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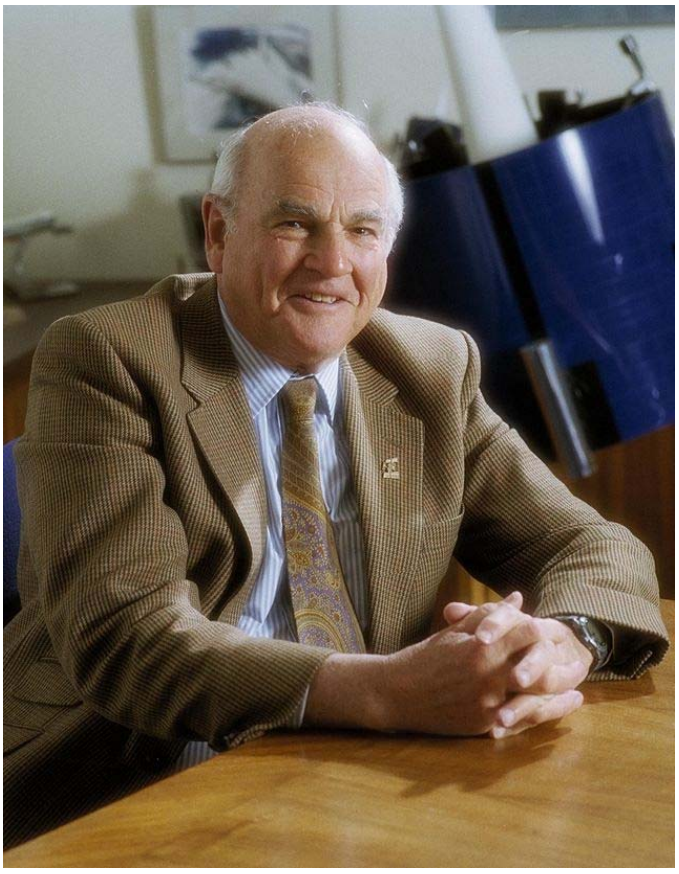

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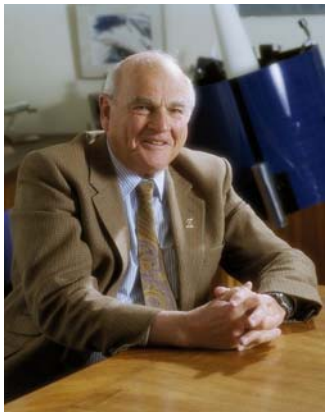

Baruch Samuel Blumberg (July 28, 1925 – April 5, 2011), known as Barry Blumberg, was an American physician and geneticist, and co-recipient of the 1976 Nobel Prize in Physiology or Medicine (with Daniel Carleton Gajdusek) for "discoveries concerning new mechanisms for the origin and dissemination of infectious diseases." Blumberg identified the hepatitis B virus and later developed its diagnostic test and vaccine. In his honour his birthday, July 28, celbreters worlwide as the World Hepatitis Day in order to provide an opportunity for education and greater understanding of viral hepatitis as a global public health problem, and to stimulate the strengthening of preventive and therapeutic measures for its control (see Editorial, pp. 567–568).

Baruch Samuel Blumberg (28. jul 1925 – 5. april 2011), poznat kao Barry Blumberg, američki lekar, genetičar, dobitnik je Nobelove nagrade za fiziologiju ili medicinu 1976. godine za otkrića u vezi sa poreklom i širenjem virusnih bolesti. On je identifikovao virus hepatitisa B i kasnije razvio dijagnostički test i vakcinu za taj virus. U njegovu čast, dan njegovog rođenja, 28. jul, obeležava se širom sveta kao Svetski dan borbe protiv hepatitisa sa ciljem da se obezbede uslovi za obrazovanje i bolje razumevanje virusnog hepatitisa kao globalnog javnozdravstvenog problema i jačanje preventivnih i terapijskih mera za kontrolu ove bolesti (vidi Uvodnik, str. 567–568).

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

UDC: 616.9:578]:616.36-002
DOI:10.2298/VSP1507567N

Viral hepatitis today

Virusni hepatitis danas

Darko Nožić

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Although hepatitis, perhaps having jaundice as clinical presentation, was recognized over 2000 years ago, back to several ceturies B.C., the knowledge made over the past few decades, with the development of molecular biology, is fundanmental for the proper classsification of viral hepatitis. Diagnostic assays, including viral molecular assays, are the fundament in the diagnosis of virus hepatitis infection and the base in monitoring therapeutic response.

Viral hepatitis is one of the most chalenging topics in medicine and a medical problem of enormous magnitude and the consequence of chronic liver diseases have significant economic implications. They are signed by the alphabet letters A, B ,C, D, and E. The most prominent problem is the consonant signed hepatitis, because of freuqent chronicity and liver cirrhosis and hepatocelullar carcinoma as a consequence.

