willings M, Coulthard P, Worthington HV. Interventions for replacing missing teeth: different times for loading dental implants. Cochrane Database Syst Rev 2007; 1:CD003878. [CrossRef] [PubMed]
9. Szmukler-Moncler S, Piattelli A, Favero GA, Dubruille JH. Considerations preliminary to the application of early and immediate loading protocols in dental implantology. Clin Oral Implants Res 2000; 11:12-25. [CrossRef] [PubMed]
10. Pearce Al, Richards RG, Milz S. Animal models for implant biomaterial research in bone: a review. Eur Cell Mater 2007; 13:1-10. [CrossRef] [PubMed]
11. Albrektsson T, Wennerberg A. The Impact of Oral Implants — Past and Future, 1966–2042. J Can Dent Assoc 2005; 71:327. [PubMed]
12. Sedlin ED, Hirsch C. Factors affecting the determination of the physical properties of femoral cortical bone. Acta Orthop Scand 1966; 37(1):29-48. [CrossRef] [PubMed]
13. Sennerby L, Merefith N. Implant stability measurements using resonance frequency analysis: biological and biomechanical aspects and clinical implications. Periodontol 2000 2008; 47:51-66. [CrossRef] [PubMed]
14. Meredith N, Book K, Friberg B, Jemt T, Sennerby L. Resonance frequency measurements of implant stability in vivo. A cross - sectional and longitudinal study of resonance frequency measurements on implants in the edentiolous and partially dentate maxilla. Clin Oral Implants Res 1997; 8:226-33. [CrossRef] [PubMed]
15. Akkocaoglu M, Uysal S, Tekdemir I, Akca K, Cehreli MC. Implant design and intraosseous stability of immediately placed implants: a human cadaver study. Clin Oral Implants Res 2005; 16:202-9. [CrossRef] [PubMed]
16. Lioubavina-Hack N, Lang NP, Karring T. Significance of primary stability for Osseointegration of dental implants. Clin Oral Implants Res 2006; 17:244-50. [CrossRef] [PubMed]

KOMPARATIVNA REZONANTNO - FREKVENTNA ANALIZA PRIMARNE STABILNOSTI KOD RAZLIČITOG DIZAJNA IMPLANTATA

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Primarna stabilnost implantata označena je kao preduslov i jedan od faktora procjene postizanja uspješne oseointegracije. Više faktora utiče na primarnu stabilnost od kojih su tri najznačajnija: dizajn implantata, hirurška tehnika ugradnje i kvalitet koštanog tkiva.

Cilj ovog rada bio je odrediti uticaj različitog makrodizajna na primarnu stabilnost implantata, kao i procjenu primarne stabilnosti u odnosu na procentualni kontakt površine implantata i kosti.

Studija je sprovedena u *in vitro* uslovima, a kao analog humane kosti u istraživanju su korišćena svinjska rebra kortikalnog sloja debljine 2 mm, cilindrični neurezujući implantati marke Nobel Biocare Replace 3,5x10 mm i samourezujući implantati marke Bredent dimenzija 3,5x10 mm. Primarna stabilnost implantata mjerena je metodom rezonantne frekvencije Osstell mentor aparatom, a za statističku obradu podataka primijenjen je Studentov t-test.

Prosječne vrijednosti primarne stabilnosti nakon tri mjerenja na dubini od 5 mm kod neurezujćih Nobel Biocare iznosila je 30 ISQ. Kod samourezujćih Bredent implantata vrednosti su bile 42 ISQ. Na dubini od 10mm izmjerene su sledeće prosječne vrijednosti primarne stabilnosti: Nobel Biocare 70 ISQ i Bredent 72 ISQ. Studentovim t-testom (p < 0,05) utvrđeno je da postoji međusobno statistički značajna razlika u vrijednostima primarne stabilnosti kod različitog dizajna implantata.

Dizajn implantata igra bitnu ulogu u postizanju adekvatne primarne stabilnosti. U ovoj studiji izmjerene su statistički značajne više vrijednosti primarne stabilnosti kod samourezujćih u odnosu na neurezujće implantate na dubini od 5 mm, što ih preporučuje kod imedijantne ugradnje.

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Ključne reči: *primarna stabilnost, rezonantna frekvencija, implantati*

INTERINDIVIDUAL AND INTRAINDIVIDUAL PHARMACOKINETIC VARIABILITY OF TACROLIMUS WITHIN THE FIRST YEAR AFTER RENAL TRANSPLANTATION: EFFECT OF CYP3A5 GENE POLYMORPHISM

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The aim of this study was to evaluate potential influence of cytochrome P450 3A5 (CYP3A5) 6986A>G gene polymorphisms on inter- and intravariability (IPV) in tacrolimus (Tac) exposure within the first year after renal transplantation. Secondary, we aimed to analyze the change in distribution of patients regarding IPV between early (<6 months) and late post-transplant period (>6 months). The study enrolled 91 renal transplant recipients, who were on Tac-based immunosuppressive protocol. Dose–adjusted concentration (C0/D) of Tac was used as a measure of Tac exposure, while coefficient of variation (CV%) and mean absolute deviation (MAD%) of C0/D as IPV parameters. Individuals carrying CYP3A5*1/*3 genotype had lower C0/D than CYP3A5*3/*3 carriers within the entire observation period (p < 0.01). The study reported higher IPV in a period of 1-6 months compared to a period of 7-12 months post-transplant, for CV% and MAD% (p < 0.05). The results showed that there was no difference in IPV regarding CYP3A5 genotype. Considering CV%, 32% and 24% of the patients had high IPV (above 30%) in the first and second half of the first post-transplant year, respectively. Analyzing the MAD%, 13% and 7% of the patients had high variability of Tac exposure in the first and second half of the first year, respectively. This study confirms that the CYP3A5 gene polymorphism contributes to the interindividual, but not in intraindividual, variability in Tac exposure within the first post-transplant year.

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Key words: *CYP3A5, renal transplantation, tacrolimus, interindividual pharmacokinetic variability, intraindividual pharmacokinetic variability*

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Introduction

Tacrolimus (Tac) is widely used as part of the most immunosuppressive protocols after renal transplantation (Tx). Its clinical use is complicated by large interindividual pharmacokinetic variability and chronic nephrotoxicity, which may lead to graft loss in the late post-transplant period (1). The attempts to replace Tac with other, equally effective but less toxic immunosuppressive drugs, have had limited success. Therefore, Tac will remain the first choice immunosuppressive agent for the next decade or so, and optimization of Tac-based immunosuppressive therapy is of utmost importance (2). Hence, therapeutic drug monitoring of Tac is a necessary tool with a view to reduce the toxicity and improve efficacy. Still, there are patients who will experience adverse effects or lack in efficacy with the Tac concentration in the optimal range, which suggests that additional investigation of the factors that may contribute to these effects is needed (3, 4). It is showed that genetics was one of the main determinants, along with demographic factors and drug–drug interactions that affects interindividual variability in Tac pharmacokinetics (4, 5). Gene polymorphism (6986 A > G) present within cytochrome P450 3A5 (CYP3A5) is assumed to be the major determinant of the Tac pharmacokinetic variability. The presence of A allele at the polymorphic site in the CYP3A5 gene suggests that an individual has a functionally active enzyme (expresser) and carries one of the two genotypes

(CYP3A5*1/*1 or CYP3A5*1/*3). Non – expressers carry CYP3A5*3/*3 genotype and do not have active enzyme (4, 6). The role of CYP3A5 gene polymorphism is well documented for the early post-transplant period (up to 3 months post-transplant), while its role in the late post-transplant period (after 6 months post-transplant) is not elucidated in its entirety (4, 7, 8).

Besides interpatient pharmacokinetic variability, tacrolimus exerts considerable intraindividual or intrapatient variability (IPV) in its pharmacokinetics. Intrapatient variability represents fluctuation in Tac concentrations within an individual patient over a certain period of time during which the Tac dose is unchanged (2). Patients with highly fluctuated Tac concentrations are at risk either for underexposure and rejection or to overexposure and Tac toxicity. Recently, it is demonstrated that high IPV in Tac exposure may be a predictor for long-term adverse outcome after Tx (9). Still, whether CYP3A5 genotype also affects Tac IPV or not, is not completely understood.

The aim of this study was to evaluate the potential influence of CYP3A5 (6986A > G) gene polymorphisms on inter- and intraindividual variability in Tac exposure within the first year after Tx. Secondary, we aimed to analyze the change in the distribution of patients regarding Tac IPV between early (< 6 months) and late post-transplant period (> 6 months).

Patients and methods

Patients

The pharmacokinetic–pharmacogenetic study was conducted at the Clinic of Nephrology, Clinical Centre Niš, Serbia and at the Research Centre for Biomedicine, Faculty of Medicine, University of Niš, Serbia between 2008-2016. The genotyping analysis included 91 patients who had transplantation surgery at Clinical Centre Niš and were monitored at the Clinic of Nephrology during the study period. The study involved a period from 1 up to 12 months after Tx, with 1564 routine controls at the Clinic and in 1515 cases Tac concentration was measured, which was valid for the analysis. Of all patients enrolled into pharmacokinetic study, 56 were men and 35 were women, mean age 40.57 ± 10.74 at the beginning of the study, 66 out of 91 patients had living donor transplantation and 25 out of 91 got their organ from deceased donors. Besides standard immunosuppressive therapy, patients also received antihypertensive drugs (beta–blockers, bisoprolol, metoprolol or carvedilol and calcium channel blockers, amlodipine in monotherapy, as well as in the combination) and omeprazole, pantoprazole or ranitidine as gastroprotective. The study was approved by the Ethics Committee of Medical Faculty Nis and fully informed written consent was obtained from each patient (01-10204-13 from 2012. and 12-2307-2/5 from 2016.).

Immunosupressive protocol

All patients started with a quaternary immunosuppressive protocol that besides Tac included intravenous methylprednisolone (MP), with an initial dose of 0.5 g/day and 2 or 3 days later it was switched to prednisone (Pre), initial dose of 1 mg/kg/day, mycophenolate mofetil (MMF), 2 g/day or mycophenolic acid (MPA), 1440 mg/day orally and 20 mg monoclonal antibody basiliximab (Bas) which was administered at the first and the fourth day after transplantation. The first oral Tac dose was administered on day 5 post-transplant at 8.00 hr before breakfast 0.05 mg/kg. Furthermore, Tac was administered twice daily (08.00 h and 20.00 h) for twice-daily Tac formulation or once daily (08.00h) for once-daily Tac formulation and the dose was adjusted according to the trough concentration of a drug in the blood, in order to maintain drug trough concen-tration (C0) in the appropriate range (5 - 15 ng/mL). Tacrolimus blood trough concentration was measured by immunoassay method according to the manufacturer's instructions (Architect, Abbott, Ab-bott Park, IL, USA).

Pharmacokinetic data

For the purpose of the analysis we used a daily dose, trough concentration (C0) and dose–adjusted concentration (C0/D) of Tac from routine controls at the Clinic (1515) during the first year after Tx. As not all patients received a constant drug dose in the observed period, we calculated C0/D (ng mL^{-1}/mg day^{-1}) of Tac as C0 (ng/mL) divided by the corresponding dose of Tac (mg/day).

Intrapatient variability

The IPV in Tac exposure were calculated in a period of 1-6 months, in a period of 7-12 months and in a period of 1-12 months post-transplant. In the conducted study we used two parameters of Tac IPV: coefficient of variation (CV%), calculated as the mean of C0/D divided by standard deviation of C0/D for the concrete observation period and mean absolute deviation (MAD%), calculated as:

$$MAD\% = \frac{1}{N}\sum_{n=1}^{N}\frac{abs(x_n - \overline{x})}{\overline{x}} \times 100$$

where $\overline{x}$ is the mean C0/D of all available samples in the period of 1-12 months after Tx; X_n is an individual value of C0/D measured in the period mentioned, and N is the number of all available values for an individual patient.

Biochemical data

A fasting blood sample was taken from each

patient during routine control at the Clinic. Of the whole blood sample, 200 µL was taken for DNA isolation. DNA was extracted from the whole blood with EDTA as an anticoagulant using Genomic DNA Purification Kit (Fermentas, Thermo Scientific, Lithuania) according to the manufacturer's instructions. Serum level of albumin (ALB), urea (URE), creatinine (CRE) and the activities of aspartate aminotransfe-rase (AST) and alanine aminotransferase (ALT) were measured by standard methods in the Biochemical laboratory at Clinic of Nephrology. Analyses were performed on the Automated random access clinical chemistry analyzer (ERBA XL – 600, ERBA Diagnostics Mannheim GmbH, Mannheim, Germany). Glomerular filtration rate was estimated (eGFR) by MDRD formula for creatinine clearance.

Table 1. Clinical and demographic characteristics of the renal transplant recipients in different post-transplant periods

PARAMETERS	Period 1-6 months after Tx	Period 7-12 months after Tx	Period 1-12 months after Tx
Number of check-ups at the Clinic	877	638	1515
Check-up per patient	8.91 ± 4.42 8 (6-10)	8.69 ± 2.08 8 (7-10)	16.65 ± 6.12 16 (13-21)
Sex (male/ female)	56/35		
Age (years)	40.57 ± 10.74 40 (32-49)		
Donor type (living/deceased)	66/25		
Body weight (kg)	72.00 ± 13.97 70 (64-80)	74.06 ± 12.95 72 (66-80)	73.10 ± 13.36 71 (65-80)
CRE (µmol / L)	146.55 ± 43.87 139 (116-177)	136.04 ± 40.79 130 (111-149)	141.65 ± 42.12 135 (113-163)
eGFR (mL/min / 1.73m^2)	50.06 ± 16.67 49 (38-58)	53.82 ± 17.88 53 (42.50-61)	51.74 ± 17.07 51 (41-60)
URE (mmol / L)	9.22 ± 4.34 9 (7-11)	8.15 ± 3.28 8 (6-10)	8.49 ± 3.72 8 (6-10)
ALB (g/L)	40.31 ± 4.15 41 (38-43)	41.23 ± 4.35 41 (39-44)	40.42 ± 4.28 41 (38-43)
ALT (U/L)	41.37 ± 38.42 28 (20-44)	38.44 ± 32.08 28 (20-44.50)	39.87 ± 35.00 28 (20-45)
AST (U/L)	22.93 ± 15.07 20 (16-25)	22.93 ± 15.07 21 (16.50-26)	24.18 ± 15.25 20.50 (16-26)
OD/TD Tac formulation	13/78		
Dose of Tac (mg/day)	6.81 ± 3.76 6.00 (4.00-9.00)	4.66 ± 2.77 4.00 (3.00-6.00)	5.71 ± 3.49 5.00 (3.00-7.00)
C0 of Tac (ng/mL)	8.82 ± 2.93 8.00 (7.00-10.00)	7.26 ± 2.41 7.00 (6.00-9.00)	7.95 ± 2.89 8.00 (6.00-10.00)
C0/D of Tac (ng mL^{-1} / mg kg^{-1} day^{-1})	1.60 ± 0.84 1.47 (0.97-2.03)	1.98 ± 1.18 1.67 (1.20-2.40)	1.77 ± 1.02 1.55 (1.08-2.15)
Dose of Pre (mg/day)	14.94 ± 8.20 10 (10–20)	10.05 ± 2.23 10 (10-10)	12.92 ± 6.85 10 (10-12)
MPA+Tac / mTOR+Tac	84/7		
Dose of MPA (mg/day)*	1059.32 ± 327.65 1080 (720-1440)	1095.61 ± 318.33 1080 (720-1440)	1119.32 ± 324.75 1080 (720-1440)
CYP3A5 genotype (*1*3 / *3*3)	13/78		

Data are expressed as median (interquartile range) or number; eGFR was calculated by MDRD formula.
*MMF dose was calculated on MPA dose
Cre – serum creatinine level; Ure – serum urea level; Tac – tacrolimus; OD/TD – once daily/twice daily; Pre – prednisone; MMF - mycophenolate mofetil ; MPA - mycophenolic acid; C0 - tacrolimus trough concentration; C0/D – tacrolimus dose - adjusted trough concentration; CRE- serum creatinne; URE-serum urea; ALB-serum albumin; ALT- alanine aminotransferase; AST- aspartate aminotransferase;

Genotyping CYP3A5 polymorphism

Genotyping was performed using TaqMan® Drug Metabolism Genotyping Assays for CYP3A5*3 (C_26201809_30) (Applied Biosystems, Carlsbad, CA, USA) on the Mx3005P Real-Time PCR System (Agilent Technologies), according to the manufacturer's instructions.

Statistical analysis

The characteristics of the study group were expressed as mean ± standard deviation and median (interquartile range) or number. We used Student t-test for normally distributed data and Mann Whitney U test for data that were not normally distributed to compare pharmacokinetic data and IPV between the groups of patients based on the CYP3A5 genotypes within same period after Tx. All analyses were performed with SPSS statistical analysis software, version 16.0 (SPSS, Chicago, IL, United States) at the significance level set at $p < 0.05$.

Results

The characteristics of the study population are given in Table 1. Of all routine controls at the Clinic (1564), 1515 was valid for the pharmacokinetic study (C0 was measured), 877 took place in a period of 1-6 months, whereas 638 took place in a period of 7-12 months post-transplant. Considering routine control per patient, approximately every patient went to the Clinic 16 times during the first year, 8 times per six months.

Table 2. Dose, trough concentration and dose–adjusted trough concentration of Tac in relation to CYP3A5 genotype up to 12 months after transplantation

Genotype	Period 1-6 months after Tx	Period 7-12 months after Tx	Period 1-12 months after Tx
CYP3A5	**Dose (mg/day)**		
*1/*3	9.23 ± 4.36 9.00 (6.00-11.00)	6.09 ± 3.65 6.00 (4.00-8.00)	7.94 ± 4.36 8.00 (5.00-10.00)
*3/*3	6.19 ± 3.22 6.00 (4.00-8.00)	4.56 ± 2.77 4.00 (3.00-6.00)	5.50 ± 2.77 5.00 (3.00-7.00)
P value	0.000	0.000	0.000
CYP3A5	**C0 (ng/mL)**		
*1/*3	8.56 ± 2.97 9.00 (7.00-11.00)	6.89 ± 2.78 7.00 (5.00-9.00)	7.87 ± 3.01 8.00 (6.00-10.00)
*3/*3	8.28 ± 2.84 8.00 (6.00-10.00)	7.18 ± 2.38 7.00 (6.00-9.00)	7.81 ± 2.71 8.00 (6.00-9.00)
P value	0.052	0.492	0.254
CYP3A5	**C0/D** **(ng mL^{-1} / mg day^{-1})**		
*1/*3	1.03 ± 0.40 0.97 (0.74-1.20)	1.36 ± 0.64 1.18 (0.90-1.68)	1.17 ± 0.54 1.05 (0.81-1.40)
*3/*3	1.62 ± 0.86 1.46 (1.03-2.03)	2.06 ± 1.31 1.67 (1.21-2.47)	1.81 ± 1.09 1.54 (1.11-2.15)
P value	0.000	0.000	0.000

Data are expressed as mean±standrad deviation and median (interquratile range)
C0 - tacrolimus trough concentration; C0/D – tacrolimus dose - adjusted trough concentration

Table 2. demonstrates comparison of Tac dose, C0 and C0/D among patients with different CYP3A5 genotype in the different periods after renal transplantation. Individuals carrying CYP3A5*1/*3 genotype had higher dose requirements and lower C0/D than CYP3A5*3/*3 carriers within the entire study period (p = 0.000). Alternatively, there was no difference in C0 in relation to CYP3A5 genotype.

Therefore, carriers of the active form of the CYP3A5 enzyme required higher daily dose of Tac to maintain optimal C0 (5-15ng/mL), not only in the early period (<6 months post-transplant), but as well in the late period after Tx (>6 months post-transplant).

Considering, Tac IPV, Graph 1. demonstrates higher IPV in a period of 1-6 months compared to a period of 7-12 months post-transplant, for both parameters, CV% (27.10 ± 11.23 vs. 23.62 ± 10.07) and MAD% (20.97 ± 9.13 vs. 18.33 ± 8.12). The IPV during the entire study period (29.76 ± 10.84 for CV% and 23.41 ± 8.67 for MAD%) was higher than in the first and second six months (for MAD%), but values were more close to the IPV in the first six months. Furthermore, there was no difference in CV% between IPV in the first six months and IPV during the entire follow-up period, suggesting that early period significantly contributes to the 12 months post-transplant Tac IPV.

The obtained results showed that there was no difference in Tac IPV regarding CYP3A5 genotype (Graph 2).

Graph 3. shows the change in distribution of patients regarding Tac IPV between the observed periods after Tx. The results of the present research demonstrated that slightly above 50% of patients had medium IPV (between 15 and 30%) in both observed periods. Considering CV%, 32% had high IPV and 12% of the patients had low IPV in the first six months post-transplant. Furthermore, in the second half of the first post-transplant year, fewer patients had high IPV (24%), while 20% of the patients were low variable regarding Tac exposure. Analyzing the MAD%, above 50% of renal transplant recipients had medium IPV, while 13% in the first and 7% in second half of the first year had high variability.
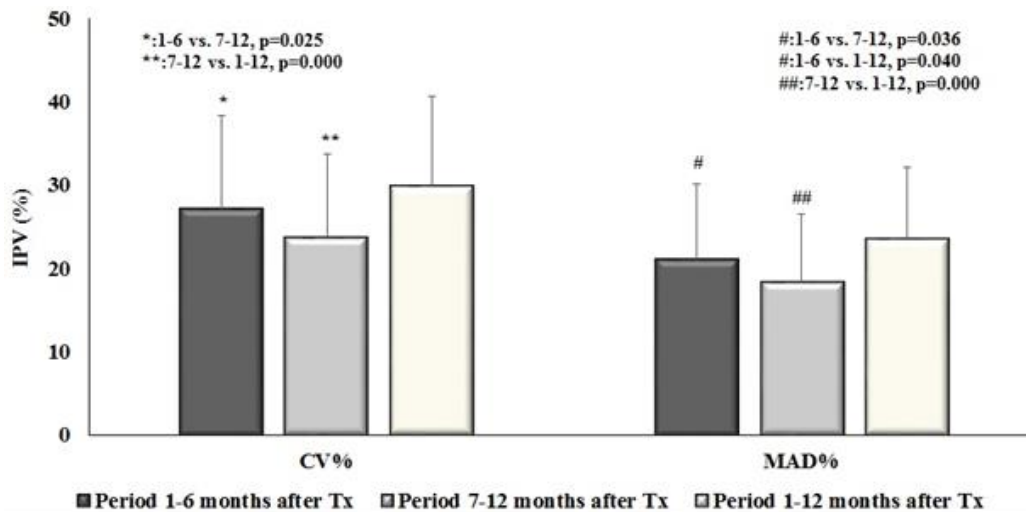
Graph 1. Intrapatient variability in Tac exposure following 12 months after Tx
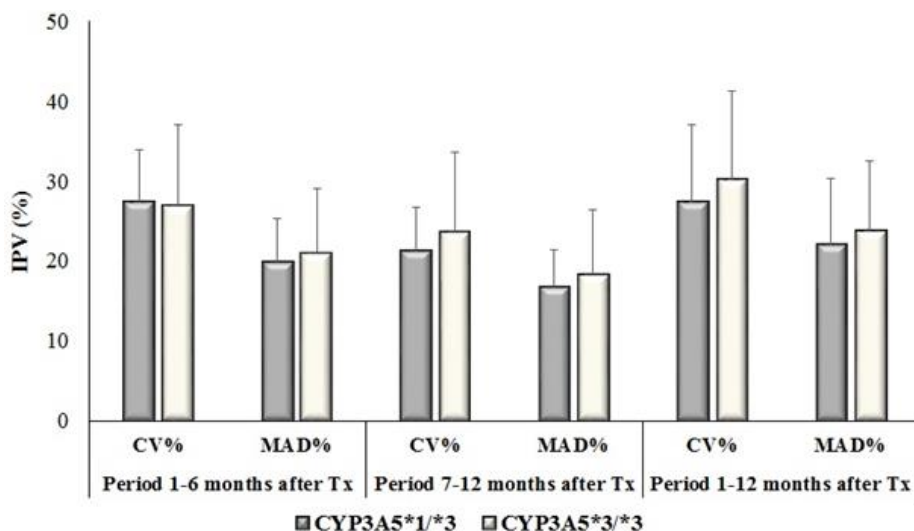
Graph 2. Intrapatient variability in Tac exposure with respect to CYP3A5 genotype
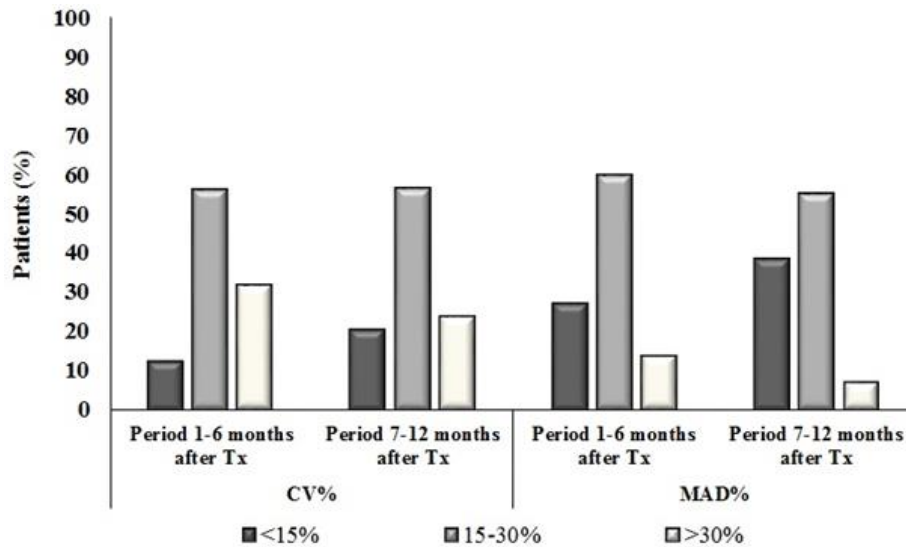
Graph 3. Distribution of Tac IPV among renal transplant recipients

Discussion

Tacrolimus is considered to be the gold standard in solid organ transplantation, but the narrow therapeutic index, genetic variations and the wide range of inter- and intraindividual pharmacokinetic variability limit its application. Considering this, there is a constant aspiration to identify the factors which contribute to this variability. Cytochrome P450 3A5 gene has 11 different polymorphisms identified to date, but 6986A>G polymorphism was the most extensively studied and indicated as the main determinant of Tac interindividual pharmacokinetic variability. It is characterized by adenine (A) to guanine (G) transition at position 6986 within intron 3 of the CYP3A5 gene and consequently the absence of the active enzyme form (4). The findings of the conducted study reported that the carriers of CYP3A5 *1/*3 genotype compared to CYP3A5 *3/*3 carriers required higher Tac daily dose to maintain drug concentration in the optimal range within one year after Tx (Table 2). The obtained reuslts were in accordance with the previous studies, which pointed genetic factor as a major predictor of Tac dose requirements (4, 10, 11). Still, the majority of the previous studies emphasized the role of CYP3A5 polymorphism in the early period after renal transplantation and have been trying to connect this polymorphism with outcomes in the early post-transplant period (12, 13, 14). This study showed that CYP3A5 polymorphism might have been a significant predictor of Tac exposure in late post-transplant periods (after 6 months), and suggested its consideration as an important contributor of Tac chronic nephrotoxiciy (15). Besides variability in CYP3A5 gene, CYP3A4*22 gene polymorphism is associated with lower CYP3A4 activity and seems to be important determinant of Tac clearance and dose requirements, especially in CYP3A5 non-expressers. Also, gene polymorphisms in ABCB1

gene, encoding P-glycoprotein (PGP), have been extensively studied, but results remained inconsistent (10, 16). Still, the later research indicated that donor CYP3A5*1 and ABCB1 TT genotype (ABCB1 3435 TT associated with lower PGP activity) may contribute to local Tac transport in transplanted kidney and therefore have a potential role in chronic nephrotoxicity of Tac (16, 17).