According to the World Hepatitis Alliance, about 500 million people are currently infected with chronic hepatitis B or C and 1 in 3 people has been exposed to one or both viruses. With 500 million people living with hepatitis B and C worldwide, 1.4 million die due to these infections every year and many more become newly infected.

About a third of the world population has been infected with hepatitis B virus at one point in their lives, including 240 million to 350 million who have chronic infections. The prevalence of HBV infection is especially high in Southeast Asia and Sub-Saharan Africa where more than 8% of population are chronic carriers. While perinatal transmission or transmission during early childhood are the reason for the very high rate of chronic infection in Asia and Africa sexual and parenteral exposure are the most important in transmission in developed countries. Among chronically infected patients approximately 15–40% develop cirrhosis, liver failure and hepatocellular carcinoma. About 1 milion people die of hepatitis B each year. More than 300,000 of these are due to liver cancer. Hepatitis B virus is responsible for at least 75% of hepatocellular carcinom. Hepatitis B currently represents 5–10% of liver transplantation.

There are no exact data in Serbia but it is estimated that more than 1,5–2% of population are the carriers of hepatitis B virus.

Because the therapy for chronic hepatitis B is very expensive and not available in many contries, prevention is a very important issue. The availability of safe and effective vaccines allowed wide immunisation programs which resulted in the significant reduction of the burden of diseases caused by hepatitis B virus with clear benefits in terms of prevention of liver cirrhosis and hepatocellular carcinoma.

Antiviral agents active against HBV are available, and have been shown to suppress HBV replication, prevent progression to cirrhosis, and reduce the risk of HCC and liver-related deaths. There are two different treatment regimes for chronic hepatitis B. The first is pegilated interferon based therapy which is limited in duration and could achieve sustained inhibition of HBV replication. The second, nucleotide/nucleozide analogs therapy, fails to eradicate the virus in most of the treated, necessitating a potentially lifelong treatment. In Serbia pegilated interferon and nucleoside analogs lamivudine and tenofovir are available and used in the therapy of chronic hepatitis B. With these medications, the treatment of chronic hepatitis B in Serbia is satisfying because all categories of patients are covered.

About 150 milions people in the world have chronic hepatitis C. The prevalence of HCV infection in the general population varies greatly in different parts of the word and is estimated to be between 0.1 and 5%. The highest prevalence is in Egypt, where 20–25% of people are infected with hepatitis C. The prevalence of HCV infection in Serbia is approximately 1% . It means that in Serbia there are about 70,000 chronic carriers of hepatitis C virus.

Infection with hepatitis C virus is one of the main causes of chronic liver disease, cirrhosis and hepatocellular carcinoma. Acute infection with hepatitis C virus in most cases is asymptomatic or mild and progress to chronic infection in about 80% of all cases. The natural history of chronic hepatitis

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C depends on many factors. Around 20–40% of patients with chronic hepatitis C will progress to end-stage liver diseases and about half of them will die due to liver-related causes, so 350,000 to 500,000 people die each year from hepatitis C-related liver diseases.

There is currently no vaccine for hepatitis C, however research in this area is ongoing, so nonspecific measures are impotant in prevention fom HCV infection.

The only solution for HCV infected patients is treatment which could help most people and stop or slow the disease progression. Thr treatment of hepatitis C is progressing especcially in a few last years. Now there are three stages of hepatitis C therapy. The first is standard and includes pegilated interferon and ribavirin. With the standard therapy about 50% of patients could be cured. In 2011, the first selective protease inhibitors boceprevir and telaprevir were approved and, when added to the standard therapy, improved cure rate up to 75%. In 2014 and 2015 many direct acting antiviral agents were approved (simeprevir, sofosbuvir, 3D, ledipasvir, daclastavir). These agents have a significant antiviral effect and the cure rate of hepatitis C in developed contries has been raised to near 100%. The problem is that these medications are very expensive and not available in most contries. The treatment of chronic hepatitis C in Serbia is very problematic. Only the standard therapy is available and about half of patients who do not respond have no other treatment option. Many of them are in the terminal stage of liver disease.

The World Hepatitis Day, observed on July 28 every year, aims to raise global awareness of hepatitis and many hepatitis groups, patients and advocates worldwide taking part in the events on that day to mark the occasion.

ORIGINAL ARTICLES

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The impact of an educational film on promoting knowledge and attitudes toward HIV in soldiers of the Serbian Armed Forces

Značaj obrazovnog filma za unapređenje znanja i stavova prema HIV infekciji pripadnika Vojske Srbije

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Abstract

Background/Aim. Millions of soldiers around the world represent one of the most vulnerable populations regarding exposure to human immunodeficiency virus (HIV) infection. The programs for HIV prevention remain the most viable approach to reducing the spread of HIV infection. Very few studies have tested the effectiveness of HIV preventive interventions undertaken in military population. The aim of this study was to determine the effectiveness of educational film to transfer knowledge about HIV infection to soldiers. **Methods.** We performed a quasi-experimental study among 102 soldiers of the Serbian Armed Forces. The experimental intervention consisted of the HIV knowledge pre-questionnaire, watching a film on HIV knowledge, then the post-HIV knowledge questionnaire. The results of pre-and post-HIV knowledge questionnaires were compared. **Results.** There were 23 questions in the test. The average total score on the questionnaire before watching the film was 18.23 and after watching it was 20.14, which was statistically significant difference ($p < 0.001$). **Conclusions.** The results of the study show that viewing a film on HIV infection is an effective method of transferring knowledge about HIV to the Serbian military population.

Key words:
military personnel; hiv infections; education; motion pictures as topic.

Apstrakt

Uvod/Cilj. Milioni vojnika širom sveta predstavljaju jednu od najrizičnijih populacija za sticanje infekcije prouzrokovane virusom humane imunodeficijencije (HIV). Preventivni programi ostaju i dalje najbolji pristup u suzbijanju pandemije HIV. U malom broju studija proveravana je efektivnost intervencija preduzetih radi prevencije infekcije HIV u vojnoj populaciji. Cilj ove studije bio je da se utvrdi efektivnost obrazovnog filma u procesu prenošenja znanja o epidemiologiji HIV infekcija vojnicima. **Metode.** Izvedena je kvazieksperimentalna studija sa 102 ispitanika, pripadnika Vojske Srbije. Eksperimentalna intervencija se sastojala u gledanju obrazovnog filma o HIV infekciji i od popunjavanja upitnika znanja o HIV infekciji pre i posle gledanja filma. Poređeni su rezultati postignuti na ova dva testa. **Rezultati.** Upitnik se sastojao iz 23 pitanja, a svaki tačan odgovor bodovan je jednim bodom. Prosečan skor na upitniku popunjavanom pre gledanja filma bio je 18,23, a na onom posle gledanja filma 20,14, što je bila statistički značajna razlika ($p < 0,001$). **Zaključak.** Rezultati studije pokazali su da je gledanje obrazovnog filma o HIV infekciji efektivna metoda prenosa znanja pripadnicima Vojske Srbije.

Ključne reči:
kadar, vojni; infekcija, hiv; obrazovanje; filmovi, tematski.

Introduction

It has been more than 30 years since the spread of human immunodeficiency virus (HIV) among humans began [1]. In the last three decades, through the professional and popular literature that has been available to everyone, both professionals and others, it has been possible to learn about epidemiology and prevention of HIV transmission, as well as about the pathology, clinical manifestations and treatment of acquired immunodeficiency syndrome (AIDS), the disease caused by this virus. However, despite the fact that HIV transmission could be successfully prevented, every year millions of people are infected

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with the virus [2]. Therefore, prevention programs remain one of the most viable approaches to reducing the transmission of HIV. Many authors have argued that preventive interventions need to be developed and implemented in a manner that is population-specific for the persons being targeted [3–6].

Health education is an important activity for controlling infectious diseases including HIV [7, 8]. Its objectives are: to give information and provide knowledge and understanding of health issues, to ensure that well-informed decisions are made and to change people's individual attitudes and behavior so that they adopt a healthy lifestyle. Health education methodologies are grouped into lectures including discussion points, individual (interview and guidance) consulting, and a combination of the two, using teaching aids such as: words, writings, posters, brochures and visual media techniques such as radio, TV and film. Using film as a teaching aid has many advantages regarding reliability in describing the action, holding one's attention and displaying certain topics in quite an extraordinary and comprehensible way. The disadvantage is the financial aspect, in particular its high production costs and special requirements necessary for its presentation (requires special technical support and cinema).

More than 22 million soldiers around the world represent one of the most vulnerable populations to contracting and transmitting HIV [9, 10]. Very few studies have tested the effectiveness of preventive interventions undertaken in military population [11]. The aim of this study was to determine the effectiveness of an educational film which was presented to soldiers of the Serbian Armed Forces (SAF) in order to evaluate their knowledge of HIV and plan appropriate future primary prevention interventions in military environment.

Methods

A quasi-experimental study was designed and performed in January 2010. The study participants were soldiers that were conveniently selected for participation during their regular military service in SAF. The design consisted of three parts: the participants completed HIV knowledge questionnaires, then they watched the educational film on HIV transmission/progression/disease and lastly, they completed the same questionnaires again. All the three activities were completed in the same day.