In addition to interindividual variability, there is a growing number of clinical studies which support the fact that intraindividual (i.e. intrapatient) variability in Tac exposure correlates with inferior long-term renal transplantation outcomes (9). The previous studies showed a wide range of Tac IPV, with some individuals having a Tac IPV of < 5%, and others having a variability of > 50%, but with an average Tac IPV between 15 and 30% (2). This is in accordance with the results of our study, which demonstrated that an average IPV was between 20 and 30%. Borra et al. showed that high IPV is closely associated with a composite end point consisting of graft loss, biopsy-proven chronic allograft nephropathy and doubling of plasma creatinine concentration (18). Different factors were assumed to affect Tac IPV, such as concomitant diet, diarrhoeal illnes, drug-drug interactions, genetic factors, substitution of different Tac formulations and non-adherence (2). The present study did not show an association between CV% or MDA% and CYP3A5 genotype, which is in accordance with the study of Spierings et al (19). and Pashaee et al. (20). Nearly all authors emphasize non-adherence as one of the dominant causes of Tac IPV, and therefore they suggested that IPV may be considered as an objective measure of adherence to immunosuppresive regimen. Some scientists have reported an improved adherence and decrease of IPV after switching from twice-daily to once-daily Tac formulation (2). Kurnatowska et al. demonstrated that conversion from twice-daily form

to once-daily form resulted in slightly lower and more stable Tac blood concentration, with a significant decrease in CV of Tac blood levels (44% versus 22%) (21). Additionally, Stifft et al. demonstrated reduced IPV after conversion from twice-daily to once-daily Tac formulation, especially in patients with the CYP3A5*1 allele. In addition, Stifft et al. suggested that decreased variability in Tac exposure is not only a consequence of improved adherence, but also intrinsic pharmacokinetic properties of prolonged-release Tac formulation (22).

Our study showed that 30% of the patients had high IPV (over 30%, expressed as CV%) within the first six months post-transplant and almost 24% of patients in the other half of the year. Rodrigo et al. used CV% as a measure of IPV, and demonstrated that more than one third of patients had a blood level CV above 30% estimated between 4 and 12 month post-transplantation. They showed that acute rejection, re-transplant and CV greater than 30% were the only variables related to de novo donor-specific antibodies, which can lead to antibody-mediated rejection and graft loss (23). Conversely, patients with high TAC IPV, expressed by MAD%, were less than 15%. Still, most of the studies which showed the association between adverse outcomes and Tac IPV used CV% as the parameter of IPV. The advantage of the MAD% over CV% is that it is less susceptible to outliers, because MAD% uses the absolute deviations from the mean, whereas CV% uses the squared deviations from the mean. The further researches should provide the appropriate and mutual parameter of Tac IPV in order to compare different studies and investigate potential determinants of Tac IPV.

Additionally, since renal transplant recipients are not on a stable Tac dose in the early post-transplant period and they often use interacting drugs [such as antibiotics and (pulse) glucocorticoids] in this period, majority of the researchers used only data on Tac exposure measured in the period of 6–12 months post-transplantation. Therefore, in this particular study, both periods were used to evaluate Tac IPV. Still, almost one quarter of the patients was high variable in period of 7-12 months post-transplant. Shuker et al. emphasize that risk of developing long-term adverse outcomes decreases with time, so patients with high IPV between 6-12 months post-transplant certainly are at a higher risk of adverse outcomes, but this risk is reduced with the time (9).

In conclusion, this study confirms that the CYP3A5 gene polymorphism contributes to the interindividual, but not in intraindividual variability in Tac dose requirements and exposure within the first post-transplant year. Still, renal transplant recipients express high Tac IPV, with one third of the patients in the first and one quarter of the patients in the second half of the first post-transplant year with CV% above 30%. Conversely, distribution of the patients considering MAD% as a measure of Tac IPV significantly differed between early and late post-transplant period, but with less highly variable patients. Therefore, appropriate measure of Tac IPV should be established. Further studies should be focused on the factors which may contribute high Tac IPV, such as non-adherence or drug formulation.

Conflict of interest statement None declared.

References

1. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet 2004; 43: 623. [CrossRef][PubMed]
2. Shuker N, van Gelder T, Hesselink DA. Intra-patient variability in tacrolimus exposure: causes, consequences for clinical management. Transplant Rev (Orlando) 2015;29: 78-84. [CrossRef][PubMed]
3. Press RR, de Fijter JW, Guchelaar HJ. Individualizing calcineurin inhibitor therapy in renal transplantation—current limitations and perspectives. Curr Pharm Des 2010; 16:176-86. [CrossRef][PubMed]
4. Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part I. Clin Pharmacokinet 2010; 49: 141-75. [CrossRef][PubMed]
5. Velickovic-Radovanovic R, Mikov M, Catic-Djordjevic A, Stefanovic N, Mitic B, Paunovic G, et al. Gender-dependent predictable pharmacokinetic method for tacrolimus exposure monitoring in kidney transplant patients. Eur J Drug Metab Pharmacokinet 2015; 40: 95-102. [CrossRef][PubMed]
6. MacPhee IA. Pharmacogenetic biomarkers: cytochrome P450 3A5. Clin Chim Acta 2012; 413:1312-7. [CrossRef][PubMed]
7. Stefanović N, Cvetković T, Veličković-Radovanović R, Jevtović-Stoimenov T, Stojanović D, Živković N. Significance of CYP3A5 gene polymorphism in Serbian renal transplant patients. Acta Medica Medianae 2013; 52: 33-8. [CrossRef]
8. Stefanović NZ, Cvetković TP, Jevtović-Stoimenov TM, Ignjatović AM, Paunović GJ, Veličković-Radovanović RM. Investigation of CYP 3A5 and ABCB1 gene polymorphisms in the long-term following renal transplantation: Effects on tacrolimus exposure and kidney function. Exp Ther Med 2015; 10: 1149-56. [CrossRef][PubMed]
9. Shuker N, Shuker L, van Rosmalen J, Roodnat JI, Borra LC, Weimar W, et al. A high intrapatient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation. Transpl Int 2016; 29: 1158-67. [CrossRef][PubMed]
10. Hesselink DA, Bouamar R, Elens L, van Schaik RH, van Gelder T. The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. Clin Pharmacokinet 2014; 53: 123-39. [CrossRef][PubMed]
11. Kurzawski M, Dąbrowska J, Dziewanowski K, Domański L, Perużyńska M, Droździk M. CYP3A5 and CYP3A4, but not ABCB1 polymorphisms affect tacrolimus dose-adjusted trough concentrations in kidney transplant recipients. Pharmacogenomics 2014 15: 179-88. [CrossRef][PubMed]
12. Cusinato DA, Lacchini R, Romao EA, Moysés-Neto M, Coelho EB. Relationship of Cyp3a5 Genotype and Abcb1 Diplotype to Tacrolimus Disposition in Brazilian Kidney Transplant Patients. Br J Clin Pharmacol 2014; 78: 364-72. [CrossRef][PubMed]
13. Han N, Ha S, Yun HY, Kim MG, Min SI, Ha J, et al. Population pharmacokinetic-pharmacogenetic model of tacrolimus in the early period after kidney transplant ation. Basic Clin Pharmacol Toxicol 2014; 114: 400-6. [CrossRef][PubMed]
14. Yaowakulpatana K, Vadcharavivad S, Ingsathit A, Areepium N, Kantachuvesiri S, Phakdeekitcharoen B, et al C. Impact of CYP3A5 polymorphism on trough concentrations and outcomes of tacrolimus minimization during the early period after kidney transplantation. Eur J Clin Pharmacol 2016;72: 277-83. [CrossRef][PubMed]
15. Kuypers DR, Naesens M, de Jonge H, Lerut E, Verbeke K, Vanrenterghem Y. Tacrolimus dose requirements and CYP3A5 genotype and the development of calcineurin inhibitor-associated nephrotoxicity in renal allograft recipients. Ther Drug Monit 2010; 32: 394-404. [CrossRef][PubMed]
16. Kuypers DR. "What do we know about tacrolimus pharmacogenetics in transplant recipients?" Pharmacogenomics 2018; 19: 593-7. [CrossRef][PubMed]
17. Knops N, van den Heuvel LP, Masereeuw R, Bongaers I, de Loor H, Levtchenko E, et al. The functional implications of common genetic variation in CYP3A5 and ABCB1 in human proximal tubule cells. Mol Pharm 2015; 12: 758-68. [CrossRef][PubMed]
18. Borra LC, Roodnat JI, Kal JA, Mathot RA, Weimar W, van Gelder T. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation. Nephrol Dial Transplant 2010; 25: 2757-63. [CrossRef][PubMed]
19. Spierings N, Holt DW, MacPhee IA. CYP3A5 genotype had no impact on intrapatient variability of tacrolimus clearance in renal transplant recipients. Ther Drug Monit 2013; 35: 328-31. [CrossRef][PubMed]
20. Pashaee N, Bouamar R, Hesselink DA, Roodnat JI, van Schaik RH, Weimar W, et al CYP3A5 genotype is not related to the intrapatient variability of tacrolimus clearance. Ther Drug Monit 2011; 33: 369-71. [CrossRef][PubMed]
21. Kurnatowska I, Krawczyk J, Oleksik T, Nowicki M. Tacrolimus dose and blood concentration variability in kidney ransplant recipients undergoing conversion from twice daily to once daily modified release tacrolimus. Transplant Proc 2011; 43: 2954-6. [CrossRef][PubMed]
22. Stifft F, Stolk LM, Undre N, van Hooff JP, Christiaans MH. Lower variability in 24-hour exposure during once-daily compared to twice-daily tacrolimus formulation in kidney transplantation. Transplantation 2014; 97: 775-80. [PubMed]
23. Rodrigo E, Segundo DS, Fernández-Fresnedo G, López-Hoyos M, Benito A, Ruiz JC, et al. Within-Patient Variability in Tacrolimus Blood Levels Predicts Kidney Graft Loss and Donor-Specific Antibody Development. Transplantation 2016; 100: 2479-85. [CrossRef][PubMed]

INTERINDIVIDUALNA I INTRAINDIVIDUALNA FARMAKOKINETIČKA VARIJABILNOST TAKROLIMUSA TOKOM PRVE GODINE NAKON TRANSPLANTACIJE BUBREGA: EFEKAT CYP3A5 GENSKOG POLIMORFIZMA

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Cilj ovog istraživanja bila je procena potencijalnog uticaja citohroma P450 3A5 (CYP3A5) 6986A>G genskog polimorfizma na interindividualnu i intraindividualnu varijabilnost (IPV) u izloženosti takrolimusa (Tac) tokom prve godine nakon transplantacije bubrega (Tx). Takođe, mi smo imali za cilj analizu promene u distribuciji bolesnika u pogledu IPV između ranog (<6 meseci) i kasnog perioda (>6 meseci) nakon transplantacije bubrega (Tx). U istraživanje je uključen 91 bolesnik sa transplantiranim bubregom. Ovi bolesnici su bili na Tac-baziranom imunosupresivnom protokolu. Koncentracija korigovana dozom (C0/D) je korišćena kao parametar izloženosti Tac, dok su koeficijent varijacije (CV%) i srednja apsolutna devijacija (MAD%) vrednosti C0/D korišćeni kao parametri IPV-a. Transplantirani bolesnici sa CYP3A5*1/*3 genotipom su imali niže vrednosti C0/D u poređenju sa nosiocima CYP3A5*3/*3 genotipa tokom sveukupnog perioda istraživanja (p < 0,01). Istraživanje je pokazalo višu IPV u periodu 1-6 meseci u poređenju sa periodom 7-12 meseci nakon Tx, za CV% i MAD% (p < 0,05). Rezultati istraživanja su pokazali da nije bilo razlike u IPV u odnosu na CYP3A5 genotip. Razmatrajući CV%, 32% i 24% bolesnika imalo je visoku IPV (iznad 30%) u prvoj i drugoj polovini prve godine nakon Tx, redom. Analizirajući, MAD%, 13% i 7% bolesnika su imali visoku varijabilnost izloženosti Tac u prvoj i drugoj polovini prve godine, redom. Istraživanje je potvrdilo da CYP3A5 genski polimorfizam doprinosi interindividualnoj, ne intraindividualnoj, varijabilnosti u izloženosti Tac u toku prve godine nakon Tx.

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Ključne reči: *CYP3A5, transplantacija bubrega, takrolimus, interindividualna farmakokinetička varijabilnost, intraindividualna farmakokinetička varijabilnost*

DICHLOROACETATE-INDUCED NEUROPATHY IN HIGH GRADE FOLLICULAR LYMPHOMA PATIENT

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Curative therapy for follicular lymphomas (FLs) has not been established yet. FLs respond well to chemotherapy and radiation. A large number of current studies confirmed an improved overall response if rituximab was added to chemotherapy. Dichloroacetate (DCA) can be used to inhibit tumor growth. There have been reports that DCA leads to neuropathy. In non-Hodgkin's lymphoma (NHL), DCA leads to antineoplastic action against cell lines and apoptosis of tumor cells, which reduces the metabolism and the number of tumor cells. We present a patient with NHL-FL Grade 3a who took alternatively DCA therapy. In our case report, DCA did not show any treatment benefit but only serious sensorimotor neuropathy as a result of DCA therapy.

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Key words: *follicular lymphoma, dichloroacetate, neuropathy*

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Introduction

Follicular lymphomas (FLs) are the second most frequent subtype of nodal lymphoid malignancies in Western Europe. The annual incidence of this disease has rapidly increased during recent decades and has risen from 2–3/100,000 during the 1950s to 5/100,000 recently (1).

Curative therapy for FLs has not been established yet. The choice of therapy is based on clinical risk factors, symptoms and prognostic disease factors. Systemic therapy has not given results in asymptomatic patients and is appropriate for use only with the occurrence of symptomatic disease. Early initiation of rituximab (R) resulted in improved progression-free survival, but without survival benefit. FLs respond well to chemotherapy and radiation. A large number of current studies confirmed an improved overall response if rituximab was added to chemotherapy. The choice of chemotherapy is based on patient comorbidity indexes, mostly in the light of anthracycline introduction (2).

Dichloroacetate (DCA) has been used for the genetic mitochondrial diseases and treatment of cancer. There have been reports that DCA leads to neuropathy (3). DCA can be used to inhibit tumor growth. This is done by transferring the cell into oxidation and phosphorylation into mitochondria, which lead to apoptosis and the formation of oxygen radicals, superoxide, that also lead to a decrease in tumor volume (4).The mechanism of DCA action includes the inhibition of pyruvate dehydrogenase kinase (PDK), which deactivates the PDK complex. This complex blocks the activity of mitochondria, and therefore there is no mitochondrial oxidative phosphorylation, so that glycolysis is switched into cytoplasm, which leads to lactic acidosis (5). Lactic acidosis facilitates tumor growth by the degradation of an extracellular matrix that allows expansion of tumor cells and initiates their mobility, increasing their metastatic potential and activation of angiogenesis. DCA reduces lactic acidosis and indirectly reduces tumor growth (6). When the mitochondria in the tumor cell are active, it means that they are having the Krebs cycle or glucose oxidation. Being hyperpolarized, they open the mitochondrial pores from which exit cytochrome C and other pro-apoptotic factors that caused tumor cell apoptosis (7).

In non-Hodgkin's lymphoma (NHL), DCA causes antineoplastic action against cell lines and apoptosis of tumor cells, which reduces the metabolism and the number of tumor cells. A decrease in lactic acidosis in lymphoma cells leads to anti-proliferative, anti-metastatic and anti-angiogenic effects on the tumor. DCA causes a significant dose-dependent decline in tumor cell survival. DCA induces dose-dependent apoptosis in Dalton's lymphoma (DL) cells (8, 9).

Case report

We present a case of a 44-year-old Caucasion male with asymptomatic swollen lymph nodes of the neck and under the jaw. In June 2014, he noticed enlarged tonsils and vegetative-infiltrative tumor of the right tonsils was verified by examination. Bilateral tonsillectomy was performed in June 2014 with the right lateral lymph node biopsy. Histopathology indicated NHL-FL Grade 3a. Immunohistochemistry was typical: CD20+, CD79a+, BSAP (PAX5)+, CD10+, bcl2+, bcl6+, CD43+/-, MUM1-, CD3-, CD5-, CD15-, CD23-, CD30-, CD138-, CyclinD1-, EMA-, CK AE1/AE3-, EBV -, Ki67+ in range of 20% - 25 %. The patient did not have B symptoms. During the staging procedures, the following was verified: ECOG 0 KI 100. Multi-sliced computed tomography (MSCT) of the whole body was performed and it revealed: microlimphadenopathy of the right axillary lymph nodes up to 12 mm. Laboratory analysis: erythrocyte sedimentation - 15, Hct 38.1% (low), Hb 124g/L, RBC 4.17X 10^{12}/L, WBC 4.7 X 10^9/L, Neu 2.9 X 10^9/L, Ly 1.3 X 10^9/L, PLT 283 X 10^9/L. Biochemistry findings: glucose 5.4 mmol/L, urea 3.5 mmol/L, creatinine 102 µmol/L, uric acid 330.3 µmol /L, total proteins 73.9 g/L, albumine 42.9 g/L, AST 18 U/L, ALT 21 U/L, ALP 104.8 U/L, LDH 322 U/L, GGT 17.7 U/L, CRP 1.5 mg/L, total bilirubin 10 µmol/L, Fe 12.7 µmol/L, Ca 2.72 mmol/L, Na 141 mmol/L, K 4.8 mmol/L. He was HIV, HCV, HBsAg negative. Plasma immunoglobulins: IgG 10.50 g/L, IgM 0.97 g/L, IgA 2.32 g/L, β2 microglobuline 2.15 mg/L. In electrophoresis, alpha 2 fraction was elevated without pathological defects, no M component was found in plasma. The bone marrow biopsy showed no infiltration of lymphoma cells. The disease was staged as minimum II CS A, with Follicular Lymphoma International Prognostic Index (FLIPI) -0, FLIPI 2-1. Because of asymptomatic FLs, low Ki67 fraction and good prognosis index, chemoimmunotherapy was not implemented. Positron emission tomography combined with CT (PET/CT) found no pathological uptake of 18 Fluoro-deoxy-glucose (18FDG). He underwent "watch and wait" strategy and follow-up. The patient started to take DCA on his own initiative. He was taking DCA for 9 months in the dose range of 750mg with thiamine. There were no effects on lymph node size; moreover, they were enlarged during the follow-up visits. The patient was still in an asymptomatic phase and deserved no treatment initiation. However, toxic neuropathic effects on his ex-tremities occurred over time and became more and more serious. This finding was confirmed by electro-neurography and staged as grade 3 neuropathy of sensorimotor conduction. The patient ceased further DCA intake after obtaining medical advice. He is now recovering from the negative side effects of DCA and is still under the strict neurologic observation.

Discussion

Nowadays, DCA in NHL therapy is especially used in experimental preclinical studies and as an alternative therapy based on the DCA mechanism effect on NHL tumor cells. We already know that nerve toxicity is more frequent in adulthood than in children. DCA has been used only as an alternative to official oncology therapy.

There have been reports on the level of case reports only, which demonstrated a successful treatment of NHL with DCA. Flavin D published a report on a patient with NHL treated successfully with R-cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) over 3 months with PET/CT, showing a complete remission of the disease. However, relapse occurred almost a year after treatment. The patient was obtaining 10 mg/kg daily of DCA, with the addition of 750 mg of thiamine daily as a neuroprotective agent from DCA toxicity. After four months of DCA intake, PET/CT showed complete remission of NHL. However, this single observation was not associated with any follow-up period (10).

Other case report published by Strum SB et al. presented a patient with NHL who received R-CHOP therapy as well, which led to complete remission. A year after, the symptoms returned and PET/CT showed a disease relapse. The patient took DCA therapy: 1,000 mg per day in one daily dose, 55 mg of caffeine and vitamin B1 500 mg/d. One month after the initiation of the DCA protocol, the neck lymph nodes were noticeably smaller and after 2 months no nodes were palpable. Complete resolution of all systemic symptoms disappeared after 71 days of DCA protocol. The patient had reversible neuropathy and encephalopathy during DCA course which did not reverse entirely in spite of taking a neuroprotective agent. This report highlighted the low cost and minimal toxicity of DCA (11).

There were many studies where treatment with DCA has been discontinued despite the fact that thiamine was used as a prevention of nerve toxicity. DCA leads to encephalopathy and peripheral neuropathy. In Brandsma D et al.'s case report it was noticed that DCA encephalopathy and peripheral neuropathy were reversible but not entirely because small consequences remained, although the patient took the recommended dose range of thiamine. Due to the apparent toxicity of DCA, it is recommended to use it only in clinical trials. Peripheral neuropathy was more common in adults (86%) than in children (10%) (12). DCA damage the Schwann cells (SCs) and dorsal root ganglia (DRG) neurons by inhibiting the synthesis of myelin. As a consequence of DCA's direct action on the morphology of SC cells, there is an evidence-based and dose-dependent change in morphology. The partial recovery of myelination was noticed when exposed to 5 mM of DCA under the same conditions. Also, myelination was completely reversible in SC and neurons of DRG (13).