The educational film "HIV/AIDS Prevention and Control in the SAF" was produced by the Military Medical Academy (MMA) in Belgrade, Serbia, thanks to the funds raised through mutual effort of Serbia and the USA which collaborated on the Department of Defense HIV/AIDS Prevention Program (DHAPP). The content of the film was designed by the SAF to specifically address to the military population. The film lasts for approximately 17 minutes and clearly answers a number of important questions about HIV transmission, voluntary counseling and testing, and destigmatization of people living with HIV (PLHIV).

The HIV knowledge questionnaire consisted of 23 multiple choice questions (maximum total score was 23). Each question has a single correct answer. All the questions in the questionnaire can be divided into three groups, according to the three types of information given in the film: questions related to the risk of HIV transmission are from 1 to 11, questions 12–17 associated with voluntary testing and counseling and 18–23 about stigma and discrimination of people living with HIV. In addition, there were a few demographic questions.

The statistical package SPSS for Windows (ver. 16) was used for the statistical analysis. To test the significance of differences, *t*-test was used for parametric and χ^2-test for nonparametric categories. A statistical significance was accepted at the level of $p < 0.05$.

The investigation was approved by the Scientific Research Ethics Committee of Military Medical Academy (MMA).

Results

A total number of study participants that were recruited during their regular military service was 102. All of them were soldiers and male. Most of them (about 90%) were under the age of 25. The majority of the participants (about 76%) had high school education and were in a relationship (about 70%) (Table 1).

Comparing data collected before and after watching the film, the minimum score in the questionnaire was 8 and maximum 23. Three participants answered all the questions correctly

Table 1

Demographic data

Parameters	n (%)
Age (years)	
15–24	92 (89.32)
25–35	10 (10.68)
Education	
elementary	1 (0.97)
high school	78 (76.70)
college	12 (11.65)
faculty	11 (10.68)
Marital status	
married	1 (0.97)
in relationship	72 (69.91)
divorced	1 (0.97)
single	28 (28.15)
Total	102 (100)

before watching the film and nine participants answered all the questions correctly after watching film (Table 2).

The average total score in the questionnaire before watching the film was 18.23 and after watching it 20.14, which was statistically significant difference ($p < 0.001$) (Table 3).

ing 10 questions, there was an increase in the number of correct answers which was not statistically significant.

On the other hand, after viewing the film the number of correct answers decreased for 4 questions (1, 7, 12 and 15). For the question 15 this difference was statistically significant (Table 5).

Table 2

Score in the HIV knowledge questionnaire before and after watching the film

Score	Before watching film		After watching film	
	n (%)	Cumulative %	n (%)	Cumulative %
8.00	/	/	1 (1.0)	1.0
10.00	1 (1.0)	1.0	/	/
11.00	2 (2.0)	2.9	1 (1.0)	1.9
12.00	2 (2.0)	4.9	/	/
13.00	5 (4.9)	9.8	1 (1.0)	29
14.00	/	/	2 (1.9)	4.9
15.00	2 (2.0)	11.8	/	/
16.00	9 (8.8)	20.6	2 (1.9)	6.8
17.00	14 (13.7)	34.3	4 (3.9)	10.7
18.00	15 (14.7)	49.0	4 (3.9)	14.6
19.00	19 (18.6)	67.6	11 (10.7)	25.2
20.00	9 (8.8)	76.5	23 (22.3)	47.6
21.00	15 (14.7)	91.2	23 (23.3)	70.9
22.00	6 (5.9)	97.1	21 (20.4)	91.3
23.00	3 (2.9)	100.0	9 (8.7)	100.0
Total	102 (100.0)	/	102 (100.0)	/

HIV – human immunodeficiency virus.

Table 3

Average score of HIV knowledge questionnaire for the soldiers before and after watching the film

Testing	X	SD	t	*p*
Before film	18.24	2.78	4.99	0.001
After film	20.14	2.48		

HIV – human immunodeficiency virus.

Table 4

Average scores of HIV knowledge questionnaire for a different group of soldiers before and after the film

Characteristics of soldiers	Number	Average score before the film	Average score after the film	Average score difference
Age (years)				
15–25	92	18.26	20.05	1.79
25–35	10	20	21.55	1.55
Education				
elementary	1	13	14	1
high	78	18	20.2532	2.2532
college	12	19.17	19.67	0.5
faculty	11	19.36	21.09	1.73
Marital status				
married	1	17	20	3
divorced	1	13	17	4
relationship	72	18.6389	20.21	1.57
single	28	18.1429	20.36	2.21

HIV – human immunodeficiency virus.

The greatest transfer of HIV knowledge was noticed in the soldiers over the age of 25, with high school education and those who were in a relationship (Table 4).

There was a total number of 23 questions in the questionnaire and for 19 of them, there was an increase in knowledge (2–6, 8–11, 13–14, and 16–23) after watching the film. The difference in the number of correct responses was statistically significant for 9 questions (2–5, 17–20 and 23), while for the remain-

Discussion

Some studies suggest that HIV in soldiers detrimentally affects military readiness and national and regional security [12, 13]. Providing soldiers with basic HIV education enables them to protect themselves from becoming infected. A study in the Ethiopian army showed that military personnel who had inaccurate knowledge about HIV transmission and

Table 5

Human immunodeficiency virus (HIV) knowledge questionnaire, correct answers before and after watching the film

No	Question	Before film n (%)	After film n (%)	χ^2-test	*p*
1	Can only one sexual intercourse transfer HIV?	97 (95.1)	95 (92.2)	0,30	0.57
2	Is HIV transmission possible from the mother to the child during pregnancy, delivery and breastfeeding?	54 (52.9)	93 (90.3)	33.42	0.001
3	Is it possible that any of your sexual partners is HIV infected and that he/she does not know that?	90 (88.2)	103 (100)	10.82	0.001
4	Can HIV infection be transmitted through oral sex?	42 (41.2)	63 (61.2)	7.41	0.006
5	Can HIV infection be transmitted using a common accessory for personal hygiene (razors)?	77 (75.5)	91 (88.3)	4.89	0.027
6	What you should avoid if you want to reduce the risk of getting HIV infection through sex?	91 (89.2)	99 (96.1)	2.65	0.103
7	Are those who frequently change sexual partners and don't use a condom in high risk for HIV infection?	102 (100)	100 (97.1)	1.33	0.24
8	Can anyone become infected with HIV if behaves risky?	87 (85.3)	92 (89.3)	0.43	0.51
9	Can HIV be transmitted during sex with a HIV-positive person?	100 (98.0)	102 (99.0)	0.0	0.99
10	Can HIV be transmitted if body fluids of infected person come into contact with broken skin or mucous membranes of sensitive individuals?	74 (72.5)	84 (81.6)	1.87	0.172
11	Is HIV infection possible after receiving organ transplants from a HIV-positive person?	92 (90.2)	88 (85.4)	0.68	0.40
12	After infection, how long someone carries HIV?	92 (90.2)	89 (86.4)	0.39	0.53
13	Is prevention the only salvation from this incurable disease?	90 (88.2)	99 (96.1)	3.39	0.065
14	Should all persons who behave risky be tested on HIV?	97 (95.1)	102 (99.0)	1.57	0.209
15	Does HIV-positive person show symptoms of the disease from which we can distinguish him/her by healthy persons?	66 (64.7)	45 (43.7)	8.29	0.004
16	Are there people in our country suffering from HIV?	96 (94.1)	98 (95.1)	0.0	0.98
17	How long after HIV infection routine tests can prove it?	47 (46.1)	76 (73.8)	15.26	0.001
18	Can HIV infection be spread in swimming pools?	71 (69.6)	97 (94.2)	19.28	0.001
19	Can HIV infection be transmitted through food prepared by an infected person?	80 (78.4)	97 (94.2)	9.47	0.002
20	Can HIV infection be transmitted through a mosquito bite that has previously bitten an infected person?	52 (51)	96 (93.2)	43.43	0.001
21	Can HIV infection be transmitted through shaking hands, hugging and kissing?	91 (89.2)	99 (96.1)	2.65	0.103
22	Can someone get HIV infection if he/she is in the same room with an infected person?	87 (85.3)	96 (93.2)	2.57	0.109
23	Can HIV Infection be obtained from the use of public toilets?	83 (81.4)	99 (96.1)	9.75	0.002

prevention were in more than 3 times higher risk of engaging in sexual risk behaviors compared with the personnel with accurate knowledge [14]. Acquiring knowledge and skills encouraged them to avoid or reduce behaviors that involve the risk of HIV infection and stimulated them to take voluntary counseling and testing. Angolan soldiers who received HIV prevention training have reported an increased condom use and less unprotected vaginal sex in the 3 previous months, a reduced number of partners as well as a greater HIV-related knowledge in 3 and 6 months [15]. Furthermore, 74% of surveyed German soldiers considered HIV education necessary and 83.2% would like to accept relevant testing [16]. HIV education also helps reduce stigma and discrimination before they have an opportunity to grow, by dispelling false information that can lead to the sense of fear and guilt. This is crucial for prevention as stigma often makes people reluctant to be tested for HIV. A person who is not aware of being HIV infected is more likely to pass the virus on to others [17].