Our patient had DCA intake in the dose range of 750mg with thiamine neuroprotection. However, his lymph nodes have enlarged slightly. During the follow-up, the patient remained still asymptomatic. We assume this asymptomatic phase is the consequence of natural biological flow of FL and is not influenced by DCA. We have only found serious sensorimotor neuropathy as a result of DCA therapy.

In conclusion, our case presented here did not show any treatment benefit with DCA, but only serious adverse event in the sense of neuropathy. Therefore, this case provides information to medical community on the negative consequences of DCA treatment for NHL.

References

1. Mounier M, Bossard N, Remontet L, Belot A, Minicozzi P, De Angelis R, et al. Changes in dynamics of excess mortality rates and net survival after diagnosis of follicular lymphoma or diffuse large B-cell lymphoma: comparison between European population-based data (EUROCARE-5). Lancet Haematol 2015; 2(11): e481-91. [CrossRef] [PubMed]

2. Dreyling M, Ghielmini M, Rule S, Salles G, Vitolo U, Ladetto M; ESMO Guidelines Committee. Newly diagnosed and relapsed follicular lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2016; 27(5):v83-v90. [CrossRef] [PubMed]

3. Kagiava A, Theophilidis G. High concentrations of dichloroacetate have minor effects on the vitality of the mammalian nerve fibers: an ex-vivo electrophysiological study. Anti-cancer Drug 2011; 22(3):27366. [CrossRef] [PubMed]

4. Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, et al. A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 2007; 11(1):37-51. [CrossRef] [PubMed]

5. Calcutt NA, Lopez VL, Bautista AD, Mizisin LM, Torres BR, Shroads AL, et al. Peripheral neuropathy in rats exposed to dichloroacetate. J Neuropath Exp Neur 2009; 68(9):985-93. [CrossRef] [PubMed]

6. Michelakis ED, Webster L, Mackey JR. Dichloroacetate (DCA) as a potential metabolic targeting therapy for cancer. Brit J Cancer 2008; 99(7):989-94. [CrossRef] [PubMed]

7. Halestrap A. Biochemistry: a pore way to die. Nature 2005; 434(7033):578-9. [CrossRef] [PubMed]

8. Kumar A, Kant S, Singh SM. Novel molecular mechanisms of antitumor action of dichloroacetate against T cell lymphoma: Implication of altered glucose metabolism, pH homeostasis and cell survival regulation. Chem-Biol Interact 2012; 199(1):29-37. [CrossRef] [PubMed]

9. Kumar A, Kant S, Singh SM. Antitumor and chemosensitizing action of dichloroacetate implicates modulation of tumor microenvironment: a role of reorganized glucose metabolism, cell survival regulation and macrophage differentiation. Toxicol Appl Pharm 2013; 273(1):196-208. [CrossRef] [PubMed]

10. Flavin DF. Non Hodgkin's lymphoma reversal with dichloroacetate. J Oncol 2010; 2010:414726. [CrossRef] [PubMed]

11. Strum SB, Adalsteinsson O, Black RR, Segal D, Peress NL, Waldenfels J. Case Report: Sodium dichloroacetate (DCA) inhibition of the "Warburg Effect" in a human cancer patient: complete response in non-Hodgkin's lymphoma after disease progression with rituximab-CHOP. J Bioenerg Biomembr 2013; 45(3): 307-15. [CrossRef] [PubMed]

12. Brandsma D, Dorlo TP, Haanen JH, Beijnen JH, Boogerd W. Severe encephalopathy and polyneuropathy induced by dichloroacetate. J Neurol 2010; 257 (12): 2099-100. [CrossRef] [PubMed]

13. Felitsyn N, Stacpoole PW, Notterpek L. Dichloroacetate causes reversible demyelination *in vitro*: potential mechanism for its neuropathic effect. J Neurochem 2007; 100(2):429-36. [CrossRef] [PubMed]

NEUROPATIJA IZAZVANA DIHLOROACETATOM KOD BOLESNIKA SA FOLIKULARNIM LIMFOMOM VISOKOG GRADUSA

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Kurativna terapija folikularnog limfoma (FLs) još uvek nije uspostavljena. Fls odlično reaguje na hemoterapiju i radioterapiju. Veliki broj sadašnjih studija potvrdio je bolji ukupni odgovor ako se rituximab koristi uz hemoterapiju. Dihloracetat (DCA) može biti korišćen zbog inhibicije tumorskog rasta. Postoje izveštaji da DCA dovodi do neuropatije. U ne-Hočkinskom limfomu (NHL) DCA dovodi do antineoplastičkog efekta ćelijskih linija i apoptoze tumorskih ćelija, što smanjuje metabolizam i broj tumorskih ćelija. Mi predstavljamo bolesnika sa NHL-FL gradusa za koji je uzimao DCA kao alternativnu terapiju. U našem prikazu slučaja DCA nije pokazao terapijski benefit, već samo ozbiljnu senzo-motornu neuropatiju kao rezultat DCA terapije.

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Ključne reči: *folikularni limfom, dihloracetat, neuropatija*

IN SILICO EVALUATION OF SELECTED BENZIMIDAZOLE DERIVATIVES IN THE DISCOVERY OF NEW POTENT ANTIMICROBIAL AGENTS

Jelena V. Živković, Sandra G. Kalauzović, Maja R. Milosavljević, Kristina G. Kalauzović

Benzimidazoles are heterocyclic compounds that represent pharmacophores of many drugs. Apart from antimicrobial (antibacterial and antifungal) activities, benzimidazole derivatives are remarkably effective compounds possessing a wide spectrum of biological activities. Pharmacokinetic and toxicological properties of forty-two benzimidazole derivatives were calculated using Molinspiration, SwissADME and OSIRIS Data Warrior. The properties of benzimidazoles were compared to those of doxycycline, chloramphenicol and ketoconazole. The compounds met all criteria for satisfying oral bioavailability. The majority of tested benzimidazoles is considered non-toxic, and the irritating behaviour was not observed in any of the cases. Finally, the most promising nine derivatives were selected on the basis of favourable pharmacokinetic parameters and toxicological characteristics. The ability to pass through the hematoencephalic barrier is expected for all components except one, and none of the selected components is a substrate for P-glycoprotein. In addition, metabolic properties are favourable since all the selected components are expected to inhibit not more than three Cytochrome P450 isoenzymes and to be non-toxic.

Key words: *antimicrobial agents, benzimidazoles, bioavailability, in silico* study, *toxicological characteristics*

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Introduction

Antimicrobial drugs are nowadays widely used. Antimicrobial resistance compromises successful prevention and treatment of an increasing number of infections (1). To overcome these challenges, novel agents should have different chemical characteristics in relation to existing agents. Therefore, the synthesis of new compounds which have structural similarities with already known biologically active molecules is done.

Benzimidazoles are organic, heterocyclic compounds that represent pharmacophores of many drugs (2). Due to their therapeutic significance, these compounds attract the attention of many researchers. 5,6-Dimethylbenzimidazole is an integral part of the chemical structure of vitamin B12, and it has been found that other derivatives of benzimidazole have the activity similar to that of vitamin B12 (3).

Benzimidazole and its derivatives can serve as model components for purine because of the similarities in chemical structure (4). It is considered that antibacterial ability of these compounds is, among everything else, precisely the result of competition with purine bases which results in the inhibition of nucleic acid and cell wall protein synthesis (4, 5). Fungicidal mechanisms of this component are facilitated by inhibition the synthesis of estrogen, which is a component of the cell membrane of fungi (6, 7). Apart from antimicrobial properties, various substituted derivatives of benzimidazole nucleus induce anthelmintic (2), antihypertensive (8), anti-inflammatory (9), antiviral (10), antioxidative (11), anticancerogenic (12), anticoagulative effects (13), as well as many other downstream activities.

Methods of a computer - based (*in silico*) analysis can considerably accelerate the rate of discovery of new drug candidates because such methods are extremely fast, thereby reducing the need for expensive lab work and clinical trials. In the present study, *in silico* drug likeliness assessment, as well as evaluation of bioavailability and toxicological characteristics of benzimidazole derivatives, were pursued in the quest to discover new potent antimicrobial agents.

Aim

The aim of this research is to present *in silico* evaluation of physicochemical properties, pharma-

cokinetic parameters and toxicity potential of benzimidazole derivatives.

Material and methods

Data sources for in silico assessment

The structures of benzimidazole derivatives (n = 42) included in the study were selected from the literature. Compounds bearing 2-aminobenzimidazole (1-3) and hydrazone moieties (4-17) were presented in the study by Özkay et al. 2011 (14). They reported that synthesized novel benzimidazole-hydrazone exhibited low antibacterial activity, while in contrast the compound showed significant antifungal activity against *Candida* yeasts. 1,3-Disubstituted-benzimidazol-2-imines (18-22) and 2-substituted-1,3-thiazolo [3,2-a] benzimidazolones (23-28) were studied with respect to their antibacterial activity against Gram-positive and Gram-negative bacteria

(15). 1,3-Diphenylpropyl-5-methyl-1,3-dihydro-2H-benzimidazol-2-imine (22) possessed significant activity against *B. subtilis, S. aureus, S. abony* and *E. coli.* The group of thiazolobenzimidazolones did not express their antibacterial activity against tested strains. 2-Methylbenzimidazole (29), 1-substituted-2-methylbenzimidazoles (30-35), 2-aminobenzimidazole (36) and 1-substituted-2-aminobenzimidazoles (37-42) were previously reported to express effective antifungal activity against *Saccharomyces cerevisiae* (6).

The properties of benzimidazoles were compared to antibacterial drugs doxycycline and chloramphenicol, as well as an antifungal agent – ketoconazole. The chemical structure of the compounds was drawn using ChemDraw (16) (Figure 1), and their Simplified Molecular Input Line Entry System (SMILES) notations were generated.
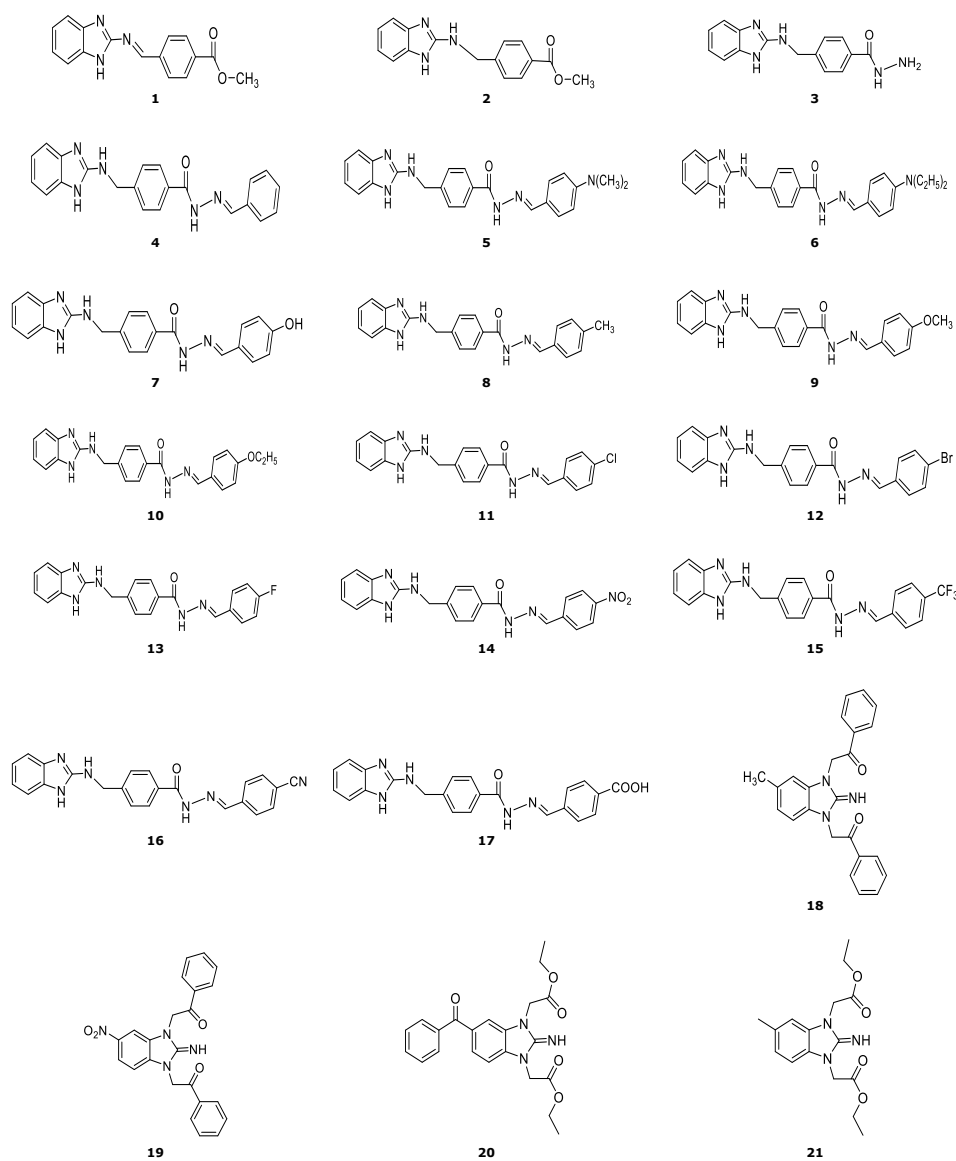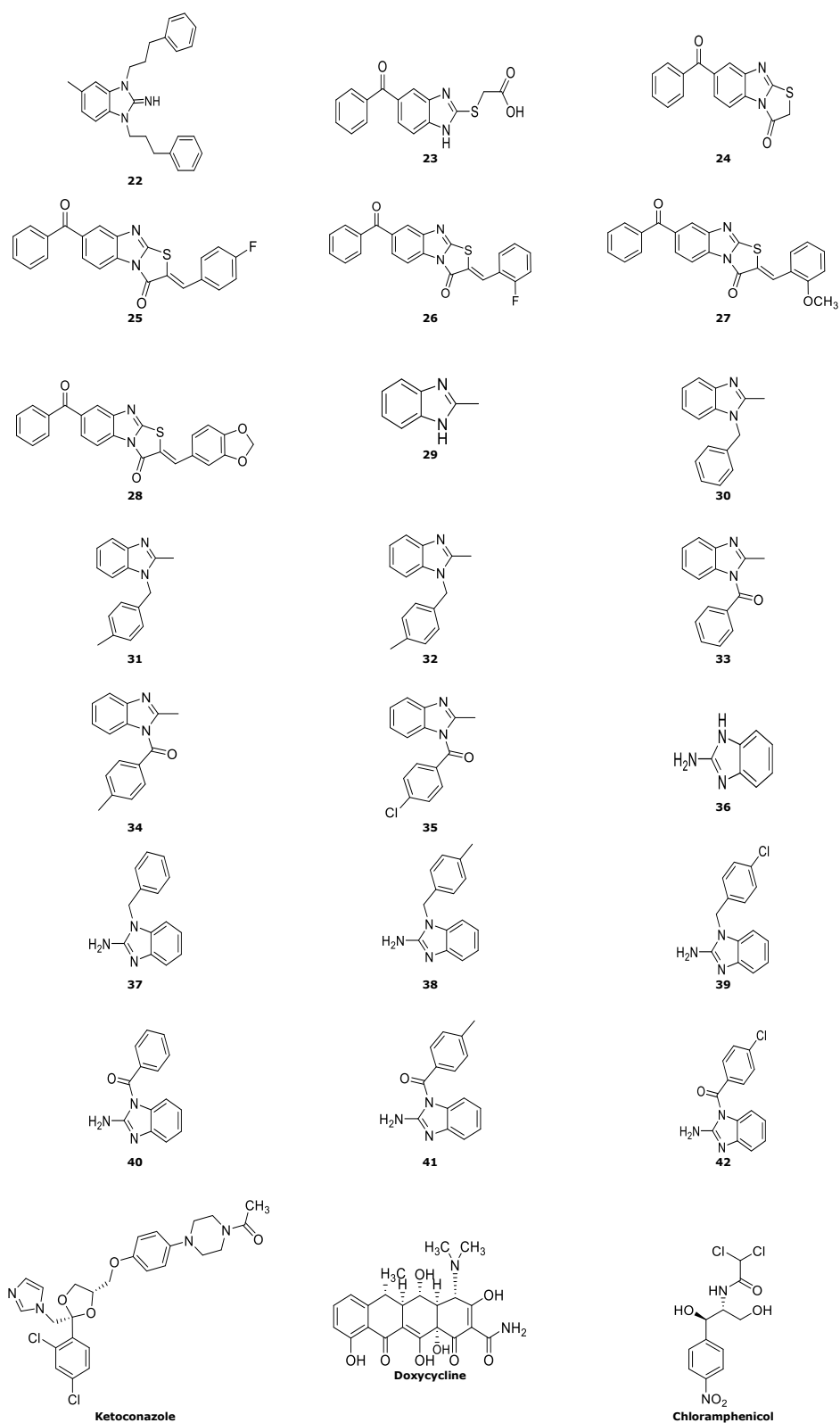
Figure 1. Chemical structure of benzoimidazoles derivatives and standards

In silico evaluation of drug-likeness, pharmacokinetic parameters and toxicological characteristics

SMILES notations of the selected compounds were fed in the Molinspiration software version 2011.06 (17) for calculating drug-likeness properties (logarithm of the partition coefficient between n-octanol and water, total polar surface area, number of hydrogen bond donors and acceptors, molecular weight, etc.) and the prediction of bioactivity score for drug targets (G protein-coupled receptor (GPCR) and nuclear receptor ligands, ion channel modulators, as well as protease, kinase and enzyme inhibitors). A molecule having bioactivity score more than 0.00 is most likely to exhibit considerable biological activities, while values -0.50 to 0.00 are expected to be moderately active. If the score is less than -0.50, the molecule is presumed to be inactive (13).

Table 1. Drug likeness score for compounds predicted by Molinspiration (17)

Compound	miLogP[a]	TPSA[b] (Å^2)	natoms[c]	Mw[d] (g/mol)	nON[e]	nOHNH[f]	nviolations[g]	nrotb[h]	volume[i] (Å^3)
1	1.78	56.40	11	147.13	3	0	0	2	127.80
2	3.18	67.02	21	281.31	5	2	0	5	254.01
3	1.39	95.83	21	281.32	6	5	0	4	252.15
4	4.17	82.17	28	369.43	6	3	0	6	335.53
5	4.27	85.41	31	412.50	7	3	0	7	381.44
6	5.02	85.41	33	440.55	7	3	1	9	415.04
7	3.69	102.40	29	385.43	7	4	0	6	343.55
8	4.62	82.17	29	383.45	6	3	0	6	352.09
9	4.22	91.41	30	399.45	7	3	0	7	361.08
10	4.60	91.41	31	413.48	7	3	0	8	377.88
11	4.84	82.17	29	403.87	6	3	0	6	349.07
12	4.98	82.17	29	448.32	6	3	0	6	353.42
13	4.33	82.17	29	387.42	6	3	0	6	340.46
14	4.13	128.00	31	414.43	9	3	0	7	358.87
15	5.06	82.17	32	437.43	6	3	1	7	366.83
16	3.92	105.96	30	394.44	7	3	0	6	352.39
17	4.08	119.47	31	413.44	8	4	0	7	362.53
18	2.83	67.86	29	383.45	5	1	0	6	352.02
19	2.34	113.69	31	414.42	8	1	0	7	358.79
20	2.02	103.40	30	409.44	8	1	0	10	367.73
21	1.01	86.33	23	319.36	7	1	0	8	293.89
22	5.18	33.72	29	383.54	3	1	1	8	381.26
23	3.02	83.05	22	312.35	5	2	0	5	261.19
24	3.59	51.97	21	294.33	4	0	0	2	242.71
25	5.30	51.45	29	400.43	4	0	1	3	329.67
26	5.25	51.45	29	400.43	4	0	1	3	329.67
27	5.15	60.68	30	412.47	5	0	1	4	350.28
28	5.03	69.92	31	426.45	6	0	1	3	348.66
29	1.52	28.68	10	132.117	2	1	0	0	125.42
30	3.18	17.83	17	222.29	2	0	0	2	214.02
31	3.63	17.83	18	236.32	2	0	0	2	230.58
32	3.86	17.83	18	256.74	2	0	0	2	227.55
33	3.06	34.90	18	236.27	3	0	0	1	216.20
34	3.51	34.90	19	250.30	3	0	0	1	232.76
35	3.73	34.90	19	270.72	3	0	0	1	229.74
36	1.23	54.71	10	133.15	3	3	0	0	120.15
37	2.90	43.85	17	223.28	3	2	0	2	208.75
38	3.34	43.85	18	237.31	3	2	0	2	225.31
39	3.57	43.85	18	257.72	3	2	0	2	222.28
40	2.77	60.92	18	237.26	4	2	0	1	210.93
41	3.22	60.92	19	251.29	4	2	0	1	227.49
42	3.45	60.92	19	271.71	4	2	0	1	224.46
KTC	3.77	69.08	36	531.44	8	0	1	7	452.47
DOX	-0.43	181.61	32	446.44	10	7	1	2	377.79
CHL	0.73	115.38	20	323.13	7	3	0	6	249.16

a Logarithm of the partition coefficient between n-octanol and water (miLogP); b Topological polar surface area (TPSA);
c Number of nonhydrogen atoms; d Molecular weight; e Number of hydrogen bond acceptors (O and N atoms);
f Number of hydrogen bond donors (OH and NH groups); g Number of Rule of 5 violations;
h Number of rotatable bonds (n-rotb); i Molecular volume; j KTC – ketoconazole; k DOX – doxycycline
l CHL – chloramphenicol.

The list of SMILES was then entered in Swiss-ADME (18) for generating the prediction of gastrointestinal absorption, brain penetration and substrates for P-glycoprotein. In addition, the analysis of ADMET absorption properties was done to check whether those compounds were inhibitors of five isoforms of Cytochrome P450 (CYP) family. Further, OSIRIS Data Warrior was used for the prediction of the compounds' mutagenic and tumorigenic risks, reproductive and irritant effects (19).

Results and discussion

Calculated physicochemical properties of the studied molecules using Molinspiration (17) are presented in Table 1.

Based on the obtained results, all examined compounds are likely to have good biological availability given the fact that they do not violate more than one of Lipinski's rules (Table 1) (20, 21). Lipinski's 'Rule of Five' (RO5) is a rule of thumb used to evaluate drug-likeness. RO5 is commonly used by medicinal chemists in drug design as a simple set of physicochemical parameter ranges associated with 90% of orally active drugs that achieved Phase II status (22). Lipinski specifically states that RO5 only holds for compounds that are absorbed by passive mechanisms (23). All examined benzimidazole derivatives are lipophilic drugs (log P > 1) (Table 1).

Compounds 6, 15, 22, 25-28 have (log P > 5) and consequently show one violation of RO5. Ketoconazole and chloramphenicol are lipophilic, whereas doxycyline (log P = -0.43) is a hydrophilic compound which affects its bioavailability and way of administration.

Topological polar surface area (TPSA) is very much correlated with hydrogen bonding of a molecule and is a very good indicator of the bioavailability of a drug molecule. Veber et al. 2002. (21) defined a rule for drug-likeness on strains as nrotb ≤ 10 and TPSA ≤ 140 Å^2. In addition, a drug can be absorbed over 90% if the TPSA value is < 60 Å^2 (24).

The number of rotatable bonds (nrotb) higher than 10 was not determined in neither of the components, which is considered another sign of expected good oral bioavailability (Table 1). TPSA of benzimidazole derivatives was observed in the range of 17.83-128.00 Å^2 and is well below the limit of 140 Å^2. The TPSA value for antifungal drug doxycycline is 181.61 Å^2, while the other two antibacterial agents have values below 140 Å^2.

The molecular weight of forty-two benzimidazole derivatives was found to be less than 500 g/mol and thus these molecules are expected to be easily transported, diffused and absorbed in comparison to large molecules. Among standard antimicrobial drugs, only ketoconazole had a higher Mw (531.44 g/mol).