In our investigation, we chose to use a film as a teaching aid in health education with the aim of transferring knowledge. In our population, young people consider film more interesting and films get more of their attention than the traditional lecture format. Some other investigations in different armies, as we will discuss, also came to similar conclusions. For example, the servicemen in China and Turkey mainly gain their knowledge about HIV through different kinds of media such as newspapers, magazines and extracurricular books [18, 19]. An investigation carried out in 1996 by the Institute of Microbiology and Epidemiology of the Academy of Military Medical Sciences, Beijing also indicated that comprehensive and proper publicity and education could play an active role in the prevention of HIV infection [18].

This study was designed to determine the effectiveness of educational film, but also to assess the knowledge of HIV in military personnel in order to plan appropriate future primary prevention interventions. The research on voluntary blood donors from the SAF showed that the most frequent risky sexual behavior in this study group was inconsistent condom use and sexual contact with partners who had engaged in high risk sexual activities [20]. As a re-

sult of this study, we tried to find out if the lack of HIV knowledge is one of the reasons for such risky behavior.

The average score in the HIV knowledge questionnaire was about 18 out of 23 points for all the participants before watching the film, that the majority of soldiers had already had what we believe to be sufficient knowledge about HIV infection. Still, some of our participants answered to almost half of HIV-related questions incorrectly. Depending on age, education and marital status of participants, the greatest transfer of knowledge was noticed among single soldiers with high school education and above the age of 25. A larger transfer of knowledge was expected among older soldiers, as well as among single ones. Based on our previous study in the SAF (unpublished data), it appears that single military personnel have more sexual partners than those who are married. As a result, they may be more likely to use condoms and are aware of the transmission of HIV infection. Also, as it was expected, participants with the highest level of education (University) had the highest average score in the questionnaire before watching the film (19.36). Soldiers with high school education had the lowest score on the pre-film questionnaire (18.0), but considering their education level, they managed to enhance their knowledge by watching the film and increased their average total score to about 20. The number of soldiers with elementary school education and soldiers who were married or divorced was too small for reliable conclusions about the transfer of knowledge in those groups.

The soldiers in our study showed a good level of knowledge. In total, more than 80% of the questions were answered correctly by about 65% of soldiers before watching the film and about 90% after the film. Nevertheless, only 9 (8.7%) soldiers answered all the questions correctly after watching the film. The results of our are similar to the results of studies performed in the South African National Defense Force on a group of military recruits (aged 18 to 24) who appropriately responded in more than 80% of the cases [21]. In addition to this, 8.5% of servicemen in China answered all the questions listed in the questionnaire correctly [18].

The lack of knowledge showed by our participants could be explained by misconceptions and myths about HIV prevention and transmission which are widespread, especially among low-income heterosexuals [22]. In our participants, we found two opposite poles of knowledge regarding HIV infections. Some soldiers have excessive fear of HIV infection (for example, they think that just being in the same room with someone who is HIV infected is risky). On the other hand, there are soldiers who do not fear of HIV infection at all. They simply ignore and misunderstand scientific knowledge regarding HIV infections and most of them think that HIV infection is reserved only for homosexuals and prostitutes. The latter are similar to servicemen in China, the majority of whom thought they had very little, or no chance to contract the illness [18]. Furthermore, a survey of the knowledge of HIV infection in recruits in the German military showed that 4% of German Army considered their own knowledge of HIV very good and 7% considered it insufficient [16].

Although the overall average HIV knowledge score in our study was high (18 out of 23 points), 58.8% of respondents believed that HIV could not be transmitted by oral sex. This was the most widespread misconception among the participants, which is consistent with the investigation among blood donors in the SAF where more than half of them reported unprotected oral sex [20].

The educational film helped in education and elimination of several misconceptions. Firstly, in the group of questions about the risk of HIV transmission, transfer of knowledge occurred for 8 questions and it was significant for 4 of them. A large number of participants learned from the film that HIV infection could be transmitted from the infected mother to the child during pregnancy, childbirth and breastfeeding (52.9 *vs* 90.3%), that there was a possibility that any of their sexual partners could be HIV positive (88.2 *vs* 100%), that HIV infection could be transmitted through oral sex (41.2 *vs* 61.2%), as well as by using a common accessory for personal hygiene such as razors (75.5 *vs* 88.3%). Few of them (85.3 *vs* 89.3%) also learned that anyone could become HIV infected if she/he behaved in a risky maner and that most important risky behaviors are the frequent change of sexual partners, sex with unknown people and the inconsistent use of condoms (89.2 *vs* 96.1%). All of this is very important from the perspective of the results of the investigation on blood donors from the SAF which showed that only 29.7% of them always use condoms, while about 17% never or almost never use condoms [20].

A lower number of correct answers after watching the film occurred for 3 questions, but it was significant for none of them. The largest increase of wrong answers occurred for the question about the risk after organ transplantation (14.6 *vs* 9.8%). Since we gave information about mandatory HIV testing for all blood, cell, tissue and organ donors, we assume that some of the participants understood no more risk for transplant procedures. Secondly, in the group of questions about voluntary testing and counseling, the knowledge increased in 4 of the 6 questions, there was a significant result of the question about routine tests for HIV: more than half of the participants thought that it was possible to test HIV-positive on the same day or a day after the exposure occurred. After watching the film, about 75% of soldiers learned that it was more likely that someone would test HIV-positive 2–6 weeks after the exposure.

For the other three questions from this group, the increase in knowledge was present but not significant. On the other hand, there were two questions that the participants answered incorrectly after viewing the film, one of which (about the symptoms of HIV infection) was significant. The number of soldiers who knew that a HIV positive person usually did not show any symptoms of disease dropped from 65% to 44% of the participants. However, this result should be put into perspective because after the film some of the participants might have confused HIV with AIDS. Further investigation of the film itself should be evaluated for accuracy and possible participant confusion.

Finally, the largest transfer of knowledge was in the group of questions related to the stigmatization of PLHIV. This group consisted of six questions, four of which showed a significant increase of knowledge. For example, the number of soldiers who thought that HIV infection could be

transmitted through a mosquito bite dropped from 49% to less than 7%. A significant increase in knowledge was also seen for other three questions. The greatest increase of knowledge was for the number of soldiers who had believed that HIV infection could be spread in a swimming pool (it dropped from 30.4 to 5.8%). The transfer of knowledge occurred but was not significant for other two questions from this group.

We believe that elimination of the prejudice toward HIV transmission is very important. Prejudice leads to fear, which again leads to stigmatization and more importantly, to discrimination of PLHIV. For example, investigation on recruits of the German Army shows that 25% of soldiers think that each HIV-infected soldier should be discharged from military service and almost 20% think that the entire barracks should be informed of such a case. Totally 36% of soldiers were in favor of obligatory registration of all HIV infected by name and more than half of them also supported a continued observation of the person infected with HIV [16].

Our study has two limitations. Firstly, findings may not be generalized to all military personnel in the SAF since the sample was selected only from soldiers. Secondly, although women are increasingly involved in the SAF, we were not able to make a meaningful gender comparison because we did not have any female participants in this study. Future studies on risky behaviors in the military environment should also include female military personnel, in order to determine their knowledge and the extent to which females in the Serbian Armed Forces are vulnerable to HIV infection. In addition, a 6-month follow-up of the same participants, with the same questionnaire would also strengthen the study and show if long-term transfer of knowledge occurred and would be considered for future studies.

Conclusion

The growing epidemic of HIV requires targeted interventions in populations which are at risk of infection. This study confirms that many soldiers lack the knowledge and have some misconceptions about human immunodeficiency virus infection and that there is room to increase the knowledge about HIV prevention, transmission, and voluntary counseling and testing in order to decrease risky sexual behaviors.

The results of the study show that the film effectively transferred the knowledge about HIV to the military population. Most of the study participants gained knowledge from watching this educational film. The soldiers learned about different aspects of HIV, but the best results were achieved in elimination of misconceptions that may lead to stigma and discrimination of people living with HIV. Watching films is good for transferring knowledge among many levels of military personnel, especially in the groups of single, high school educated persons above the age of 25.

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Treatment of stable and unstable intertrochanteric fractures with selfdynamisable internal fixator (concept of double dynamisation)

Lečenje stabilnih i nestabilnih intertrohanternih preloma samodinamizirajućim unutrašnjim fiksatorom (koncept duple dinamizacije)