Table 2. Bioactivity score of compounds according to Molinspiration (17)

Drug targets	Activity	Compound
GPCR ligand	CST[a] active	*6* (22, 35, 39-42) and KTC
GPCR ligand	Mod.[b] active	*30* (1-21, 23, 24, 30-34, 37, 38) and DOX, CHL
GPCR ligand	Inactive	*6* (25-29, 36)
ICM[c]	CST active	*5* (6, 9, 36, 37, 39)
ICM	Mod. active	*28* (1-5, 7, 8, 10, 11, 13-17, 20, 22, 23, 29-35, 38, 40-42) and KTC, DOX, CHL
ICM	Inactive	*9* (12, 18, 19, 21, 24-28)
Kinase inhibitor	CST active	*7* (2, 3, 5, 7, 13, 15, 16)
Kinase inhibitor	Mod. active	*30* (1, 4, 6, 8-12, 14, 17-28, 30-38) and KTC, CHL
Kinase inhibitor	Inactive	*5* (29, 39-42) and DOX
NRL[d]	CST active	*1* (1)
NRL	Mod. active	*16* (2, 15, 17, 20, 22-24, 30-35, 40-42) and KTC, DOX, CHL
NRL	Inactive	*25* (3-14, 16, 18, 19, 21, 25-29, 36-39)
Protease inhibitor	CST active	/
Protease inhibitor	Mod. active	*30* (2-7, 9-11, 13, 15-17, 18-20, 22, 23, 30-35, 37-42) and KTC, DOX, CHL
Protease inhibitor	Inactive	*12* (1, 8, 12, 14, 21, 24-29, 36)
Enzyme inhibitor	CST active	*12* (3, 4, 23, 33-35, 37-42) and KTC
Enzyme inhibitor	Mod. active	*29* (1, 2, 5-22, 24-28, 30-32, 36) and CHL
Enzyme inhibitor	Inactive	*1* (29) and DOX

a CST – Considerable; b Mod. – moderate; c ICM – Ion channel modulator; d NRL– Nuclear receptor ligand

Table 3. Absorption properties of compounds predicted by SwissADME (18)

Absorption properties	Compound
High HIA[a]	*40* (1-7, 9-13, 15-42) and KTC, CHL
Low HIA	*2* (8,14) and DOX
BBB[b] permeant	*19* (1, 2, 18, 22, 24, 29-42) and KTC
Non-BBB permeant	*23* (3-17, 19-21, 23, 25-28) and DOX, CHL
Substrates for P-gp[c]	*6* (3, 8, 22, 37-39) and DOX
Non-Substrates for P-gp	*36* (1, 2, 4-7, 9-21, 23-36, 40-42) and KTC, CHL

a HIA – human intestinal absorption; b BBB – blood brain barrier; c P-gp – P-glycoprotein

Table 4. Metabolic properties of compounds according to SwissADME (18)

Metabolic properties	Compound
CYP1A2 inhibitor	*34* (1-5, 7, 9-13, 15-17, 22-27, 29-42)
CYP2C19 inhibitor	*36* (1, 2, 4-7, 9-20, 22, 24-28, 30-35, 37-42) and KTC
CYP2C9 inhibitor	*27* (1, 4-7, 9-20, 22-28, 34, 35, 42) and KTC
CYP2D6 inhibitor	*18* (2, 4-7, 9-13, 16, 22, 30-32, 37-39) and KTC
CYP3A4 inhibitor	*19* (4-6, 9-16, 18, 22, 27, 30, 31, 37-39) and KTC

The hydrogen bonding capacity of benzimidazoles, described by the number of hydrogen bond donors and acceptors, differs significantly. They show 2 to 9 hydrogen bond acceptors and 0 to 5 hydrogen bond donors. In the group of standard drugs, ketoconazole shows 10 hydrogen bond acceptors and 7 hydrogen bond donors and consequently manifests one RO5 violation.

The volume of molecules determines the transport of molecules through the intestinal and hematoencephalic barrier. By increasing the volume of molecules, which can be considered a valid parameter of the assessment of oral bioavailability, the number of rotatable bonds also increases, as well as the number of hydrogen bonds (17).

None of the molecules show volume of more than 500 Å^3. The presented results of physicochemical properties indicate that good intestinal absorption is predicted for all investigated compounds.

The bioactivity scores of listed compounds for drug targets were also predicted by Molinspiration (17) (Table 2).

The obtained results (Table 2) clearly demonstrate that the physiological actions of benzimidazole derivatives might involve multiple mechanisms and, which could be the result of interactions with GPCR ligands, nuclear receptor ligands, inhibit protease, kinase and other enzymes. The bioactivity score of compounds is suggestive of most components of mainly moderate interactions with all drug targets. However, the compounds are assumed to exhibit the weakest biological activities with nuclear receptor ligands, because twenty-five benzimidazoles are predicted to be inactive. The most promising twenty-nine compounds which are predicted to act by more than four mechanisms are identified as 1-7, 9-13, 15-17, 20, 22, 23, 30-35, 37-39, 41, 42. Ketoconazole and chloramphenicol may have effects on all proposed drug targets, while doxycycline is a little less active due to the lack of kinase and enzyme inhibitors' activity.

Absorption and metabolic properties of the compounds were predicted by SwissADME (18) (Table 3 and Table 4).

SwissADME enables predictions for passive human gastrointestinal absorption (HIA) and blood-brain barrier (BBB) permeation of compounds, as well as the assessment of whether a compound is a substrate of P-gp and an inhibitor of CYP isoenzyme family (18, 23). The knowledge about compounds being substrates or non-substrates of P-gp is essential for the evaluation of active efflux through biological membranes. A crucial role of P-gp is to prevent intracellular accumulation of toxic compounds and to protect the central nervous system (CNS) from xenobiotics (25, 26).

The analysis of benzimidazole derivatives using SwissADME shows that all compounds, except for 8 and 14, are expected to have good gastrointestinal (GIT) absorption. The ability to pass through the hematoen-cephalic barrier is predicted for nineteen compounds. Furthermore, it was determined that only six compounds can be substrates for P-glycoprotein. Ketoconazole and chloramphenicol are predicted to have high HIA, permeant BBB is expected for ketoconazole, and doxycycline is expected to be a substrate for P-gp (Table 3).

The superfamily of CYP isoenzymes is crucial for drug elimination through phase I of metabolic biotransformation. CYP and P-gp are likely to metabolize small molecules synergistically to improve protection of tissues and organisms. Therapeutic molecules are substrates of five major isoforms of CYP enzyme family: CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. The inhibition of these isoenzymes is certainly a major cause of pharmacokinetics-related drug-drug interactions, which could induce toxic or other undesirable adverse effects (26).

Seven compounds, 4, 9-13 and 22, are predicted to inhibit all the listed CYP isoenzymes. The least numbers of compounds, eighteen, will probably inhibit isoenzymes CYP2D6. Ketoconazole is predict-

ed to inhibit 4 enzyme subfamilies, whereas doxycy-cline and chloramphenicol are not predicted to have an inhibitory effect on CYP enzymes (Table 4).

Toxicological properties of the compounds predicted by OSIRIS Data Warrior (19) are shown in Table 5.

Table 5. Predicted toxicological properties of compounds by OSIRIS Data Warrior (19)

Toxicological properties	Level	Compound
Mutagenic risk	High	14 (6, 14, 18-22, 24-29, 36) and CHL
Tumorigenic risk	High	5 (3, 5, 14, 18, 20) and CHL
	Low	8 (6, 21, 22, 24-28)
Reproductive Effect	High	3 (28, 29, 36) and CHL
	Low	5 (18, 24-27)
Irritant Effect	Low	/
	High	CHL

The majority of tested benzimidazole deriva-tives is considered non-toxic, and the irritating beha-viour was not observed in any of the cases (Table 5). Compounds 14, 18 and 20 are predicted to have high mutagenic and tumorigenic risks, whereas com-pounds 28, 29 and 36 are predicted to have a high mutagenic risk and a high reproductive effect. Com-pounds 6, 19, 21, 22, 24-27 are predicted to have a high mutagenic risk, whereas compounds 3 and 5 are predicted to have a high tumorigenic risk. Chloram-phenicol is likely to exhibit all four toxic effects, where as ketoconazole and doxycycline are consider-ed safe to use.

Based on *in silico* evaluation of physicoche-mical properties, pharmacokinetic parameters and toxicity potential of benzimidazole derivatives, it was determined that the following nine compounds out of forty-two benzimidazole derivatives showed the best characteristics: 1, 2, 17, 32-35, 41, 42. The selected compounds, as well as all benzimidazole derivatives, met Lipinski's RO5 (Table 1); therefore, they express good oral availability. Their bioactivity score indicates high activity since they are predicted to react with more than four drug targets (Table 2). The absorp-tion properties of compounds are satisfactory given the fact that all the mentioned components are expected to have good GIT absorption. The ability to pass through the hematoencephalic barrier is ex-pected for all components except component 17, and none of the selected components is a substrate for P-gp (Table 3). Furthermore, metabolic properties are favourable since all the selected components are expected to inhibit not more than three CYP enzy-mes (Table 4), and to be non-toxic (Table 5). These are benzimidazoles that have high antifungal acti-vity, proven by previous tests of antimicrobial acti-vities (6, 14). Carboxy substituted (17) benzimida-zole-hydrazone (Figure 1) exhibits two-fold better potency (MIC = 25 µg/mL) than the reference drug ketoconazole (MIC = 50 µg/mL) against all analysed *Candida* fungal strains. On the other hand, their antibacterial activity is low (14). 1-Substituted-2-methylbenzimidazoles (32, 33, 34, 35) and 1-sub-stituted-2-aminobenzimidazoles (41, 42) show com-parable antifungal activity to that of ketoconazole and amphotericin against *S. cerevisiae* (6). More-

over, authors conclude that antifungal activity ex-hibited by the tested compounds is governed by the partition coefficient, log P.

Fundamental physiochemical properties of the central nervous system (CNS) drugs are evaluated on the basis of their ability to penetrate the blood-brain barrier and to exhibit CNS activity. Lipophilicity and TPSA of drugs significantly influence their ability to penetrate the blood-brain barrier.

A drug targeting the CNS should ideally have a log P value around 2. Benzimidazoles with log P values between 2 and 3, i.e. molecules which are considered to be able to pass through the hemato-encephalic barrier due to their optimal lipophilicity, are compounds 18-20, 37 and 40 (Table 1). Among these compounds, 1-benzylbenzimidazol-2-amine (37) and 2-amino-3H-benzimidazol-1-yl)-phenylmethano ne (40) (Figure 1) can be considered non-toxic (Table 5). Compounds 37 and 40 are expected to show high activity because they are predicted to react with more than four drug targets (Table 2). Also, both components are expected to have good GIT absorption and to be able to pass through the hematoencephalic barrier, whereas only compound 37 is predicted to be a prospective substrate for P-gp (Table 3). Compound 37 is expected to potentially inhibit four CYP enzymes, whereas compound 40 was expected to inhibit only CYP1A2 and CYP2C19 isoenzyme subfamilies. (Table 4). Those compounds were previously reported to express good antifungal activity against S. cerevisiae (6).

The drugs can be targeted to the CNS with a PSA less than 60 $Å^2$ (24). Sixteen compounds, 1, 22, 24-26, 29-39, have slightly lower TPSA values below 60 $Å^2$, which indicates that they could pass through the hematoencephalic barrier (Table 1). Among ben-zimidazoles with lower TPSA values, ten compounds, 1, 30-35 and 37-39, can be considered non-toxic (Table 5). The properties of compounds 1 and 32-35 have already been discussed because they belong to the group of nine benzimidazoles with the best *in silico* analysis results. In addition to having high GIT, the rest of the selected compounds, 30, 31, 37-39, are predicted to pass through the BBB, whereas the components 37-39 are P-gp substrates as well (Table 3). The selected CNS-permeable compounds

are expected to inhibit four CYP isoenzymes, therefore, side effects are likely to appear (Table 4).

Conclusion

Based on the calculated physicochemical properties, we can conclude all of the benzimidazole derivatives have good oral bioavailability since they do not violate more than one RO5. Also, most of them (40 out of 42) have good intestinal absorption. Less than half of the compounds are expected to pass through the BBB, and a limited number of them is predicted to be P-gp substrates. The obtained results clearly reveal that the physiological actions of the analysed derivatives might involve multiple mechanisms, possibly the result of interactions with GPCR ligands, nuclear receptor ligands, inhibit protease, kinase and other enzymes. The most promising twenty-nine benzimidazoles are predicted to act by more than four mechanisms. Ketoconazole and chloramphenicol could influence on all tested drug targets, while doxycycline is a little less active due to the lack of kinase and enzyme inhibitors' activity. Seven compounds are predicted to inhibit all the listed CYP isoenzymes. The majority of tested benzimidazole derivatives is considered safe to use, and the irritating behaviour was not observed in any of the cases. Chloramphenicol is likely to exhibit all four toxic effects, whereas ketoconazole and doxycycline are considered non-toxic. Methods of a computer based (*in silico*) analysis could be very useful in the discovery of new drug candidates.

Acknowledgments

References

1. Bayrak H, Demirbas A, Karaoglu SA, Demirbas N. Synthesis of some new 1, 2, 4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities. Eur J Med Chem 2009; 44(3):1057-66. [CrossRef] [PubMed]

2. Bansal Y, Silakari O. The therapeutic journey of benzimidazoles: A review. Bioorg Med Chem 2012; 20 (21):6208-36. [CrossRef] [PubMed]

3. Yadav G, Ganguly S. Structure activity relationship (SAR) study of benzimidazole scaffold for different biological activities: A mini-review. Eur J Med Chem 2015; 97:419-43. [CrossRef] [PubMed]

4. Arjmand F, Parveen S, Afzal M, Shahid M. Synthesis, characterization, biological studies (DNA binding, cleavage, antibacterial and topoisomerase I) and molecular docking of copper(II) benzimidazole complexes. J Photochem Photobiol B 2012; 114:15-26. [CrossRef] [PubMed]

5. Bandyopadhyay P, Sathe M, Ponmariappan S, Sharma A, Sharma P, Srivastava AK, et al. Exploration of in vitro time point quantitative evaluation of newly synthesized benzimidazole and benzothiazole derivatives as potential antibacterial agents. Bioorg Med Chem Lett 2011; 21(24):7306-09. [CrossRef] [PubMed]

6. Podunavac-Kuzmanovic SO, Barna DJ, Cvetkovic, DD. Quantitative structure-activity relationships to predict antibacterial effect of some benzimidazole derivatives. Acta Period Technol 2008; 39:181-91. [CrossRef]

7. Kilcig GA, Altanlar N. Synthesis and antifungal activity of some benzimidazole derivatives. Turk J Chem 2006; 30(2):223-8.

8. Kubo K, Kohara Y, Yoshimura Y, Inada Y, Shibouta Y, Furukawa Y, et al. Nonpeptide angiotensin II receptor antagonists. Synthesis and biological activity of potential prodrugs of benzimidazole-7-carboxylic acids. J Med Chem 1993; 36(16):2343-9. [CrossRef] [PubMed]

9. Sabat M, VanRens JC, Laufersweiler MJ, Brugel TA, Maier J, Golebiowski A, et al. The development of 2-benzimidazole substituted pyrimidine based inhibitors of lymphocyte specific kinase (Lck). Bioorg Med Chem Lett 2006; 16(23):5973-7. [CrossRef] [PubMed]

10. Fonseca T, Gigante B, Marques MM, Gilchrist TL, De Clercq E. Synthesis and antiviral evaluation of benzimidazoles, quinoxalines and indoles from dehydroabietic acid. Bioorg Med Chem 2004; 12(1):103-12. [CrossRef] [PubMed]

11. Gurer-Orhan H, Orhan H, Suzen S, Püsküllü MO. Buyukbingol E. Synthesis and evaluation of *in vitro* antioxidant capacities of some benzimidazole derivatives. J Enzyme Inhib Med Chem 2006; 21(2):241-7. [CrossRef] [PubMed]

12. Refaat HM. Synthesis and anticancer activity of some novel 2-substituted benzimidazole derivatives. Eur J Med Chem 2010; 45(7):2949-56. [CrossRef] [PubMed]

13. Zhao Z, Arnaiz DO, Griedel B, Sakata S, Dallas JL, Whitlow M, et al. Design, synthesis, and in vitro biological activity of benzimidazole based factor Xa inhibitors. Bioorg Med Chem Lett 2000; 10(9):963-6. [CrossRef] [PubMed]

14. Özkay Y, Tunalı Y, Karaca H, Işıkdağ I. Antimicrobial activity of a new series of benzimidazole derivatives. Arch Pharm Res 2011; 34(9):1427-35. [CrossRef] [PubMed]

15. Mavrova A, Yancheva D, Anastassova N, Anichina K, Zvezdanovic J, Djordjevic A, et al. Synthesis, electronic properties, antioxidant and antibacterial activity of some new benzimidazoles. Bioorg Med Chem 2015; 23(19):6317-26. [CrossRef] [PubMed]

16. ChemDraw. http://www.cambridgesoft.com/software/overview.aspx

17. Molinspiration Cheminformatics, 2016. Molinspiration property engine v2016.09. http://www.molinspiration.com/cgi-bin/properties

18. SwissADME. http://www.swissadme.ch/

19. OSIRIS Data Warrior v.4.7.2 http://www.openmolecules.org/datawarrior/

20. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001; 46 (1-3):3-26. [CrossRef] [PubMed]

21. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 2002; 45(12):2615-23. [CrossRef] [PubMed]

22. Keller TH, Pichota A, Yin Z. A practical view of "druggability". Curr Opin Chem Biol 2006; 10(4):357-61. [CrossRef] [PubMed]

23. Cortes C, Vapnik V. Support vector machine. Mach Learn 1995; 20(3):273-297. [CrossRef]

24. Prasanna S, Doerksen RJ. Topological polar surface area: a useful descriptor in 2D-QSAR. Curr Med Chem 2009; 16(1):21-41. [CrossRef] [PubMed]

25. Montanari F, Ecker GF. Prediction of drug-ABC-transporter interaction-Recent advances and future challenges. Adv Drug Deliv Rev 2015; 86:17-26. [CrossRef] [PubMed]

26. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017; 7:42717. [CrossRef] [PubMed]

IN SILICO ISPITIVANJE ODABRANIH DERIVATA BENZIMIDAZOLA U POTRAZI ZA NOVIM POTENTNIM ANTIMIKROBNIM AGENSIMA

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Benzimidazoli su heterociklična jedinjenja koja predstavljaju farmakofore mnogih lekova. Pored antimikrobne (antibakterijske i antifungalne) aktivnosti, derivati benzimidazola su izuzetno efikasna jedinjenja koja pokazuju širok spektar bioloških aktivnosti. Korišćenjem kompjuterskih programa Molinspiration, SwissADME i OSIRIS Data Warrior, ispitivane su farmakokinetičke i toksikološke osobine četrdeset dva derivata benzimidazola. Jedinjenja su ispunila sve kriterijume za zadovoljavajuću oralnu bioraspoloživost. Za većinu je predviđeno da su netoksična, a potencijalno iritantno delovanje nije utvrđeno ni u jednom slučaju. Na kraju, na osnovu povoljnih farmakokinetičkih parametara i toksikoloških karakteristika selektovano je devet najobećavajućih derivata. Sposobnost prolaska kroz hematoencefalnu barijeru se očekuje za sve komponente osim jedne, a nijedna od odabranih nije supstrat za P-glikoprotein. Metaboličke karakteristike selektovanih jedinjenja su takođe povoljne, jer se predviđa inhibicija ne više od tri citohrom P450 izoenzima i odsustvo toksičnosti.

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Ključne reči: *antimikrobni agensi, benzimidazoli, bioraspoloživost, in silico* ispitivanje*, toksikološke osobine*

THE ROLE OF VITAMIN D IN TREATING PATIENTS WITH TYPE 2 DIABETES MELLITUS

Milena M. Cojić

Vitamin D is a steroid hormone the primary role of which is to maintain adequate blood levels of calcium and phosphorus needed for the normal bone mineralization process. Receptors for vitamin D active form and enzymes involved in its activation have been found in many other body tissues, leading to a conclusion that vitamin D deficiency is connected with the development of many chronic diseases such as hypertension, multiple sclerosis, certain malignant tumors and type 2 diabetes mellitus (T2 DM). Numerous observational studies have shown that patients with T2 DM have lower blood levels of vitamin D compared to healthy subjects. This indicates that vitamin D could play an important role in the pathogenesis of this chronic non-communicable disease. By monitoring parameters related to glycemic status, insulin secretion and insulin resistance, many researchers tried to answer the question whether vitamin D supplementation could help patients with diabetes better control their disease and prevent the complications. The results were contradictory and failed to provide enough solid evidence for recommending vitamin D supplementation as a therapeutic measure for these patients. However, patients who might benefit from supplementation are those with the increased T2 DM risk or those at the beginning of the disease. In order to assess which group of patients could benefit from such a supplementation, it is necessary to provide well-designed, long-term experimental studies with precisely defined groups of patients (e.g. prediabetes, early T2 DM, etc.), supplemented with sufficiently high vitamin D doses in relevant monitoring periods.
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Introduction

Vitamin D is a steroid hormone the primary role of which in bone metabolism is widely acknowledged. Owing to this hormone, optimal concentration of calcium and phosphate necessary for bone mineralization process is maintained in blood. In addition to its "classical" role, more attention is being paid to its possible "non-classical" effects in prevention and treatment of numerous chronic non-communicable diseases (1, 2). The reason is in the fact that the receptors for active D vitamin metabolite (1,25-dihydroxy vitamin D), as well as the enzyme involved in its activation, are found not only in tissues related to bone metabolism, but also in 38 other tissues (brain, prostate, breasts, etc.) in which the hormone regulates the processes of cell proliferation, differentiation, apoptosis and angiogenesis (3, 4). Vitamin D deficiency is therefore connected with the increased risk of hypertension, multiple sclerosis, significant number of malignant tumors, type 2 diabetes mellitus (T2 DM) (4-6). Essential role of vitamin D in the emergence of T2 DM is proven by results of numerous prospective studies which have shown that low vitamin D blood level is linked to the increased risk of this disease and a disturbed glucose metabolism. However, the nature of this link is still not completely clear (6-9). If vitamin D was one of the causal factors, instead of being a consequence of specific pathophysiologic processes responsible for the disease, we would have a natural, cheap and easily available means, which could be compensated for taking an important step towards prevention and treatment of this chronic non-communicable diseases and its complications (10).

The goal of this paper is to summarize the existing knowledge on the possible role and effects of vitamin D supplementation in treating patients with T2 DM.

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Vitamin D

Vitamin D implies two forms: vitamin D2 (ergosterol) and vitamin D3 (cholecalciferol) (11, 12). Vitamin D2 is converted from sterols by ultraviolet (UV) radiation in mushrooms exposed to sunlight and enters the body solely through food. Although it can be found in food, the greatest source of vitamin D for people is its endogenous production during skin exposure to sunlight. Namely, the cells of epidermis and dermis contain 7-dehydrocholesterol, a cholesterol derivative which absorbs UVB rays during sun exposure (at wavelengths between 290-320 nm) and transforms into provitamin D3, which then isomerizes into a thermally more stable form of vitamin D3 (cholecalciferol) (13, 14). Upon synthesis in the skin, or absorption from the digestive system if taken through food, vitamin D (D2, D3 or both) is biologically inactive. Two enzyme-mediated reactions of hydroxylation have to take place to activate inactive cholecalciferol. The first hydroxylation occurs in the liver catalyzed by the enzyme 25-hydroxyvitamin D hydroxylase and produces 25-hydroxyvitamin D3 (calcidiol). The second reaction of hydroxylation takes place in the kidneys, catalyzed by enzyme 25-hydroxyvitamin D-1α-hydroxylase, producing the active form of vitamin D, i.e. 1,25-dihydroxyvitamin D3 (calcitriol). This active form goes to target tissues where it binds to vitamin D-specific receptor. In the intestinal tissue it is responsible for the increased intestinal absorption of calcium and phosphorus and increased reabsorption of calcium in the kidneys respectively. As soon as blood calcium level becomes low, parathyroid glands secrete parathyroid hormone (PTH) which stimulates the production of vitamin D's active form in the kidneys, which further increases the calcium level presumably via increased intestinal resorbtion. If this is insufficient, vitamin D stimulates the osteoclasts function and consequent bone resorption process in coordination with PTH (15-17). Lowered calcium intake may lead to bone damaging, but this is rarely the case. A considerably more frequent reason for inadequate bone metabolism is the vitamin D deficiency. The metabolite generated after the first hydroxylation in the liver, 25-hydroxyvitamin D3 (25(OH)D3), is used for estimating blood vitamin D concentration, since its half-life in circulation is longer (around 2-3 weeks) in comparison to the active metabolite 1,25(OH)2 D3 generated in the kidneys, with the half-life of around 4 hours. Additionally, circulating concentrations of vitamin D's active form are 1000 times lower than 25(OH)D3 and are mostly normal or moderately elevated, even in vitamin D deficiency due to secondary hyperparathyroidism (3, 4).

Recommendations regarding the optimal blood level of vitamin D metabolite are still not completely harmonized. This is due to a great number of factors influencing the vitamin D production in skin and its food intake, as well as due to a great number of clinical tests for determining 25(OH)D3 levels, whose values are very variable. Moreover, optimal levels for maintaining an adequate bone metabolism and health in general could be different (18, 19).

According to the guidelines for treatment and prevention of vitamin D deficiency published by The Endocrine Society, the optimal level of circulating vitamin D should be above 30 ng/ml (75 nmol/L), because PTH values in blood are minimal above that level (4, 5). Level of 20-30 ng/ml (50-75 nmol/L) implies vitamin D insufficiency, since PTH levels are elevated; however, vitamin D level is sufficient for an increased intestinal absorption of calcium and phosphorus. Vitamin D deficiency occurs when its blood level is below 20 ng/ml (50 nmol/L) and this condition is connected with malabsorption of Ca and phosphorus, as well as with a disturbed mineralization, i.e. bone resorption, in order to provide sufficient quantity of Ca and P in blood. This leads to rickets in children and osteomalacia in adults (3, 4). Therefore, many experts suggest that normal bone metabolism requires maintaining the blood level of 25(OH)D3 above 20 ng/ml (50 nmol/L), whereas its optimal value should be above 75 nmol/L so that it could contribute to general health improvement. On the other hand, lower values may lead to immunity impairment, myopathy, DM and increased risk of some types of carcinoma (20).

These recommendations are not fulfilled with most people. It is estimated that around 1 billion of world population has vitamin D insufficiency or deficiency (21). The primary reasons are seldom and inadequate sun exposure, as well as a diet lacking in vitamin D (5, 13, 22). Its quantity in the body is influenced by other factors as well, e.g. birth year, race, season, latitude, use of sun protection creams and use of some medications (anticonvulsants, corticosteroids etc.) (4, 13, 22, 23).

Vitamin D and type 2 diabetes mellitus

Numerous observation studies have shown that decreased levels of 25(OH)D3 in blood may have a role in pathogenesis of this disease (24-27).

Meta-analysis, which included 21 prospective studies with 4996 patients suffering from T2 DM and 76 220 subjects without this diagnosis, has shown inverse correlation between vitamin D blood level and the risk of developing T2 DM (RR 0,62 [95% CI 0.54–0.70]) (7). However, the mechanisms and the causes of this relationship are still incompletely clarified. It is not yet established whether these low levels are the cause of diabetes, or they are only a reflection of impaired health (28-30). The three processes which play an important role in T2 DM pathogenesis and could be influenced by vitamin D are the following: insulin secretion (IS), insulin resistance (IR) and inflammation (31).

Vitamin D and insulin secretion

Pancreatic beta-cells responsible for insulin secretion, contain not only receptors for vitamin D active form, but also the 25-hydroxyvitamin D-1α-hydroxylase enzyme, which is responsible for its activation. In addition to the direct influence, vitamin D could also have an indirect influence on IS by increasing the concentration of intracellular calcium in beta-cells, bearing in mind that insulin secretion is a process dependant on calcium. This means that sufficient quantity of vitamin D in blood would facilitate an adequate response of beta-cells to glucose stimu-

lation, whereas its deficiency would decrease IS. Vitamin D has no impact on other pancreatic hormones, or on basal insulinemia (32, 33).

Vitamin D and insulin resistance

Modified cell response to insulin action is an important factor which contributes to T2 DM pathogenesis and which can also be under the direct or indirect influence of vitamin D. Directly, vitamin D influences the expression of insulin receptors on target organs' cells, which improves the cell response to insulin, whereas indirectly it can contribute to the increased concentration of intracellular calcium, which can raise the glucose transport in insulin-dependent tissues. Taking these facts into account, it is clear that the lack of vitamin D could lead to an increased cell resistance to insulin (34, 35).

Vitamin D and inflammation

Inflammation per se, through the activation of inflammatory network factors (fibrinogen, interleukin-6, C-reactive protein) can raise the risk for developing T2 DM (36). These factors can also have an impact on IR and can contribute to damage to beta-cells, causing their apoptosis. Vitamin D could manifest its favorable effect by reducing the production of these cytokines and by modulating their effects (37).

On that account, by examining the effect of vitamin D supplementation with or without calcium, some trials have shown that the intake of this vitamin could be useful in prevention and even delay the onset of T2 DM (38-40). However, the question remains what happens to patients who are already suffering from this disease.

Effects of vitamin D supplementation in patients with T2 DM

Studies conducted in order to explore the role of vitamin D in treating patients with diabetes have shown opposing results (41). Most of them monitored the glycemic status, IS and IR as the final outcome of disease control.

One of the meta-analyses conducted by Pittas et al. tried to provide an answer to that question by summing up the results of intervention studies which had investigated supplementation effects on glucose metabolism. Among the the analyzed studies four of them were short-term and included a small number of patients, whereas the two other were long-term, primarily designed to explore the effect of supplementation on bones. Some studies incorporated only patients with T2 DM, while others included patients with prediabetes, as well as healthy patients. Considering that these studies were distinctively designed and that various forms and doses of vitamin D were used with different patient groups, this meta-analysis could not provide concrete answers on the effect of vitamin D supplementation in patients with T2 DM, but in conclusion, it indicated that a combination of vitamin D and Ca supplements could provide favorable results in regulating glucose metabolism, especially in patients at risk. Conclusion presented by Pittas et al. is mostly based on results of a randomized, double-blind, placebo-controlled study which included a group of 314 patients older than 65, who had been administered with a combination of 700 international units (IU) of vitamin D3 and 500 mg of calcium or a placebo over the period of 3 years. The study was primarily designed to investigate the effects of supplementation on bones. However, *post hoc* analysis of morning glycemia and HOMA-IR index (*Homeostasis model assessment of insulin resistance index*) revealed that patients with primarily irregular values of fasting glycemia had a significant improvement in controlling glucose metabolism after 3 years of vitamin D supplementation (0,4 vs. 6,1 p = 0,04). In patients with normal values of fasting glycemia no effect was documented (37).

Several years later, a systematic review and meta-analysis encompassing a much larger number of studies were conducted for the purpose of providing an answer whether vitamin D supplementation with or without Ca could favorably affect the IR, C-peptide level, morning glycemia and glycosylated hemoglobin (HbA1c), as well as the development of microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (heart attack, stroke, peripheral vascular disease) complications. Inclusion criteria were fulfilled by 15 studies of different quality; some of them included patients with T2 DM, or patients with impaired glucose tolerance, or even healthy subjects. Various forms of vitamin D in doses less than 2000 IU per day were used for supplementation. Some studies combined calcium with vitamin D, but also with placebo. Majority of studies were conducted on a small sample with an average duration of several months.

Out of 15 analyzed studies, 8 explored the influence of vitamin on fasting glycemia level, whereas 4 out of those 8 included subjects with normal fasting blood glucose levels. The analysis revealed a small, but significant decline of fasting glycemia in patients with T2 DM or with impaired glucose tolerance who were administered with some form of vitamin D in comparison to those who were administered with placebo (mean value of difference -0.32 mmol/l, 95% confidence interval, -0.57 to -0.07, p = 0,01).

Vitamin D supplementation also led to IR improvement (measured by using HOMA-IR model or the relation between morning insulinemia and C-peptide values) in patients with impaired glucose tolerance (mean value of difference -0.25, 95% confidence interval, -0.48 to -0.03, p = 0,03).

The impact of vitamin D on the level of HbA1c was explored in 4 studies in which vitamin D supplementation in patients with diabetes or impaired glucose tolerance did not cause decrease in the level of HbA1c compared to placebo (0,03%, 95% confidence interval, -0,18% to 0,23%). In patients with normal glycemic values there was no significant difference in values of these parameters. Moreover, there was no sufficient data to make a conclusion on the effect of supplementation on micro- and macrovascular complications (42).

Nigil Haroon et al. conducted a systematic review encompassing 17 randomized and controlled studies and 7 longitudinal surveys. Tracking period

for all studies was longer than a month; therefore, studies with tracking period of up to 3 months were considered short-term (total of 16 studies), while other studies were long-term ones with tracking period between 4-18 months. As opposed to previous studies, this systematic review comprised studies in which more than 70% of subjects were T2 DM patients. In most cases, cholecalciferol was administered as supplement in the dose of 400 IU to 5700 IU per day. A single-dose intramuscular injection of vitamin D3 was given in 5 studies. The number of patients varied from 10 to 204. Parameters investigated were the following: HbA1c, HOMA-IR, HOMA-B for glycemia control estimate, IR and IS (function of pancreatic beta-cells).

Majority of short-term studies (total of 10) revealed improvement in HbA1c, HOMA-IR and HOMA-B, whereas most of long-term studies did not document any significant effects (43).

Despite great expectations, numerous studies provided different results; hence, the role of vitamin D in treating this chronic non-communicable disease cannot be precisely determined (Table 1). Before proceeding with further research of the matter, one should bear in mind the reasons why surveys conducted so far produced different and inconsistent results. First of all, some studies included heterogeneous patient groups with regard to gender, age, BMI, ethnic affiliation and the existence of an impaired

glucose metabolism, considering that some of them were conducted simultaneously among healthy subjects, those at risk, as well as among those who are already T2 DM patients (37). There are also great discrepancies in the form and method of supplement dosage. Different vitamin D forms were used (inactive and active), different doses and routes of administration (oral, intramuscular). Additionally, various effects in elevating the blood level of 25OHD occurred. Most patients in many studies reported 25OHD3 levels lower than 75 nmol/L, which was probably insufficient to demonstrate favorable effects on glucose metabolism, since some studies showed favorable effects on IR only after the 25 OHD3 blood levels had reached values between 80 and 119 nmol/l (34,58), or even between 100 and 150 nmol/L (59). Therefore, overall effect might have failed to produce results with regard to IR, but the patients who corrected the vitamin D deficiency the most were the only ones who demonstrated a decrease in IR, despite the fact that there was no significant improvement of this parameter in the entire group (45). All of the above mentioned implies the need to define the optimal 25 OH vitamin D blood level, which would have a favorable influence on health in general, and not solely on the bones, as well as to define the necessary dosage and length of supplementation period which would suffice to achieve and maintain the optimal level reached.

Table 1 Randomized clinical research (N > 30) during which vitamin D supplementation effect was tested with or without calcium on glucose metabolism in patients with T2 DM

Study lead author, year	Patients and sample size (N)	Sex M/F	Age, years [a]	Type, dose of used supplement	Time period	Vitamin D level[a]		Result and comment (↔, ↑, ↓) [b]
						Before intervent.	After intervention	
Ljunghall et al. 1987 (44)	Prediabetes and T2 DM [c] (N=65)	M	61-65	1(OH)D3 0,75 mcg/day (N=33); Control group: placebo (N=32)	12 weeks	25(OH)D3 38 ng/ml	NF [d]	↔ HbA1c [e] (values before and after intervention (%):6,46-5,90 vs 6,28-5,70, P<0,01) ↔ IR [*] after IVGTT [**] (I/G [***] values before and after intervention: 0 min. 1,84-1,98 vs. 2,35-1,98, p<0,05; 60. min. 3,56-2,58 vs. 3,90-3,56, p<0,01)
Sugden et al. 2008 (45)	T2 DM (N=34)	M and F	64	Ergocalciferol 100 000 IU [#] in a single dose (N=17); Control group: placebo (N=17)	8 weeks	25(OH)D3 38 nmol/l	25(OH)D3 61 nmol/l in group admin. with ergocalciferol	↔ HbA1c (change compared to basal value (%): 0,01 vs. -0,05 p=0,74) ↑ IS_HOMA [##] with patients with 25(OH)D3 level increased by value which is ≥ 11 nmol/l, HOMA +15 vs. -98 p=0,003
Jorde, Figenschau 2009 (46)	T2 DM (N=32)	M and F	56	D3 40 000 IU/week (N=16); Control group: placebo (N=16)	6 months	25(OH)D3 59 nmol/L	25(OH)D3 57 nmol/L	↔ HbA1c (change compared to basal value (%):-0,2 vs.-0,2, p=0,90) ↔ IR_HOMA [###] (change compared to basal value: 0,3 vs.-0,2, p=0,58) ↔ IS_HOMA (change compared to basal value: 10 vs.63, p=0,99)
Witham et al. 2010 (47)	T2 DM (N=61)	M and F	66	D3 100 000 IU in a single dose (N=19); D3 200 000 IU in a single dose (N=17); Control group: placebo (N=22)	16 weeks	25(OH)D3 45 nmol/L	25(OH)D3 65 nmol/L	↔ HbA1c (change compared to basal value (%):0,1_(100 000 IU) vs. 0,3 _(200 000IU) vs. -0,1_(placebo), p=0,65_(placebo vs. 100 000 IU); p=0,87_(placebo vs. 200 000 IU)) ↔ IR_HOMA (change compared to basal value: 2,4_(100 000 IU) vs. -1,4_(200 000IU)) vs. -8,1_(placebo), p=0,95_(placebo vs. 100 000 IU); p=0,11_(placebo vs. 200 000 IU))

Nikooyeh et al. 2011 (48)	T2 DM (N=90)	M and F	51	D3 1000 IU/day+Ca 300 mg/day (N=30); D3 1000IU/day+ Ca 500 mg/day (N=30); Control group: Ca 300 mg/day (N=30)	12 weeks	25(OH)D3 43,5 nmol/L	25(OH)D3 63 nmol/L	↓HbA1c (%):−0.4 % (p < 0.001) ↓ IR$_{HOMA}$ 3.3 vs. 2.7 (p=0,001)
Shab-Bidar et al. 2011 (49)	T2 DM (N=100)	M and F	52,5	D3 1000 IU/day+ Ca 240 mg/day (N=50); Control group: Ca 240 mg/day (N=50)	12 weeks	25(OH)D3 38 nmol/L	25(OH)D3 53 nmol/L	↓ HbA1c (change compared to basal value in vitamin D group (%):-0,9, p=0,001) ↑ QUICKI [†] (change compared to basal value in vitamin D group:0,01, p=0,001)
Eftekhari et al. 2011 (50)	T2 DM (N=70)	M and F	54	Calcitriol 0,25 μg/day (N=35); Control group: placebo (N=35)	12 weeks	25(OH)D3 40,5 ng/ml	25(OH)D3 32,5 ng/ml	↑ HbA1c (change compared to basal value (%):0,82 vs. 1,56, p<0,005) ↑ IS$_{HOMA}$ (values before and after intervention in vitamin D group:3.4 vs. 4.8, p<0,005) ↑ IR$_{HOMA}$ (values before and after intervention in vitamin D group: 3.6 vs. 4.8, p=0,02)
Heshmat et al. 2012 (51)	T2 DM (N=42)	M and F	56	D3 300 000 IU in a single dose (N=21); Control group: placebo (N=21)	3 months	NF	25(OH)D3 78 ng/ml	↔ HbA1c (change compared to basal value (%):-0,01 vs. -0,2, p=0,495) ↔ IR$_{HOMA}$ (change compared to basal value:0,2 vs. -0,9, p=0,017)
Soric et al. 2012 (52)	T2 DM (N=31)	M and F	54	D3 2000 IU/day (N=16); Control group: vitamin C (N=15)	12 weeks	NF	NF	↓ HbA1c (change compared to basal value (%):-1,4 vs. 0,2, p=0,013), statistically significantly decreased value in patients with HbA1c>9,0 %
Yiu et al. 2013 (53)	T2 DM (N=100)	M and F	65	D3 5000 IU/day (N=50); Control group: placebo (N=50)	12 weeks	NF	25(OH)D3 87 nmol/L	↔ HbA1c (change compared to basal value (%):7,35 vs. 7,20, p=0,008)
Breslavsky et al. 2013 (54)	T2 DM (N=47)	M and F	67	D3 1000 IU/day (N=24); Control group: placebo (N=23)	12 months	NF	NF	↔ HbA1c (values before&after intervention in vit. D group (%): 7,0 vs. 7,3, p=0,212) ↔ IR$_{HOMA}$ (values before&after intervention in vit. D group: 4,2 vs. 6,1, p=0,243) ↔ IS$_{HOMA}$ (values before&after intervention in vit. D group: 84,7 vs. 42,5, p=0,184)
Tabesh et al. 2014 (55)	T2 DM (N=118)	M and F	50	D3 50 000 IU/week (N=29); D3 50 000 IU/week + Ca carbonate 1000mg/day (N=30); Control groups: Ca carbonate 1000 mg/day (N=29); Placebo (N=30)	8 weeks	25(OH)D3 16 ng/ml	25(OH)D3 35,1 ng/ml in vitamin D group	↓ HbA1c (change compared to basal value in vitamin D + Ca group (%):−0,70 ± 0,19, p = 0,02) ↓ IR$_{HOMA}$ (change compared to basal value in vitamin D + Ca group:−0,46 ± 0,20, p = 0,001)
Jehle et al. 2014 (56)	T2 DM (N=55)	M and F	67	D3 300 000 IU in a single dose (N=29); Control group: placebo (N=26)	6 months	25(OH)D3 32 nmol/L	25(OH)D3 84,9 nmol/L in vitamin D group	↓ HbA1c (change compared to basal value (%): 2,9 vs. 6,9, p=0,041) ↓ IR$_{HOMA}$ (change compared to basal value:-12,8 vs. 10, p=0,032
Strobel et al. 2014 (57)	T2 DM (N=86, 14 given up)	M and F	30-78	D3 1902 IU/day N=39; Control group: placebo (N=33)	6 months	25(OH)D3 11,9 ng/ml	25(OH)D3 35 ng/ml in vitamin D group	No effect on the values of metabolic parameters. Patients with the level of 25(OH)D3>20 ng/ml had significantly lower value of HbA1c at the beginning and at the end of the survey compared to group with the level of 25(OH)D3≤20 ng/ml. (HbA1c (mmol/mol Hb):48 vs. 52, p=0,008; 54 vs. 50, p=0,009)

[a] data presented as a mean value or a range; [b] ↔ no statistically significant difference; [c] T2 DM – type 2 diabetes mellitus;
[d] NF – not familiar; [e] HbA1c – glycolized hemoglobin;
↑ statistically significantly increased values;
↓ statistically significantly decreased values;
* IR – insulin resistance;
** IVGTT - intravenous glucose tolerance test;
*** I/G – relation between values of basal insulinemia and glycemia;
[#] IU-international unit;
[##] ISHOMA- homeostasis model assessment of basal insulin secretion;
[###] IRHOMA- homeostasis model assessment of insulin resistance index;
[†] QUICKI-quantitative insuline sensitivity check index

Many studies failed to take into account various therapeutic regimes, physical activity, diet, season and other relevant factors which may influence the rise of vitamin D blood level (60). For example, obese patients require larger doses, due to vitamin D's ability to store in adipose tissue (61). Diet and physical activity can influence both the glycemia control and the level of 25(OH)D3 (35). During physical activity, the time spent outdoors and UV rays can cause the increase in vitamin D level, both in patient group and in control group (40). It is, therefore, essential to consider all relevant factors which might have an impact on the lack of manifestation of differences in supplementation effects in various groups of patients. Additionally, it should be noted that not all studies in which the subjects demonstrated high levels of 25(OH)D3 showed benefits in the case of glycemia control, IS and inflammation (62). The question is to what extent vitamin D can help patients with advanced disease when beta-cells have already been exhausted (35). Some studies reported that the major benefit from vitamin D supplementation would be for patients with an increased risk for T2 DM, and for those patients in early stages of the disease (35, 37, 40). Finally, all studies comprised a small sample and mostly had a short tracking period (shorter than a year).

Conclusion

At the present moment, there is no sufficient evidence to recommend vitamin D to patients with T2 DM as a therapeutic tool for better metabolic control. Therefore, it is necessary to conduct well-designed research which would include a sufficient number of precisely defined patient target groups (who are at risk or already affected in the same disease period), with clearly determined doses of the relevant vitamin D form and a sufficiently long follow-up period, in order to clarify to what extent and at which stage of the disease the patients may have benefit from vitamin D supplementation.

References

1. Wacker M, Holick MF. Vitamin D-effects on skeletal and extraskeletal health and the need for supplementation. Nutrients 2013; 5(1): 111-48. [CrossRef][PubMed]
2. Rosen CJ, Adams JS, Bikle DD, Black DM, Demay MB, Manson JE, et al. The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. Endocr Rev 2012; 33(3): 456-92. [CrossRef][PubMed]
3. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357: 266-81. [CrossRef][PubMed]
4. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96(7): 1911-30. [CrossRef][PubMed]
5. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006; 81(3): 353-73. [CrossRef][PubMed]
6. Forouhi NG, Ye Z, Rickard AP, Khaw KT, Luben R, Langenberg C, et al. Circulating 25-hydroxyvitamin D concentration and the risk of type 2 diabetes: results from the European prospective investigation into cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies. Diabetologia 2012; 55: 2173-82. [CrossRef][PubMed]
7. Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C, Manson JE, et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. Diabetes Care 2013; 36(5): 1422-8. [CrossRef][PubMed]
8. Afzal S, Bojesen S, Nordestgaard BG. Low 25-hydroxyvitamin D and risk of type 2 diabetes: a prospective cohort study and meta-analysis. Clin Chem 2013; 59(2): 381-91. [CrossRef][PubMed]
9. Kostoglou-Athanassiou I, Athanassiou P, Gkountouvas A, Kaldrymides P. Vitamin D and glycemic control in diabetes mellitus type 2. Ther Adv Endocrinol Metab 2013; 4(4): 122-8. [CrossRef][PubMed]
10. Ye Z, Sharp SJ, Burgess S, Scott RA, Imamura F, InterAct Consortium, et al. Association between circulating 25-hydroxyvitamin D and incident type 2 diabetes: a mendelian randomisation study. Lancet Diabetes Endocrinol 2015; 3(1): 35-42. [CrossRef][PubMed]
11. Vieth R. Why "Vitamin D" is not a hormone, and not a synonym for 1,25-dihydroxy-vitamin D, its analogs or deltanoids. J Steroid Biochem Mol Biol 2004; 89-90(1-5): 571-3. [CrossRef][PubMed]
12. Zhang R, Naughton DP. Vitamin D in health and disease: Current perspectives. Nutr J 2010; 9: 65. [CrossRef][PubMed]

13. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 2004; 80(6): 1678S-88S. [CrossRef][PubMed]

14. Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF. Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? J Am Coll Cardiol 2008; 52(24): 1949-56. [CrossRef][PubMed]

15. De Luca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004; 80 (6): 1689S-96S. [CrossRef][PubMed]

16. Christakos S, Lieben L, Masuyama R, Carmeliet G. Vitamin D endocrine system and the intestine. Bonekey Rep 2014; 3: 496. [CrossRef][PubMed]

17. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009; 19(2): 73-8. [CrossRef][PubMed]

18. Binkley N, Ramamurthy R, Krueger D. Low vitamin D status: definition, prevalence, consequences and correction. Endocrinol Metab Clin North Am 2010; 39(2): 287-301. [CrossRef][PubMed]

19. Holick MF. The use and interpretation of assays for vitamin D and its metabolite. J Nutr 1990; 120(11): 1464-9. [CrossRef][PubMed]

20. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. Mayo Clin Proc 2010; 85(8): 752-8. [CrossRef][PubMed]

21. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol 2014; 144: 138-45. [CrossRef][PubMed]

22. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr 2005; 135(2): 317-22. [CrossRef][PubMed]

23. Norman A. Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: integral components of the vitamin D endocrine system. Am J Clin Nutr 1998; 67(6): 1108-10. [CrossRef][PubMed]

24. Liu E, Meigs JB, Pittas AG, Economos CD, McKeown NM, Booth SL, et al. Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring Study. The Am J Clin Nutr 2010; 91(6): 1627-33. [CrossRef][PubMed]

25. Knekt P, Laaksonen M, Mattila C, Härkänen T, Marniemi J, Heliövaara M, et al. Serum vitamin D and subsequent occurrence of type 2 diabetes. Epidemiology 2008; 19(5): 666-71. [CrossRef][PubMed]

26. Pittas AG, Nelson J, Mitri J, Hillmann W, Garganta C, Nathan DM, et al. Plasma 25-hydroxyvitamin D and progression to diabetes in patients at risk for diabetes; An ancillary analysis in the Diabetes Prevention Program. Diabetes care 2012; 35(3): 565-73. [CrossRef][PubMed]

27. Isaia G, Giorgino R, Adami S. High prevalence of hypovitaminosis D in female type 2 diabetic population. Diabetes Care 2001; 24(8): 1496. [CrossRef][PubMed]

28. Mezza T, Muscogiuri G, Sorice GP, Prioletta A, Salomone E, Pontecorvi A, et al. Vitamin D deficiency: a new risk factor for type 2 diabetes? Ann Nutr Metab 2012; 61(4): 337-48. [CrossRef][PubMed]

29. Ozfirat Z, Chowdhury TA. Vitamin D deficiency and type 2 diabetes. Postgrad Med J 2010; 86(1011): 18-25. [CrossRef][PubMed]

30. Scragg R. Vitamin D and type 2 Diabetes: are we ready for a prevention trial? Diabetes. 2008; 57(10): 2565-6. [CrossRef][PubMed]

31. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: The Medical Research Council Ely Prospective Study 1990 - 2000. Diabetes 2008;57(10):2619-25. [CrossRef][PubMed]

32. Harinarayan CV. Vitamin D and diabetes mellitus. Hormones 2014; 13(2): 163-81. [CrossRef][PubMed]

33. Mathieu C, Gysemans C, Giulietti A, Bouillon R. Vitamin D and diabetes. Diabetologia 2005; 48(7): 1247-57. [CrossRef][PubMed]

34. Sung CC, Liao MT, Lu KC, Wu CC. Role of vitamin D in insulin resistance. J Biomed Biotechnol 2012; 2012: 634195. [CrossRef][PubMed]

35. Alvarez JA, Ashraf A. Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol 2010; 2010: 351385. [CrossRef][PubMed]

36. Wang X, Bao W, Liu J, OuYang YY, Wang D, Rong S, et al. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care 2013; 36(1): 166-75. [CrossRef][PubMed]

37. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes: a systematic review and meta-analysis. J Clin Endocrinol Metab 2007; 92(6): 2017-29. [CrossRef][PubMed]

38. Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care 2006; 29(3): 650-6. [CrossRef][PubMed]

39. Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in non-diabetic adults. Diabetes Care 2007; 30(4): 980-6. [CrossRef][PubMed]

40. Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: a systematic review. Eur J Clin Nutr 2011; 65(9): 1005-15. [CrossRef][PubMed]

41. Oosterwerff MM, Eekhoff EMW, Van Schoor NM, Boeke AJP, Nanayakkara P, Meijnen R, et al. Effect of moderate-dose vitamin D supplementation on insulin sensitivity in vitamin D–deficient non-Western immigrants in the Netherlands: a randomized placebo-controlled trial. Am J Clin Nutr 2014; 100(1): 152-60. [CrossRef][PubMed]

42. George PS, Pearson ER, Withm MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. Diabet Med 2012; 29(8): e142-50. [CrossRef][PubMed]

43. Nigil Haroon N, Anton A, John J, Mittal M. Effect of vitamin D supplementation on glycemic control in patients with type 2 diabetes: a systematic review of interventional studies. J Diabetes Metab Disord 2015; 14(1): 3. [CrossRef][PubMed]

44. Ljunghall S, Lind L, Lithell H, Skarfors E, Selinus I, Sorensen OH, et al. Treatment with one-alpha-hydroxycholecalciferol in middle-aged men with impaired glucose tolerance-a prospective randomized double-blind study. Acta Med Scand 1987; 222(4): 361-7. [CrossRef][PubMed]

45. Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. Diabet Med 2008; 25(3): 320-5. [CrossRef][PubMed]

46. Jorde R, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. Eur J Nutr 2009; 48(6): 349-54. [CrossRef][PubMed]

47. Witham MD, Dove FJ, Dryburgh M, Morris AD, Struthers AD. The effect of different doses of vitamin D3 on markers of vascular health in patients with type

2 diabetes: a randomised controlled trial. Diabetologia 2010; 53(10): 2112-9. [CrossRef][PubMed]

48. Nikooyeh B, Neyestani TR, Farvid M, Alavi-Majd H, Houshiarrad A, Kalayi A, et al. Daily consumption of vitamin D or vitamin D calcium-fortified yogurt drink improved glycemic control in patients with type 2 diabetes: a randomized clinical trial. Am J Clin Nutr 2011; 93(4): 764-71. [CrossRef][PubMed]

49. Shab-Bidar S, Neyestani TR, Djazayery A, Eshraghian MR, Houshiarrad A, Gharavi A, et al. Regular consumption of vitamin D-fortified yogurt drink (Doogh) improved endothelial biomarkers in subjects with type 2 diabetes: a randomized double-blind clinical trial. BMC Med 2011; 9: 125. [CrossRef][PubMed]

50. Eftekhari MH, Akbarzadeh M, Dabbaghmanesh MH, Hasanzadeh J. Impact of treatment with oral calcitriol on glucose indices in type 2 diabetes mellitus patients. Asia Pac J Clin Nutr 2011; 20(4): 521-6. [CrossRef][PubMed]

51. Heshmat R, Tabatabaei-Malazy O, Abbaszadeh-Ahranjani S, Shahbazi S, Khooshehchin G, Bandarian F, et al. Effect of vitamin D on insulin resistance and anthropometric parameters in Type 2 diabetes; a randomized double-blind clinical trial. Daru 2012; 20 (1): 10. [CrossRef][PubMed]

52. Soric MM, Renner ET, Smith SR. Effect of daily vitamin D supplementation on HbA1c in patients with uncontrolled type 2 diabetes mellitus: a pilot study. J Diabetes 2012; 4(1): 104-5. [CrossRef][PubMed]

53. Yiu YF, Yiu KH, Siu CW, Chan YH, Li SW, Wong LY, et al. Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. Atherosclerosis 2013; 227(1): 140-6. [CrossRef][PubMed]

54. Breslavsky A, Frand J, Matas Z, Boaz M, Barnea Z, Shargorodsky M. Effect of high doses of vitamin D on arterial properties, adiponectin, leptin and glucose homeostasis in type 2 diabetic patients. Clin Nutr 2013; 32(6): 970-5. [CrossRef][PubMed]

55. Tabesh M, Azadbakht L, Faghihimani E, Tabesh M, Esmaillzadeh A. Effects of calcium-vitamin D co-supplementation on metabolic profiles in vitamin D insuf-

ficient people with type 2 diabetes: a randomised controlled clinical trial. Diabetologia 2014; 57(10): 2038-47. [CrossRef][PubMed]

56. Jehle S, Lardi A, Felix B, Hulter HN, Stettler C, Krapf R. Effect of large doses of parenteral vitamin D on glycaemic control and calcium/phosphate metabolism in patients with stable type 2 diabetes mellitus: a randomised, placebo-controlled, prospective pilot study. Swiss Med Wkly 2014; 144: w13942. [CrossRef][PubMed]

57. Strobel F, Reusch J, Penna-Martinez M, Ramos-Lopez E, Klahold E, Klepzig C, et al. Effect of a randomised controlled vitamin D trial on insulin resistance and glucose metabolism in patients with type 2 diabetes mellitus. Horm Metab Res 2014; 46(1): 54-8. [CrossRef][PubMed]

58. Sadiya A, Ahmed SM, Carlsson M, Tesfa Y, George M, Ali SH, et al. Vitamin D supplementation in obese type 2 diabetes subjects in Ajman, UAE: a randomized controlled double-blinded clinical trial. Eur J Clin Nutr 2015; 69(6): 707-11. [CrossRef][PubMed]

59. Talaei A, Mohamadi M, Adgi Z. The effect of vitamin D on insulin resistance in patients with type 2 diabetes. Diabetol Metab Syndr 2013; 5(1): 8. [CrossRef][PubMed]

60. Hyppönen E, Power C. Vitamin D status and glucose homeostasis in the 1958 British birth cohort the role of obesity. Diabetes Care 2006; 29(10): 2244-6. [CrossRef][PubMed]

61. Alkharfy KM, Al-Daghri NM, Sabico SB, Al-Othman A, Moharram O, Alokail MS, et al. Vitamin D supplementation in patients with diabetes mellitus type 2 on different therapeutic regimens: a one-year prospective study. Cardiovasc Diabetol 2013; 12: 113. [CrossRef][PubMed]

62. Ryu OH, Lee S, Yu J, Choi MG, Yoo HJ, Mantero F. A prospective randomized controlled trial of the effects of vitamin D supplementation on long-term glycemic control in type 2 diabetes mellitus of Korea. Endocr J 2014; 61(2): 167-76. [CrossRef][PubMed]

ULOGA VITAMINA D U LEČENJU BOLESNIKA OBOLELIH OD DIJABETESA MELITUSA TIPA 2

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Vitamin D je steroidni hormon čija je osnovna uloga da održava adekvatnu koncentraciju kalcijuma i fosfora u serumu potrebnu za proces mineralizacije kostiju. Otkriće receptora za aktivni oblik ovog vitamina, kao i enzima koji učestvuje u njegovoj aktivaciji u mnogim drugim tkivima u organizmu, dovelo je do toga da se njegov nedostatak povezuje sa nastankom mnogih hroničnih bolesti kao što su hipertenzija, multipla skleroza, neke vrste malignih tumora i dijabetes melitus tip 2. Mnogobrojne opservacione studije su pokazale da oboleli od dijabetesa melitusa tipa 2 imaju niže vrednosti vitamina D u krvi u odnosu na zdrave ispitanike. To je navelo na pomisao da bi vitamin D mogao igrati bitnu ulogu u pato-genezi ove hronične nezarazne bolesti. Istraživanja koja su pokušala da odgovore na pitanje da li bi suplementacija vitaminom D mogla pomoći ovim bolesnicima da bolje kontrolišu svoju bolest i spreče nastanak komplikacija, kao krajnji ishod pratila je parametre vezane za glikemijski status, sekreciju insulina i insulinsku rezistenciju. Dobijeni rezultati su oprečni i nisu dali dovoljno čvrstih dokaza na osnovu kojih bi mogli preporučiti vitamin D kao terapijsko sredstvo. Međutim, benefit od suplementacije bi mogli imati bolesnici koji su u riziku ili oni na početku bolesti. Da bi procenili koja grupa bolesnika može imati dobiti od suplementacije ovim vitaminom, potrebne su dobro dizajnirane eksperimentalne studije sa precizno definisanim grupama ispitanika (onih koji su u riziku ili već oboleli sa jednakim stažom bolesti), dovoljno visokim dozama vitamina D i dovoljno dugim periodom praćenja.

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Ključne reči: *vitamin D, suplementacija, efekat, dijabetes melitus tip 2*

BENCE JONES PROTEIN – THE FIRST TUMOUR MARKER IN HISTORY OF MEDICINE

Nenad Govedarović

Bence Jones protein is generally accepted term for protein described in urine of myeloma patients. Today, qualitative, as well as quantitative determination of Bence Jones protein serve as routine analyses for diagnosis and screening of myeloma and skeletal affections. Although Bence Jones is not the first to recognize the characteristics of the urine of the diseased, his merit is that he recognized the importance of this protein in patients with myeloma. He is considered as the pioneer in medical chemistry and is one of the first doctors who emphasized the importance of chemical analysis for the diagnosis of the disease. His discovery of proteinuria in multiple myeloma has long been the only biochemical test for cancer until the seventies of the twentieth century, with the discovery of carcinoembrion antigen (CEA) and alpha-fetoprotein (alpha-FP) It can rightly be said that a protein named after him is the first tumor marker in the history of medicine.

Key words: *Bence Jones protein, multiple myeloma, history of medicine*

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Uvod

Termin Bens Džonsov protein (Bence Jones) je danas opšteprihvaćen naziv za belančevinu opisanu u mokraći bolesnika sa multiplim mijelomom. Zanimljivo je da Bens Džons nije prvi dao opis ovog proteina, ali je prepoznao njegov značaj u dijagnostici mijeloma.

Priča o ovom epohalnom otkriću odigrala se u prvoj polovini devetnaestog veka. Glavni akteri bili su doktor Vilijem Mek Intajer, doktor Henri Bens Džons (Henry Bence Jones) i doktor Džon Delrajmpli (John Dalrymple), baš kao i Tomas Aleksander Mek Bin (Thomas Alexander McBean), prvi jasno dokumentovani bolesnik sa multiplim mijelomom.

Tajanstvena bolest gospodina Mek Bina

Tokom 1843. godine, Aleksander Tomas Mek Bin, ugledni četrdesetčetvorogodišnji trgovac iz Londona, počeo je da se pojačano zamara što ga je pri-

moravalo da zastaje pri dužem hodu. Primetio je da ima i češći nagon za mokrenjem, kao i da mu je „veš stalno natopljen mokraćom iako nije mokrio" (1).

Dinamične prirode, Mek Bin septembra 1844. godine odlazi na višednevni boravak u Škotsku, u prirodu. Pri pokušaju izlaska iz jedne podzemne pećine zadobija oštar bol, „kao da mu je nešto prsnulo unutar grudi", što ga je nateralo da legne na tlo i neko vreme ostane ležeći nepokretan. Nakon prespavane noći u obližnjem odmaralištu, bol se smanjio. Po dolasku, Mek Bin se javlja svom ordinirajućem lekaru, Tomasu Votsonu, lekaru opšte prakse, koji je radio u jednoj državnoj bolnici u Londonu. Doktor Votson zaključuje da se radi o upali plućne maramice, te bolesniku aplikuje gipsani zavoj oko grudnog koša, donekle mu olakšavajući bolove koji su se javljali pri disanju i pri najmanjem pokretu ruku (2). Ovo je Mek Binu omogućilo da se koliko toliko, vrati svom svakodnevnom poslu. Mesec dana kasnije bolovi su se ponovo javili te je, od strane mesnog hirurga lečen „pijavicama i flebotomijom radi prečišćavanja krvi" (3).

U proleće 1845, Mek Bin je usled višekratnog „terapijskog ispuštanja krvi" postao adinamičan, bled, a uskoro su mu se pojavili i otoci lica i gležnjeva. Ponovo je posetio svog lekara, dr Votsona, koji mu je prepisao terapiju sa smešom gvožđa citrata i kinina i opijumom. Nakon ovoga, opšte stanje i apetit Mek Bina su se značajno popravili, te ponovo odlazi u Škotsku gde provodi leto.

Septembra 1845, Mek Bin se ponovo vraća u London, sada već jako iscrpljen, sa oslabljenim apetitom, nadutošću i epizodama dijareje, kao i otocima nogu i bolovima u grudima i krsno-slabinskom poja-

su kičme. Ponovo biva sagledan od strane doktora Votsona, koji uključuje terapiju toplim kupkama, amonijakom i kamforom. Budući da stanje bolesnika postaje sve lošije, 30. oktobra 1845. godine, konsultovan je doktor Vilijem Mek Intajer, u to vreme pedesettrogodišnji lekar – konsultant iz lečilišta Metropoliten i Zapadne opšte bolnice (Metropolitan Convalescent Institution , Western General Dispensary) u delu Londona Sveti Meriliboun (St. Marylebone) (4).

Doktor Mek Intajer je saslužao žalbe obolelog na malaksalost i bolove u kostima i konstantovao izražene periferne edeme. Razmatra mogućnost da se radi o mogućem oboljenju bubrega, uzeo je uzorak urina za analizu. Analizom urina nije dokazao šećer, ali je primetio da je urin zamućen, kiseo i velike specifične težine (1035). Zatim je ispitivao prisustvo albumina. U to vreme, standardni test za dokazivanje albumina bio je zagrevanje urina do temperature nešto niže nego što je tačka ključanja, a zatim rashlađivanje istog uzorka. Zagrevajući urin, primetio je da pri tremperaturi nešto višoj od 50C stepeni dolazi do stvaranja precipitata. Ovo je bila temperatura niža nego što je potrebno za taloženje albumina. Daljim zagrevanjem, ovaj precipitat se rastvarao. Mek Intajer nije imao ideju o čemu se radi, ali je zapazio da nije reč o albuminu (4, 5).

Izgleda da doktor Votson pri svojim prethodnim pregledima nije ispitivao urin gospodina Mek Bina, već je bio prisutan kad je doktor Mek Intajer analizirao urin ili mu je to bilo saopšteno. Slično današnjim čestim dupliranjem laboratorijskih analiza, i doktor Votson je nezavisno poslao jedan uzorak urina na analizu, tada tridesetjednogodišnjem doktoru Bensu Džonsu, tada već poznatom „lekaru i hemičaru", u bolnicu Sent Džordž. U propratnom pismu napisanom u subotu, 1. novembra 1845. godine, doktor Votson kaže:

„Dragi doktore Džons,
(...) U epruveti se nalazi urin jako visoke specifične težine. Zagrevanjem postaje lako zamućen. Dodavanjem azotne kiseline postaje penušav i poprima crvenkasti odsjaj i razbistri se, ali daljim hlađenjem opet postaje zamućen. Novim zagrevanjem se ponovno rastvara. Šta je to ?" (1, 3, 4, 6, 7).

Henri Bens Džons je ponovio test sa uzorkom urina i dobio identičan rezultat. Primetio je još da dodavanjem azotne kiseline nastaje precipitat koji se zagrevanjem rastvara, a zatim ponovnim hlađenjem stvara fenomen koji su opisali doktor Votson i Mek Intajer. Džons je obavio opsežne analize i naposletku zaključio da je reč o specifičnom oksidu albumina, tj. „hidratisanom dioksidu albumina". Procenio je da na 1000 zapreminskih delova urina dolazi 66,97 delova dioksida albumina i da je ovaj odnos ekvivalentan udelu albumina u normalnoj krvi. U skladu sa ovakvom procenom, izvesna količina ove „albuminske materije se prelivala u urin", pri čemu je njegova koncentracija i u krvi i u mokraći ostajala nepromenjena. Doktor Bens Džons je izračunao da je bolesnik gubio oko 60 grama belančevina dnevno putem urina, što predstavlja količinu koja se ne može kompenzovati nikakvim unosom putem hrane. O ovome

je sačinio izveštaj i prosledio ga doktoru Mek Intajeru, navodeći da bolesnik ima „albumozuriju" (5, 7). Nekoliko dana nakon ovih događaja, oboleli Mek Bin je preminuo.

Slika 1. Henry Bence Jones (1813-1873)

Neobjašnjiv nalaz na obdukciji

Osim saznanja o bolovima u kostima, na zahvaćenost skeleta se nije sumnjalo tokom trajanja bolesti. Trideset i šest časova nakon smrti Mek Bina, izvršena je obdukcija. Obdukcija je sprovedena od strane doktor Šoa (Shaw) u prisustvu njegovih kolega, Votsona, Ben Džonsa i Mek Intajera. „Rebra su se ugibala pod ošticom skalpela. Toliko su bila meka i krhka da su se lako mogla seći nožem. Unutrašnjost rebara bila je ispunjena glatkom želatinoznom supstancom krvavo crvene boje. Sternum je bio mekan, krhke građe i pucketao je pri pomeranju. Grudni i slabinski kičmeni pršljenovi su bili slične građe kao i rebra. Štaviše, bubrezi su makroskopski i mikroskopski izgledali normalni" (8, 9).

Na mogućnost sekundarne amiloidoze u sklopu mijeloma ukazivala je dijareja, slabost, uvećanje jetre, gasovi, gubitak apetita, otoci gležnjeva, nadutost lica i masivna proteinurija u urinu. Ipak, obdukcijski nalaz normalne građe srca i bubrega i „uve-

ćane jetre koja je uredne strukture", činila je dijagnozu amiloidoze neizvesnom. Izgleda neverovatno da je amiloidoza previđena, budući da su u to vreme voštane promene karakteristične za amiloidozu jetre redovno bile sagledavane.

Doktor Mek Intajer je skromno zaključio „da njegov udeo u traženju prave istine ovde postaje nedovoljan", predlažući da se slučaj prikaže drugim istraživačima „koji imaju dar i kvalifikacije da sprovedu istraživanje na višem nivou, kako bi se identifikovalo očigledno do sada nezabeleženo patološko stanje mokraće" (10).

Treći čovek, Džon Delrajmpli

Doktor Džon Delrajmpli, tada hirurg u Kraljevskoj očnoj bolnici (Royal Ophthalmic Hospital) i član Udruženja mikroskopista, dobio je zadatak da ispita dva lumbalna pršljena i rebro, koji su pripadali preminulom Mek Binu.

Utvrdio je da bolest nastaje u šupljikavom delu kosti, stvarajući crvenkaste formacije ovalnog ili nepravilnog oblika koje su se providile kroz periost.

Šupljine ovih kostiju bile su ispunjene želatinoznom masom čiji su najveći udeo činile krupne ovalne ćelije, dva puta veće od prosečnog eritrocita. Ove ćelije su često imale po dva jedra u kojima se jasno razaznavalo jedarce. Postojale su i pojedinačne krupne ćelije sličnih karakteristika koje su imale po tri jedra. Delrajmpli je zaključio da su opisane ćelije „odgovorne za razmekšanje kostiju (mollities osssium), da su kratkog života i da se nakon razgrađivanja izbacuju putem bubrežne cirkulacije". Kako Delrajmpli, tako je i Mek Intajer, verovao da je reč o malignoj bolesti kostiju (9).

Neposredno nakon ovoga, u toku 1846. godine, Delrajmpli objavljuje rad sa opisom postmortem nalaza u jednom patohistološkom časopisu. Henri Bens Džouns 1847. godine objavljuje svoja zapažanja u vidu kratkog pisma u Lancetu. Godinu dana kasnije on objavljuje i definitivni rad sa detaljnim opisom laboratorijskog nalaza i tehnike koju je koristio za analiziranje urina. Konačno, 1850. godine, Mek Intajer objavljuje svoj izveštaj, sagledan iz kliničkog ugla gledanja.
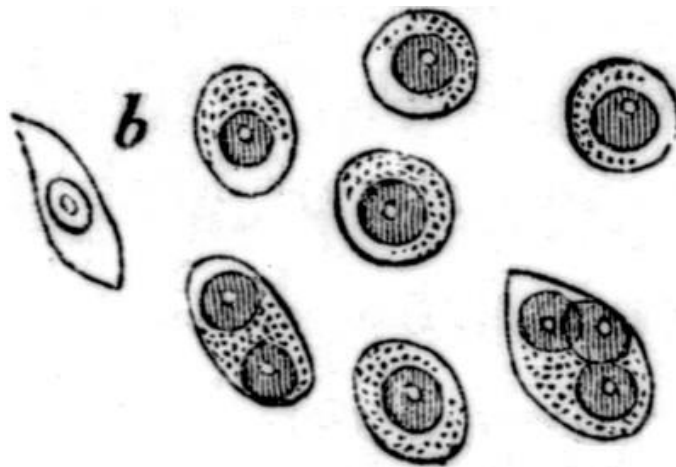
Slika 2. Crtež Džona Delrajmplija na kome je skicirao plazma ćelije viđene na mikroskopskom uzorku

Gledano sa strane, nije poznato šta je sprečilo tri eminentna lekara da objave jedinstveni koautorski izveštaj. U istoriji medicine nije poznat nijedan slučaj izveštavanja o istom bolesniku sačinjen od troje ljudi različitih specijalnosti (patolog, hemičar i kliničar), objavljen u tri različita časopisa, u periodu od četiri godine (9). Ipak, stalna kooperativnost i intenzivna razmena mišljenja ovih lekara umnogome podseća na preteču današnjih konferencija u istraživačkoj medicini.

Prva otkrića iz histologije mijeloma

Termin „plazma ćelija" prvi put je upotrebio Valdejer (Waldeyer) 1875. godine. On je opisao krupne ćelije sa granuliranom citoplazmom, ali nije po

menuo njihovo ekscentrično postavljeno jedro, niti perinuklearno rasvetljenje koje je sadržalo Goldžijev aparat (Golgi apparatus). Vrlo verovatno je da je Valdejer opisao mast ćeliju tkiva. Detaljan opis plazma ćelije srećemo u izveštajima Ramona Kahala (RamoÂn y Cajal) iz 1890. godine koji se bavio studijom sifilističnog kondiloma. Kahal je bio uverenja da su plazma ćelije bile normalni konstituenti vezivnog tkiva (3).

Rajt (Wright) 1900. godine objavljuje izveštaj o pedesetčetvorogodišnjem bolesniku sa tumorom grudnog koša i bolovima u kostima. Radiografija (rendgenski zraci su otkriveni svega tri godine ranije) je pokazala rasvetljenja na 5. i 8. rebru sa leve strane i 7. i 8. rebru sa desne strane grudnog koša. Ujedno je postojala „albumosurija" i anemija. Pri ob

dukciji opisan je tumor koji se sastojao od ćelija sa ekscentrično položenim jedrom i jako bojivim hromatinom, pri čemu su neke od ćelija sadržale i po dva jedra. Zaključuje da je reč o plazma ćelijama ili njihovim neposrednim potomcima. Rajt je takođe opisao i plazma ćelije u normalnoj koštanoj srži i naglasio da je multipni mijelom „neoplazma koja potiče iz crvene kostne srži i vodi poreklo isključivo od plazma ćelija" (11).

Prepoznata je nova bolest

Multipli mijelom je verovatno u ljudskoj vrsti prisutan vekovima. Morze (Morse) je sa saradnicima 1974. godine objavio izveštaj o četiri moguća slučaja mijeloma, opisujući promene na skeletima američkih Indijanaca koji datiraju iz 200 godine posle n.e. Morze je opisao jasno ograničene litičke lezije na kostima bez okolne skleroze niti znakova stvaranja nove kosti (12).

Prvi publikovani opis mijeloma u Americi potiče od Vebera (Weber) i saradnika iz 1898. godine. Izveštaj opisuje bolesnicu sa progresivnim bolovima u kostima i kifozom, koja je umrla od mijeloma, a par godina kasnije (1903) izveštava o bolesniku sa sličnim tegobama i proteinurijom od 15 grama dnevno. Autori su pretpostavili da je mesto produkcije Bens Džonsovog proteina koštana srž, da nastaje iz rezidua citoplazme nakon lize ćelija, te da je njegovo prisustvo od „fatalnog značaja" ukazujući skoro uvek, ako ne i uvek, da bolesnik boluje od multipnog mijeloma. U svom zaključku, Veber navodi važnost upotrebe rendgenovih zraka pri sumnji na zahvaćenost skeleta.

Dijagnoza mijeloma je umnogome olakšana uvođenjem aspiracione punkcije koštane srži. U svojim izveštajima, Arinkin (Arinkin, 1929) i Rozental i Vogel (Rozenthal et Vogel, 1938) naglasili su važnost sternalne punkcije kod bolesnika sa anemijom nejasnog uzroka i poremećajima skeleta (3).

Dalja saznanja o Bens Džonsovom proteinu

Brojni istraživači ostali su zapisani u priči o Bens Džonsovom proteinu. Bredšo (Bradshaw) je 1898. godine utvrdio da hrana nema uticaj na količinu ovog proteina izlučenog u dnevnom urinu. Voters (Walters) 1921. godine daje izveštaj o studiji na tri bolesnika sa mijelomom, da dnevni proteinski unos nema uticaja na količinu proteinurije, da ne postoji diurnalna varijacija, odnosno da je stepen ekskrecije ovog proteina prilično ujednačen tokom dana. Voters zaključuje da je Ben Džonsov protein endogenog porekla i da verovatno nastaje u krvi koja sekretuje abnormalne ćelije kostne srži (3, 8).

Bejn Džons i Vilson (Bayne-Jones et Wilson) 1922. godine dokazuju da se Bens Džonsov protein sastoji od dve grupe sličnih, ali ne identičnih proteina, označivši ih kao grupa I i grupa II. Tek 1956. godine, sa napretkom laboratorijske tehnike, Korngold & Lipari pokazuju da antiserum na Bens Džonsov protein reaguje i sa ovim mijelomskim proteinima. U čast ovih istraživača, dve klase Bens Džonsovih proteina označene su kao kappa i lambda.

Sto sedamnaest godina od prvog opisa proteina, Edelma i Gouli (Edelmman et Gally, 1962) pokazuju da laki lanci dobijeni iz monoklonskog proteina IgG i Bens Džonsovog proteina iz urina istog bolesnika poseduju identični redosled aminokiselina, identičnu molekulsku masu, slično ponašanje pri spektroflourometriji i identičan prikaz pri hromatografiji na celuloznoj traci i elektroforezi na gelu. Ovim je utvrđeno da Bens Džonsov protein vodi poreklo od lakih lanaca imunoglobulina.

Vilson (Wilson) 1964. godine daje opis metode imunofiksacije ili direktne imunoelektroforeze, kada aplikuje antiserum na površinu agara neposredno po završetku elektroforeze. Imunofiksacija je korisna u prepoznavanju malih monoklonskih lakih lanaca koji se ne mogu dokazati standardnom imunoelektroforezom.

Napretkom nauke i usavršavanjem laboratorijskih tehnika, omogućeno je precizno kvantitativno i kvalitativno detektovanje monoklonske belančevine i podtipa lakih lanaca, čime je zaokružena dijagnostika mijeloma.

Zaključak

Identitet bolesnika u ovom prvom dokumentovanom opisu mijeloma ostao je nepoznat skoro čitav vek. Doktor Mek Intajer je u svojim prepiskama sa kolegama bolesnika uvek oslovljavao kao „gospodin M.", a u svojim izveštajima doktor Bens Džons ga nije identifikovao po imenu. Tek 1967. godine, pažljivom i mukotrpnom pretragom Registra umrlih lica za područje Londona, za prvi kvartal 1846. godine, kao i eliminacijom zasnovanom na podacima o prvom slovu imena, starosti, zanimanju i uzroku smrti, pronađen je posmrtni list i utvrđen identitet bolesnika. Bio je to gospodin Tomas Aleksander Mek Bin, preminuo 1. januara 1846. godine, dok je kao uzrok smrti navedena „atrofija zbog albuminurije" (10).

Postojala su mišljenja nekih autora da bi, gledano sa istorijske distance, prikladniji naziv za mijelom bio „Mek Binova bolest sa Mek Intajerovom proteinurijom" (McBean's disease with Macintyre's proteinuria) (5). Termin „multipni mijelom", prvi put je upotrebio von Rustizky 1873. godine u svom opisu multipnih zona tumora koji je zahvatio koštanu srž. Ipak, trebalo je da prođe čitavih 16 godina pa da Oto Kaler (Otto Kahler) u svojim radovima o mijelomu iz 1889. godine uvede termin „H. B. Jones" proteinurija (7).

Danas kvalitativno i kvantitativno određivanje Bens Džonsovog proteina praktično predstavlja deo rutinskih analiza za dijagnostiku i skrining bolesnika sa mijelomom i bolestima skeleta. Iako Henri Bens Džons nije taj koji je prvi prepoznao svojstva urina obolelog, njegova zasluga je u tome da je prepoznao važnost ovog proteina kod bolesnika sa mijelomom. Smatra se pionirom u medicinskoj hemiji i jedan je od prvih lekara koji je naglašavao važnost hemijskih analiza za dijagnozu bolesti. Njegovo otkriće proteinurije u multipnom mijelomu dugo je važilo za jedini biohemijski test za kancer, sve do sedamdesetih godina dvadesetog veka, sa otkrićem karcinoembrionskog antigena (CEA) i alfa fetoproteina (alfa-FP) (9). S pravom se može reći da protein nazvan po njegovom imenu predstavlja prvi tumorski marker u istoriji medicine.

Kratke crtice iz biografije

Vilijem Mek Intajer (William MacIntyre; 1791–1857)

Vilijem Mek Intajer je radio kao lekar u ordiniciji smeštenoj u području Merliboun (84 Harley Street, Marylebone) u središnjem delu Londona. Danas, ovaj urbani deo odiše elegancijom i nosi tradiciju najbolje britanske konsultativne medicine.

Interesantno je da je Mek Intajer podjednako bio posvećen humanitarnom radu, budući da je kao lekar radio u dvema ustanovama za „nemoćne i bolesne" (Metropolitan Convalescent Institution i Western General Dispensary). Uslovi rada u ovakvim bolnicama podrobno su opisani u delu „Oliver Tvist" Čarlsa Dikensa, objavljenog 1838. godine (1).

Džon Delrajmpli (John Dalrymple; 1804-1852)

Rođen u Norviču, u Engleskoj, (Norwich), završio medicinu na Univerzitetu u Edinburgu, Škotska (Edinburgh), a kasnije postaje oftalmolog, odnosno „hirurg za oko" u Kraljevskoj očnoj bolnici (Royal Ophthalmic Hospital) u Londonu. U medicini ostaje zapamćen po svom opisu histopatološkog nalaza kod obolelih od mijeloma, u članku naslovljenom kao „O mikroskopskim odlikama razmekšanja i krhkosti kostiju" (On microscopic characteristics of mollitas and fragilitas ossium) (5).

Henri Bens Džons (Henry Bence Jones; 1813-1873)

Henri Bens Džons je rođen u Safoku, u Engleskoj (Suffolk), 31. decembra 1813. godine. Nakon završenog crkvenog koledža u Kembridžu (Cambridge), odlučuje da se ne pridruži crkvi, već 1837. godine odlazi u bolnicu Sveti Džordž (Saint George) kako bi učio za apotekarskog pomoćnika. Kasnije će za ovaj period života Bens Džons tvrditi da mu je podario najdragocenije iskustvo za sav njegov radni vek (1).

Godinu dana kasnije, podstaknut saznanjima i novinama koje se primenjuju u svetu medicine, Bens Džons se upisuje u novoosnovanu medicinsku školu pri bolnici. Sa širokim interesovanjima pristupa učenju, od Faradejevih lekcija o elektricitetu, do toga kako koristiti u to vreme tek uveden u praksu, ali još uvek kontraverzan, stetoskop.

Nakon završenog koledža, odlazi na šestomesečne studije u Gesen (Giessen), u Nemačku, na šestomesečno usavršavanje u hemijskoj laboratoriji Libig (Liebig). U to vreme, u medicinskim školama se malo pažnje poklanjalo hemizmu tkiva, već se držalo teorije da se dijagnoza postavlja na osnovu simptomatologije i nalaza na pojedinom organu. Iskustvo stečeno u radu u ovoj laboratoriji stvorilo je u njemu doživotnu želju za proučavanjem ljudskog hemizma i primenom hemije u medicini. Nije preterano reći da, zajedno sa drugim učenicima laboratorijske škole u Libigu, Bens Džouns postaje okosnica nečega iz čega će se, kao posebna specijalnost, izroditi klinička hemija (7).

References

1. Rathore R, Coward RA, Woywodt A. What's in a name? Bence Jones protein. Clin Kidney J 2012; 5: 478–83. [CrossRef][PubMed]
2. Kyle RA, Rajkumar SV. Multiple Myeloma. Blood 2008; 111(6): 2962–72. [CrossRef][PubMed]
3. Kyle RA. Multiple Myeloma: an odyssey of discovery. Br J Haematol 2000; 111(4): 1035-44. [CrossRef][PubMed]
4. Kyle RA. Multiple Myeloma: how did it begin? Mayo Clin Proc 1994; 69(7): 680-3. [CrossRef][PubMed]
5. Kyle RA. Henry Bence Jones - Physician, chemist, scientist and biographer: a man for all seasons. Br J Haematol 2001; 115(1): 13-8. [CrossRef][PubMed]
6. Kahn SN. Dear Dr. [Bence] Jones... Clin Chem 1991; 37(9): 1557-8. [CrossRef][PubMed]
7. Rosenfeld L. Henry Bence Jones (1813-1873): the Best „Chemical Doctor" in London. Clin Chem 1987; 33(9): 1687-92. [CrossRef][PubMed]
8. Stone MJ. Henry Bence Jones and his protein. J Med Biogr 1998; 6(1): 53-7. [CrossRef][PubMed]
9. Hajdu SI. A Note from history: the First biochemical test for detection of cancer. Ann Clin Lab Sci 2006; 36(2): 222-3. [CrossRef][PubMed]
10. Clamp JR. Some aspects of the first recorded case of multiple myeloma. Lancet 1967; 2(7530): 1354-6. [CrossRef][PubMed]
11. Wright JH. A case of multiple myeloma. Transactions of the Association of American Physicians 1900; 15: 137-47.
12. Morse D, Dailey RC, Bunn J. Prehistoric multiple myeloma. Bull N Y Acad Med 1974; 50(4): 447-58. [CrossRef][PubMed]

BENS DŽONSOV PROTEIN – PRVI TUMORSKI MARKER U ISTORIJI MEDICINE

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Termin Bens Džonsov protein (Bence Jones) danas je opšteprihvaćen naziv za belančevinu opisanu u mokraći bolesnika sa multiplim mijelomom. Danas, kvalitativno i kvantitativno određivanje Bens Džonsovog proteina praktično predstavlja deo rutinskih analiza za dijagnostiku i skrining bolesnika sa mijelomom i bolestima skeleta. Iako Henri Bens Džons nije bio taj koji je prvi prepoznao svojstva urina obolelog, njegova zasluga je u tome da je prepoznao važnost ovog proteina kod bolesnika sa mijelomom. Smatra se pionirom u medicinskoj hemiji i jedan je od prvih lekara koji je naglašavao važnost hemijskih analiza za dijagnozu bolesti. Njegovo otkriće proteinurije u multipnom mijelomu dugo je važilo za jedini biohemijski test za kancer, sve do sedamdesetih godina dvadesetog veka i otkrića karcinoembrionskog antigena (CEA) i alfa fetoproteina (alfa-FP). S pravom se može reći da protein nazvan po njegovom imenu predstavlja prvi tumorski marker u istoriji medicine.

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Ključne reči: *Bens Džonsov protein, multipni mijelom, istorija medicine*

THE EFFECT OF TEMPERATURE TREATMENT OF XENOGENEIC BONE SUBSTITUTE ON THE TISSUE RESPONSE – A MINI REVIEW

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In general, it has been revealed that interaction of bone substitute material with the host immune system is dependent upon their physico-chemical properties. In the case of xeno-grafts, different purification methods are applied to process the precursor tissue. One purification method that differs the most is the applied temperature. Materials treated with low and high temperatures are available. In this context, the question remains as to the influence of the different temperature treatments on the physical and chemical material properties and, thus, on the tissue reactions during the healing processes. It has been hypothesized that materials that induce mononuclear cells induce physiological healing processes, while a pathological reaction is accompanied with the induction of multinucleated giant cells (MNGCs). In this mini-review, the focus is on the comparison of preclinical research into tissue reactions to sintered and non-sintered bovine-derived xenograft. Interpretation of this data showed that an induction of higher numbers of MNGCs by sintered xenograft also induced a higher implant bed vascularization. Finally, the higher number of MNGCs and increased vascularization presumably resulted in a higher expression of anti-inflammatory molecules that may support the process of bone remodeling.

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Key words: *bone substitute, xenograft, multinucleated giant cells, implant bed vascularization, inflammation*

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Introduction

Bone tissue is a hard tissue and a type of dense connective tissue which has the ability to grow and heal itself in the case of minor defects. However, more pronounced bone defects and bone augmentation sites require a scaffold as a platform for bone regeneration. Bone substitution means the implantation of substitute materials into bone defects with the aim of allowing defect regeneration, ideally up to the condition of *restitution ad integrum*, i.e., the complete bone defect healing. A large variety of bone substitute materials are nowadays available on the market. Bone grafts can generally be classified based on their origin. Bone substitute materials can originate from autografts, allografts, xenografts and synthetic grafting materials. An autogenic graft is harvested from the patient itself, i.e., most often from the iliac crest bone. However, its harvesting is often accompanied with the effects of a surgical intervention, such as pain or infections at the donor side (1, 2). Furthermore, an allograft is derived from the individuals of same species, i.e., most often living human donors. Xenografts are derived from non-human species, i.e., mostly animal sources such as bovines. In contrast, synthetic grafting materials are manufactured mostly based on calcium phos-phates such as hydroxyapatite (HA) or beta-trical-cium pho-

sphate (β-TCP) as these compounds are parts of the natural mineral component of bone tissue (3).

In general, an optimal bone graft should be easy to handle and should become incorporated, re-vascularized and integrated (4). Additionally, it should be biocompatible, non-immunogenic, physiologically stable and in simple words, it should be acceptable by patient and without the risk of disease transmission (4).

Interestingly, it has already been revealed that both "natural" bone substitute materials such as bovine-based xenografts and synthetic grafting materials induce an immune response within the implantation bed of the recipient, called a "foreign body reaction to biomaterials" (5, 6). In this cascade, macrophages and their fused relative cell type, the so-called multinucleated giant cell (MNGC), have mani-

foldly shown to be involved (5). It has been revealed that both these cell types are regulatory elements of the tissue reaction cascade as they express pro- and anti-inflammatory molecules that guide the cascade and, thus, the bone healing process (Figure 1.) (5, 7). In this context, it has been shown in more detail that the severity and the inflammatory alignment of the material-associated tissue reaction cascade is mainly influenced by different physical and chemical properties of bone substitute materials, such as their chemical composition, the granule size or the granule porosity, amongst others (8-10). Interestingly, these physicochemical properties of a bone substitute have also shown to have importance for the clinic as these factors have influence on the bony regeneration process (5, 11).
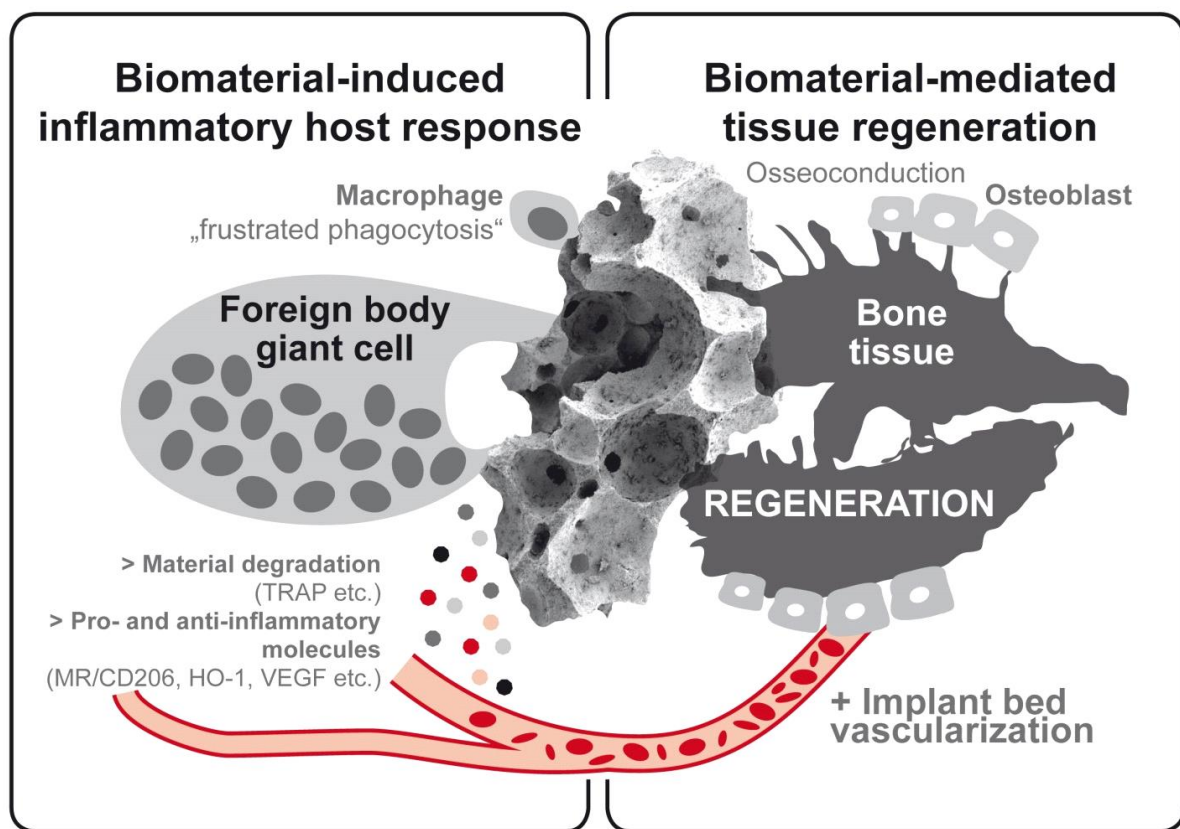
Figure 1. Schematic illustration of the correlation between cellular and inflammatory processes caused by bone materials, the process of implant bed vascularisation and the process of bone tissue regeneration

In case of both allo- and xenografts, the donor tissue has to be purified from immunologically effectual components such as cells or different proteins prior to their application as a bone graft material. Xenografts based on bovine donor tissue or bovine hydroxyapatite (BHA) are widely used and researched bone substitute materials due to their similar physiochemical properties compared to human bone, their osteoconductivity potential and availability (12). Two of the most popular and commonly used bovine-derived xenografts are Bio-Oss™ (Geistlich Bio-

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materials, Wolhusen, Switzer-land) and cerabone® (botiss biomaterials, Berlin, Germany). Although it has been shown that both these bovine-derived bone substitutes provide acceptable regenerative potential, there are still essential differences in their purification processes (13, 14). The most prominent variation in these processes is the treatment of the precursor bone tissue at different temperatures. While Bio-Oss™ undergoes a low heat treatment with temperatures around 300 °C, cerabone® becomes treated at temperatures of up to 1250 °C (so-called

"sintering") (15, 16). Based on the different temperature treatments, it is presumable that there are differences in the material structure, subsequent tissue reactions and maybe in the healing capacity of both materials. The present mini-review aims to compare the tissue reactions to these two xenogeneic bone substitute materials and gives an overview of preclinical results.

The preparation processes of the xenogeneic bone substitute materials

In order to have a successful bone substitute produced from natural sources, it is extremely crucial to carry out physical and/or chemical treatments in order to remove all organic material and immunologically active contents, such as pathogens and cells. Most often, only the mineral content of the former bone tissue remains and should function as a bone substitute. Interestingly, different purification methods are applied for manufacturing of the available xenogeneic bone substitute materials.

In case of Bio-Oss™, an initial purification step that includes a heat treatment with temperatures up to 300 °C and a further cleansing step by means of a strongly alkaline agent, sodium hydroxide (NaOH) are applied (17). In this context, it has been stated that the treatment of the bovine bone matrix at lower temperatures, as in case of Bio-Oss™, leads to the preservation of the mineral crystals of the bone matrix (18). However, it has been revealed that the crystallinity changes during the heat treatment, although the bone substitute material consists of phase-pure hydroxyapatite (HA) (19). In contrast to human bone, the heat-treated HA causes an increase of the crystal size by 200 – 300%, quantified via transmission electron microscopy (TEM) and X-ray diffraction (XRD) measurement (19).

For the synthesis of cerabone®, a two-stage heat-based process, including an initial oxidative combustion at temperatures around 800 °C and a second heat treatment at higher temperatures of up to 1,250 °C (sintering), is applied (20). Although cerabone® also consists of 100% HA, further differences in the crystallinity have been revealed (19). A larger increase of the crystal size by 500-1000% and a higher crystal density in comparison to human bone have been measured, which leads to the conclusion that cerabone® is comparable to a ceramic-based material (21).

Results of preclinical in vivo studies

The inflammatory tissue reactions to both xenogeneic materials have comparatively been analyzed using the subcutaneous implantation model and established histomorphometrical methods (7-10, 13, 14, 16, 21-26). Different numbers of multinucleated giant cells (MNGCs), which showed partial expression of the lytic enzyme tartrate-resistant acid phosphatase (TRAP), have been found besides a large number of mononucleated cells such as macrophages (26). The comparative measurements showed initially that larger numbers of (TRAP-positive) MNGCs were found in the case of Bio-Oss™, which was related to the smaller material particles trig-gering the tissue reaction even at early study time points, while their numbers significantly decreased at later time points. In contrast, comparatively high numbers of MNGCs were found within the implantation beds of cerabone® starting after 10 days post *implantationem*. However, the MNGC numbers did not decrease with time and remained at a comparable level up to 60 days post *implantationem*. Interestingly, implant bed vascula-rization also differed: while a fast and continuously high implantation bed vascularization was measured for Bio-Oss™, vascularization was initially low and increased over time to a high level in case of cerabone®.

Moreover, it has been shown that the MNGCs in the implant bed of Bio-Oss™ seem to be foreign body giant cells (FBGCs), as also found in case of a synthetic hydroxyapatite-based bone substitute, which indicates that the different treatments based on different physical and chemical methods lead to a conversion of the former bone matrix in the direction of a foreign material (7). In this context, it is possible that the MNGCs found in the implant beds of cerabone® are also FBGCs. However, it has been shown that this cell type is not restricted to express only pro-inflammatory molecules but also anti-inflammatory mediators such as the vascular endo-thelial growth factor (VEGF) or the mannose receptor (MR, CD206), which leads to a related increased implant bed vascularization (22). Thus, it is pres-umable that a higher induction of MNGCs also might also cause a better bone regeneration, as implant bed vasculari-zation is a key component for (bone) tissue regeneration (27, 28). Interestingly, the first results of a new study also confirm this theory, as it could be shown that a higher severity of a material-related inflammatory process, including MNGCs, supports directly and indirectly the bony regene-ration process (unpublished data by Barbeck et al.).

Different preclinical implantation studies have been conducted to evaluate the material-related bone growth by means of Bio-Oss™ and cerabone® (Table 1) (29-39). In the case of cerabone®, only a few preclinical *in vivo* studies quantitatively analyzing bone regeneration have been conducted (Table 1) (29, 30). Interestingly, these studies report very diverse results. The studies give the range of newly built bone using cerabone® at different time points to be; 0 and 40% for between 21-28 days, 14-78% between 42-84 days and finally 21–30% for up to 168 days (Table 2) (29, 31, 39). In contrast, a variety of *in vivo* studies have been carried out to analyze the bone regeneration capacities of Bio-Oss™ (Table 1) (32-38, 39). A comparable variety of histomorphometrical results have been presented as in case of Bio-Oss™ (Table 1). Altogether, percent values of newly built bone tissue are between 8 and 34% for a time frame between 14-30 days, 4-57% for a time frame between 42-84 days and finally 39-47% for the time frame between 112-168 days have been found (Table 2) (32-38, 39). Altogether, the comparison of these preclinical data shows comparable bone healing capacities for both bone substi-tute materials (Table 2). However, even in case of cerabone®, more studies are necessary to evaluate the healing properties of this xenograft treated at high temperatures.

Table 1. Overview of preclinical *in vivo* studies analyzing the bone healing capacities of both xenogeneic bone substitutes

Implantation model	Time point(s)	Bone growth	Authors
Cerabone			
Calvarian critical size defect model, rat	28 and 56 days	28 days (42.10%) 56 days (77.60 %)	Shakir *et al.* (31)
Calvarian critical size defect model, rabbit	60 days	55%	Huber *et al.* (29)
Periapical implantation model, cat	84 and 168 days	30.2% 5.7% at the grafted membrane-protected sites	Artzi *et al.* (30)
Bio-Oss, Cerabone			
Calvarian critical size defect model, rabbit	21 and 42 days	cerabone® 60.6% new bone growth for BioOss® 52.1% new bone growth for	Institute of Bone Scienc, Seoul, Korea
Bio-Oss			
Calvarian critical size defect model, rabbit	14 and 28 days	14 days (8.6 3.1%) 28 days (15.7 5.4%)	Park *et al.* (c) (34)
Calvarian critical size defect model, rabbit	28 days	11.7 2.4 %	Rokn, Khodadoostan (35)
Calvarian critical size defect model, rabbit	28 and 56 days	28 days (12.9 5.8%) 56 days (14 7.2%)	Park *et al.* (b) (33)
Calvarian critical size defect, rat	30 and 60 days	30 days (54.05% 5.78) 60 days (63.58% 5.78)	Oliviera *et al.* (36)
Calvarian critical size defect, rat	42 and 84 days	42 days (6.4 4.3%) 84 days (8.2 3.9%)	Park *et al.* (a) (32)
Calvarian critical size defect model, sheep	84 and 168 days	84 days (21 ± 1.2 %) 168 days (39 ± 3.3 %)	Scarano *et al.* (38)
Calvarian critical size defect, rat	112 days	47.4 7.1 %	Mah *et al.* (39)
Calvarian critical size defect model, rabbit	8 weeks	57.76 ± 7.75 %	Takauti *et al.* (37)

Table 2. Comparison of the preclinical in vivo data

	Bio-Oss	**cerabone**
14 – 30 days	**8 – 34% (18.69%)**	**0 – 40% (20%)**
42 – 84 days	**4 – 57% (23.76%)**	**14 – 78 % (46,56%)**
112 – 168 days	**39 – 47% (43.2%)**	**21 – 30% (25,9)**

Conclusion

The sintering temperature of bone substitutes including bovine hydroxyapatite based materials has shown to be an important parameter that can affect the properties of HA. In this context, the sintering temperature has influence on phase stability, densification behavior, crystallinity and porosity of HA. The data outlined in the present mini-review show that the heat treatment at different temperatures influence the tissue response to the bone matrix based bone substitute materials. Although it has been shown that both Bio-Oss™, which is purified at temperatures of 300 °C, and cerabone® with a treat-ment at 1250 °C, allow for comparable outcomes of bone healing, the number of the MNGCs and the related implant bed vascularization seem to be influenced by the material differences, induced by the different temperature treatments. Thus, it is also conceivable that variations in the expression of pro- and anti-inflammatory molecules by both macrophages and MNGCs are induced by these material differences. Thus, the question arises as to how the temperature treatment affects material properties to be more favorable for optimal bone tissue regeneration.

Conflicts of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References

1. Hill NM, Geoffrey Horne J, Devane PA. Donor site morbidity in the iliac crest bone graft. Aust N Z J Surg 1999; 69(10):726-8. [CrossRef] [PubMed]
2. Arrington DE, Smith WJ, Chambers HG, Bucknell AL, Davino NA. Complications of iliac crest bone graft harvesting. Clin Orthop Relat Res 1996; (329):300-9. [CrossRef] [PubMed]
3. Vallet-Regí M, González-Calbet JM. Calcium phosphates as substitution of bone tissues. Prog Solid State Ch 2004; 32(1-2):1-31. [CrossRef]
4. Damien CJ, Parsons JR. Bone graft and bone graft substitutes: A review of current technology and applications. J Appl Biomater 1991; 2(3):187-208. [CrossRef] [PubMed]
5. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol 2008; 20 (2):86-100. [CrossRef] [PubMed]
6. Nuss KMR, von Rechenberg B. Biocompatibility issues with modern implants in bone - A review for clinical orthopedics. Open Orthop J 2008; 2:66-78. [CrossRef] [PubMed]
7. Barbeck M, Booms P, Unger R, Hoffmann V, Sader R, Kirkpatrick CJ, et al. Multinucleated giant cells in the implant bed of bone substitutes are foreign body giant cells-New insights into the material-mediated healing process. J Biomed Mater Res A 2017; 105(4):1105-11. [CrossRef] [PubMed]
8. Barbeck M, Najman S, Stojanović S, Mitić Ž, Živković JM, Choukroun J, et al. Addition of blood to a phycogenic bone substitute leads to increased *in vivo* vascularization. Biomed Mater 2015; 10(5):055007. [CrossRef] [PubMed]
9. Ghanaati S, Barbeck M, Detsch R, Deisinger U, Hilbig U, Rausch V, et al. The chemical composition of synthetic bone substitutes influences tissue reactions *in vivo*: histological and histomorphometrical analysis of the cellular inflammatory response to hydroxyapatite, beta-tricalcium phosphate and biphasic calcium phosphate ceramics. Biomed Mater 2012; 7(1): 015005. [CrossRef] [PubMed]
10. Lorenz J, Barbeck M, Sader RA, Kirkpatrick CJ, Russe P, Choukroun J, et al. Foreign body giant cell-related encapsulation of a synthetic material three years after augmentation. J Oral Implantol 2016; 42(3):273-7. [CrossRef] [PubMed]
11. Cheyn Z, Klein T, Murray RZ, Crawford R, Chang J, Wu C, et al. Osteoimmunomodulation for the development of advanced bone biomaterials. Mater Today 2016; 19(6):304-21. [CrossRef]
12. Campana V, Milano G, Pagano E, Barba M, Cicione C, Salonna G, et al. Bone substitutes in orthopaedic surgery: from basic science to clinical practice. J Mater Sci Mater Med 2014; 25(10):2445-61. [CrossRef] [PubMed]
13. Barbeck M, Udeabor SE, Lorenz J, Kubesch A, Choukroun J, Sader RA, et al. Induction of multinucleated giant cells in response to small sized bovine bone substitute (Bio-Oss) results in an enhanced early implantation bed vascularization. Ann Maxillofac Surg 2014; 4(2):150-7. [CrossRef] [PubMed]
14. Ghanaati S, Barbeck M, Booms P, Lorenz J, Kirkpatrick CJ, Sader RA. Potential lack of "standardized" processing techniques for production of allogeneic and xenogeneic bone blocks for application in humans. Acta Biomater 2014;10(8):3557-62. [CrossRef] [PubMed]
15. Chappard D, Fressonnet C, Genty C, Baslé MF, Rebel A. Fat in bone xenografts: Importance of the purification procedures on cleanliness, wettability and biocompatibility. Biomaterials 1993; 14(7):507-12. [CrossRef] [PubMed]
16. Barbeck M, Udeabor S, Lorenz J, Schlee M, Holthaus MG, Raetscho N, et al. High-temperature sintering of xenogeneic bone ubstitutes leads to increased multinucleated giant cell formation: *in vivo* and preliminary clinical results. J Oral Implantol 2015; 41(5):e212-22. [CrossRef] [PubMed]
17. Barbeck M, Unger R, Schnettler R, Wenisch S, Witte F. Xenogeneic bone grafting materials. Implants. International Magazine of Oral implantology 2017; 17(3): 34-6.

18. Bufler MA. Calciumphosphate Synthese, Reaktionen in Wässrigen Median und Charakterisierung von Oberflachen und Grenzflachen [dissertation]. Siegen: der Universitat Siegen; 2004.

19. Weber U. Calciumorthophosphate mit kontrollierter Kristallmorphologie und ein injizierbares, poröses Biomaterial: Materialentwicklung und Charakterisierung [dissertation]. Rostock: der Universitat Rostock; 2013.

20. Ramirez Fernandez MP, Gehrke SA, Perez Albacete Martinez C, Calvo Guirado JL, de Aza PN. SEM-EDX study of the degradation process of two xenograft materials used in sinus lift procedures. Materials (Basel) 2017; 10(5):542. [CrossRef] [PubMed]

21. Barbeck M, Dard M, Kokkinopoulou M, Markl J, Booms P, Sader RA, et al. Small-sized granules of biphasic bone substitutes support fast implant bed vascularization. Biomatter 2015; 5(1):e1056943. [CrossRef] [PubMed]

22. Barbeck M, Motta A, Migliaresi C, Sader R, Kirkpatrick CJ, Ghanaati S. Heterogeneity of biomaterial-induced multinucleated giant cells: Possible importance for the regeneration process? J Biomed Mater Res A 2016; 104(2):413-8. [CrossRef] [PubMed]

23. Lorenz J, Eichler K, Barbeck M, Lerner H, Stubinger S, Seipel C, et al. Volumetric analysis of bone substitute material performance within the human sinus cavity of former head and neck cancer patients: A prospective, randomized clinical trial. Ann Maxillofac Surg 2016; 6(2):175-81. [CrossRef] [PubMed]

24. Barbeck M, Lorenz J, Kubesch A, Böhm N, Booms P, Choukroun J, et al. Porcine dermis-derived collagen membranes induce implantation bed vascularization via multinucleated giant cells: a physiological reaction? J Oral Implantol 2015; 41(6):e238-51. [CrossRef] [PubMed]

25. Ghanaati S, Barbeck M, Lorenz J, Stuebinger S, Seitz O, Landes C, et al. Synthetic bone substitute material comparable with xenogeneic material for bone tissue regeneration in oral cancer patients: First and preliminary histological, histomorphometrical and clinical results. Ann Maxillofac Surg 2013; 3(2):126-38. [CrossRef] [PubMed]

26. Lorenz J, Kubesch A, Korzinskas T, Barbeck M, Landes C, Sader RA, et al. Trap-positive multinucleated giant cells are foreign body giant cells rather than osteoclasts: results from a split-mouth study in humans. J Oral Implantol 2015; 41(6):e257-66. [CrossRef] [PubMed]

27. Yang YQ, Tan YY, Wong R, Wenden A, Zhang LK, Rabie AB. The role of vascular endothelial growth factor in ossification. Int J Oral Sci 2012; 4(2):64-8. [CrossRef] [PubMed]

28. Michalski MN, Koh AJ, Weidner S, Roca H, McCauley LK. Modulation of osteoblastic cell efferocytosis by bone marrow macrophages. J Cell Biochem 2016; 117(12):2697-706. [CrossRef] [PubMed]

29. Huber FX, Berger I, McArthur N, Huber C, Kock HP, Hillmeier J, et al. Evaluation of a novel nanocrystalline hydroxyapatite paste and a solid hydroxyapatite ceramic for the treatment of critical size bone defects (CSD) in rabbits. J Mater Sci Mater Med 2008; 19(1):33-8. [CrossRef] [PubMed]

30. Artzi Z, Wasersprung N, Weinreb M, Steigmann M, Prasad HS, Tsesis I. Effect of guided tissue regeneration on newly formed bone and cementum in periapical tissue healing after endodontic surgery: an *in vivo* study in the cat. J Endod 2012; 38(2):163-9. [CrossRef] [PubMed]

31. Shakir M, Jolly R, Khan AA, Ahmed SS, Alam S, Rauf MA, et al. Resol based chitosan/nano-hydroxyapatite nanoensemble for effective bone tissue engineering. Carbohydr Polym 2018; 179:317-27. [CrossRef] [PubMed]

32. Park JW, Jang JH, Bae SR, An CH, Suh JY. Bone formation with various bone graft substitutes in critical-sized rat calvarial defect. Clin Oral Implants Res 2009; 20(4):372-8. [CrossRef] [PubMed]

33. Park JW, Kim ES, Jang JH, Suh JY, Park KB, Hanawa T. Healing of rabbit calvarial bone defects using biphasic calcium phosphate ceramics made of submicron-sized grains with a hierarchical pore structure. Clin Oral Implants Res 2010; 21(3):268-76. [CrossRef] [PubMed]

34. Park JW, Ko HJ, Jang JH, Kang H, Suh JY. Increased new bone formation with a surface magnesium-incorporated deproteinized porcine bone substitute in rabbit calvarial defects. J Biomed Mater Res A 2012; 100(4):834-40. [CrossRef] [PubMed]

35. Rokn AR, Khodadoostan MA, Reza Rasouli Ghahroudi AA, Motahhary P, Kharrazi Fard MJ, Bruyn HD, et al. Bone formation with two types of grafting materials: a histologic and histomorphometric study. Open Dent J 2011; 5:96-104. [CrossRef] [PubMed]

36. Oliveira MR, deC Silva A, Ferreira S, Avelino CC, Garcia IR Jr, Mariano RC. Influence of the association between platelet-rich fibrin and bovine bone on bone regeneration. A histomorphometric study in the calvaria of rats. Int J Oral Maxillofac Surg 2015; 44(5):649-55. [CrossRef] [PubMed]

37. Takauti CA, Futema F, Brito Junior RB, Abrahao AC, Costa C, Queiroz CS. Assessment of bone healing in rabbit calvaria grafted with three different biomaterials. Braz Dent J 2014; 25(5):379-84. [CrossRef] [PubMed]

38. Scarano A, Piattelli A, Pecora G, Petrizzi L, Valbonetti L, Varasano V, et al. A histomorphometric comparison of anorganic bovine bone (ABB) and calcium sulfate (CaS) used in sinus augmentation procedures: a study in sheep. J Osseointegr 2010; 2(2):38-44. [CrossRef]

39. Mah J, Hung J, Wang J, Salih E. The efficacy of various alloplastic bone grafts on the healing of rat calvarial defects. Eur J Orthod 2004; 26(5):475-82. [CrossRef] [PubMed]

Revijalni rad

EFEKAT TERMIČKOG TRETMANA KSENOGENIH KOŠTANIH ZAMENIKA NA TKIVNI ODGOVOR – MINI PREGLED

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　　　　Uopšteno govoreći, otkriveno je da materijali za zamenu kosti izazivaju interakcije sa imunskim sistemom domaćina zavisno od njihovih fizičko-hemijskih osobina. U slučaju kseno-grafta, primenjuju se različite metode prečišćavanja za obradu izvornog tkiva. Jedna od naj-zastupljenijih metoda koja se primenjuje za njihovo prečišćavanje je termička, pošto se do-stupni materijali tretiraju zagrevanjem na različitim temperaturama. U ovom kontekstu ostaje pitanje kako različite temperature tretmana mogu da utiču na fizička i hemijska svojstva materijala, a time i na reakcije tkiva na njih i procese lečenja. Pretpostavljeno je da materijali čiju tkivnu reakciju karakterišu mononuklearne ćelije izazivaju fiziološke procese zarastanja, dok uz patološku reakciju ide indukcija multinuklearnih gigantskih ćelija (MNGC). U ovom mini pregledu fokus je na komparaciji tkivnih reakcija na sinterovane i nesinterovane goveđe ksenografte u pretkliničkim ispitivanjima. U tumačenju ovih podataka pokazalo se da indukcija većeg broja MNGC pomoću sinterovanog ksenografta indukuje i veću vaskularizaciju ležišta implanta. Konačno, veći broj MNGC i veća vaskularizacija, zajedno sa verovatno većom eks-presijom antiinflamatornih molekula mogu podržati proces remodelovanja kostiju.

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Ključne reči: *koštani zamenik, ksenograf, multinuklearne gigantske ćelije, vaskularizacija ležišta implantata, inflamacija*

EFEKTI KAROTIDNOG STENTINGA NA KOGNITIVNE FUNKCIJE KOD BOLESNIKA SA STENOZOM KAROTIDNE ARTERIJE

Marijana Stošić, Marija Andjelković-Apostolović, Nataša Đinđić, Dušica Ilić, Saša Ristić, Miroslava Živković, Dragan Stojanov

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U radu " EFEKTI KAROTIDNOG STENTINGA NA KOGNITIVNE FUNKCIJE KOD BOLESNIKA SA STENOZOM KAROTIDNE ARTERIJE" autora Marijana Stošić, Marija Andjelković-Apostolović, Nataša Đinđić, Dušica Ilić, Saša Ristić, Miroslava Živković, Dragan Stojanov, objavljenom u časopisu AMM za 2018. godinu broj 57 (3) na stranama od 23-32, došlo je do tehničke greške, pri kojoj je odštampan pogrešan sažetak na srpskom za ovaj rad. Ovom prilikom se izvinjavamo autorima i čitaocima. Uz saglasnost autora, u ovom broju objavljujemo ispravan spisak referenci.

Acta Medica Medianae 2019;58(1):138-139.

Stenting karotidne arterije (CAS) je značajan terapijski modalitet kod pacijenata sa stenozom karotidne arterije. Stenoza unutrašnje karotidne arterije visokog stepena dovodi do poremećaja i deficita kognitivnih funkcija, čak i kod asimptomatskih bolesnika. Potencijalni uticaj stentiranja karotidne arterije na kognitivne funkcije bolesnika sa stenozom karotidne arterije nije dovoljno istražen. Cilj ovog istraživanja bio je da se ispita uticaj karotidnog stentinga na kognitivne funkcije kod bolesnika sa stenozom karotidne arterije visokog stepena, na različite kognitivne domene, kao i na potencijalne faktore koji mogu uticati na kognitivne funkcije kod ovih bolesnika.

U studiju je uključeno 25 bolesnika sa simptomatskom i asimptomatskom stenozom karotidne arterije i 25 zdravih ispitanika. Kognitivne funkcije su evaluirane jedan dan pre procedure i tri meseca nakon procedure. Za evaluaciju kognitivnih funkcija korišćen je Montreal cognitive assessment (MoCA)-test.

Ukupan MoCA skor kod bolesnika pre intervencije bio je značajno niži u odnosu na kontrolnu grupu. Ovaj skor je značajno povišen tri meseca nakon intervencije. Značajano su se popravili rezultati za pažnju, egzekutivne funkcije i pamćenje.

Karotidni stenting može poboljšati ukupne kognitivne funkcije kao i pažnju, egzekutivne funkcije i pamćenje kod simptomatskih i asimptomatskih bolesnika sa stenozom karotidne arterije visokog stepena. Visok nivo holesterola predstavlja nezavisni faktor rizika za deficit kognitivnih funkcije pre revaskularizacije, dok nizak nivo obrazovanja predstavlja nezavistan faktor za nizak nivo kognitivnih funkcija nakon revaskularizacije.

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EFFECT OF CAROTID ARTERY STENTING ON COGNITIVE FUNCTION IN PATIENTS WITH INTERNAL CAROTID ARTERY STENOSIS

Marijana Stošić, Marija Andjelković-Apostolović, Nataša Djindjić, Dušica Ilić, Saša Ristić, Miroslava Živković, Dragan Stojanov

(Vol 57, No 3, October, 2018)

In the paper titled " EFFECT OF CAROTID ARTERY STENTING ON COGNITIVE FUNCTION IN PATIENTS WITH INTERNAL CAROTID ARTERY STENOSIS" by Marijana Stošić, Marija Andjelković-Apostolović, Nataša Djindjić, Dušica Ilić, Saša Ristić, Miroslava Živković, Dragan Stojanov, published in AMM journal in 2018, number 57 (3), there occurred a technical error on the page 32, with published wrong summary on Serbian for the paper. We hereby apologize to the authors and readers. With authors' approval, we are now publishing the correct summary.

Acta Medica Medianae 2018;57(3):23-32.

JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

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Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood–leukaemia in Europe after Chernobyl: 5 year follow-up. Br J Cancer 1996;73:1006-12.

2. Organizacija kao autor

The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. Med J Aust 1996;164:282-4.

3. Članak bez poznatih autora

Cancer in South Africa (editorial). S Afr Med J 1994;84:15.

4. Volumen sa suplementom

Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.

5. Broj sa suplementom

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1 Suppl 2):89-97.

6. Volumen sa više delova

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem 1995;32(Pt 3):303-6.

7. Broj sa više delova

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. N Z Med J 1994;107(986 Pt 1):377-8.

8. Časopisi sa brojem bez volumena

Turan I, Wredmark T, Fellander-Tsai L. Arthros-copic ankle arthrodesis in rheumatoid arthritis. Clin Orthop 1995;(320):110-4.

9. Časopisi bez volumena i broja

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. Curr Opin Gen Surg 1993:325-33.

10. Reference u obliku apstrakta ili prethodnih saopštenja

Enzensberger W, Fischer PA. Metronome in Parkinson's disease (letter) Lancet 1996;347:1337.

Clement J, De Bock R. Hematological complica–tions of hantavirus nephropathy (HVN) (abstract). Kidney Int 1992; 42:1285.

Udžbenici i monografije

11. Monografija

Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

12. Autori kao urednici

Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

13. Organizacija kao autor i izdavač

Institute of Medicine (US). Looking at the future of the Medicaid program. Washington: The Institute; 1992.

14. Poglavlje u knjizi

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

15. Conference proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

16. Conference paper

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors.

MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-5.

17. Istraživački ili tehnički izveštaji

Službeni izveštaji (Issued by funding / sponsoring agency):

Smith P, Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX): Dept. of Health and Human Services (US), Office of Evaluation and Inspections; 1994 Oct. Report No.: HHSIGOEI69200860.

Sponzorisani izveštaji (Issued by performing agency)

Field MJ, Tranquada RE, Feasley JC, editors. Health services research: work force and educational issues. Washington: National Academy Press; 1995. Contract No.: AHCPR282942008. Sponsored by the Agency for Health Care Policy and Research.

18. Magistarske i doktorske disertacije

Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

Druge vrste publikovanog materijala

Neobjavljeni materijal

19. U štampi (In press)

Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med. In press 1996.

Elektronski zapisi

20. Internet članak u elektronskom formatu

Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar "cited 1996 Jun 5"; 1(1)(24 screens). Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm

21. Monografija u elektronskom formatu

CDI, clinical dermatology illustrated (monograph on CD-ROM). Reeves JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego: CMEA; 1995.

22. Kompjuterski podaci

Hemodynamics III: the ups and downs of hemodynamics (computer program). Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

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Tekst članka: Naučni i stručni članci, kao i opšti pregledi i meta-analize ne smeju prelaziti 11 stranica sa prilozima; aktuelne teme– 6 stranica; kazuistika 6–stranica; prethodna saopštenja– 5 stranica, a izveštaji sa skupova i prikazi knjiga 2 stranice. Naučni i stručni članci obavezno treba da sadrže poglavlja: uvod, cilj, materijal i metode, rezultati, diskusija i zaključak. Zahvalnost ili komentar povodom sponzorstva rada dati na kraju teksta članka iza poglavlja "zaključak".

U tekstu naznačiti mesta priloga i obeležiti ih onako kako su obeleženi u prilogu.

Literatura se daje u posebnom poglavlju, pri čemu se navodi onim redosledom kojim se citati pojavljuju u tekstu. Broj literaturne reference se u tekstu označava arapskim brojem u zagradi. Za navođenje literature koristiti pravila Vankuverske konvencije. Strane se numerišu arapskim brojevima u donjem desnom uglu

Priloge u vidu teksta, tabela i ilustracija (grafikoni, crteži i dr.) ne unositi u tekst članka, već na kraju teksta, na posebnim stranicama obeleženim u gor-njem levom uglu sa "Tabela, Grafikon, Ilustracija" i arapskim brojem redosledom pojavljivanja u tekstu (npr. Tabela 1, Grafikon 1 i dr.) i svakoj se daje kratak naslov. Kratka objašnjenja i skraćenice daju se u fusnoti. Za fusnotu koristiti sledeće simbole: *, **, ***, #, ##, ###, …itd. Tabele, grafikone i ilustracije treba praviti korišćenjem nekog od programa iz Microsoft Office paketa. Izbegavati upotrebu boja kod izrade grafika.

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The second page should contain only research article title, abstract and key words without names of author(s) and institution(s). Abstract for research and professional articles, review articles and meta-analyses should have up to 350 words while abstract for all other types of publications should consist of 250 words. Key Words section should have up to 5 key words or phrases related to a submitted article. It is desirable that authors use corresponding descriptors from Medical Subject Heading (MeSH) that can be found on Index Medicus list for key words. The first and the second page should not be numbered.

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