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Abstract

Bacground/Aim. Intertrochanteric fractures of the femur are the third most common fractures among all bone fractures. Today in everyday orthopedic practice a number of different methods of treatment of trochanteric fractures of the femur are applied. Despite the improvement in the development of new implants, the percentage of serious complications of the treatment of these fractures remains very high, varying from 10% to 20%. One of the most serious complications of internal fixation of intertrochanteric fractures is nonunion of fractures due to the lack of additional axial dynamisation of implants. The aim of this study was to determine the efficacy of double dynamisation in stable and unstable intertrochanteric fractures treatment using the self dynamisable internal fixator. **Methods.** During the period from 2000 to 2009 we analyzed the use of selfdynamisable internal fixator (SIF implant) in the treatment of 247 patients with stable and unstable intertrochanteric fractures. Fracture types were classified according to the AO Fracture Classification/Orthopaedic Trauma Association Scheme. Salvati and Wilson scoring systems were used for functional assessment considering pain, walking ability and hip movements of operated patients. **Results.** Of the total number of treated patients, 134 were males and 113 females, aged 19 to 90 (average 49.6) years. More than a half of the patients were older than 50 years. Monitoring of the patients after the operation was carried out clinically and radiographically for a period of three to six months in all the patients, whereas a 2-year follow-up was conducted in 176 (71.2%) patients. The average duration of surgery was 47 min, the average blood loss 145 mL, and the average fluoroscopy time was 16 sec (8–97 sec). The average time for union was 3.7 months (3–6.5 months). Double dynamisation (dynamisation along the neck and shaft of the femur) was observed in 85 (34.4%) patients, and was on average 4.3 mm (1.5–8 mm). All fractures managed with dynamisation implants healed completely within no later than six months after the surgery. In 17 cases there was a cut-out phenomenon of implant, while in seven cases there was mechanical implant failure. Complications were detected within 3 to 6 weeks after the surgery, and treated by the method of intramedullary fixation. During the study, there were no cases of infecton and thromboembolic complications detected. **Conclusion.** The concept of double dynamisation improves the fracture healing in the stable and unstable intertrochanteric fractures using the selfdynamisable internal fixator. This biological method of fixation provides healing of intertrochanteric fracture in the optimum period of time, significantly reducing the risk for mechanical failure.

Key words:
hip fractures; ortopedic procedures; internal fixators; fracture healing; treatment outcome.

Apstrakt

Uvod/Cilj. Intertrohanterni prelomi femura treći su po učestalosti prelomi od svih preloma koštanozglobnog sistema. Danas se u svakodnevnoj ortopedskoj praksi primenjuju različite metode fiksacije intertrohanternih preloma femura. Uprkos napretku u razvoju novih implantata, procenat ozbiljnih komplikacija lečenja ovih preloma i dalje je veoma visok i kreće se u opsegu od 10% do 20%. Jedna od najtežih komplikacija unutrašnje fiksacije intertrohanternih preloma je nesrastanje preloma usled nedostatka dodatne aksijalne dinamizacije implantata. Cilj rada bio je da se utvrdi efikasnost koncepta dvostruke dinamizacije (korišćenjem samodinamizirajućeg unutrašnjeg fiksatora) u lečenju stabilnih i nestabilnih intertrohanternih preloma. **Metode.** U periodu od 2000 do 2009. godine analizirali smo primenu samodinamizirajućeg unutrašnjeg fiksatora u lečenju 247 bolesnika sa stabilnim i nestabilnim intertrohanternim prelomima femura. Prelomi su klasifikovani na osnovu sistema klasifikacije AO/OTA. Sistemi bodovanja (ocenjivanja) Salvati-Vilson korišćeni su za funkcionalnu procenu, uzimajući u obzir bol, sposobnost samostalnog hoda i pokretljivost kuka operisanih bolesnika.

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Rezultati. Od ukupnog broja lečenih bolesnika, bilo je 134 muškaraca i 113 žena, starosti od 19 do 90 (prosečno 49,6) godina. Više od polovine ispitanih bolesnika bilo je starije od 50 godina. Praćenje bolesnika nakon operacije sprovedeno je klinički i radiološki za period od tri do šest meseci kod svih ispitanika, dok je dvogodišnje praćenje sprovedeno kod ukupno 176 (71,2%) bolesnika. Prosečno vreme trajanja operacije iznosilo je 47 min, uz prosečan gubitak krvi od 145 mL, dok je prosečno vreme fluoroskopije iznosilo 16 (8–97) sec. Prosečno vreme zarastanja preloma iznosilo je 3,7 (3–6,5) meseci. Dupla dinamizacija (dinamizacija duž ose vrata i dijafize butne kosti) zabeležena je kod 85 (34,4%) bolesnika, i iznosila je u proseku 4,3 (1,5–8) mm. Svi prelomi kod kojih je došlo do dinamizacije implantata u potpunosti su sanirani najkasnije za šest meseci od operacije. Kod 17 bolesnika primećen je *cut-out* fenomen implantata (izvlačenje klinova iz glavenovratnog dela femura, sa dezintegracijom preloma), dok je kod sedam bolesnika došlo do mehaničkog loma implantata. Komplikacije su uočene u roku od 3 do 6 nedelja nakon operacije, a bolesnici su lečeni metodom intramedularne fiksacije *Gamma* klinom. Tokom studije nisu otkrivene infekcije i tromboembolijske komplikacije. **Zaključak.** Korišćenjem samodinamizirajućeg unutrašnjeg fiksatora koncept dvostruke dinamizacije značajno unapređuje lečenje stabilnih i nestabilnih intertrokanternih preloma. Ovaj biološki metod fiksacije pruža zarastanje intertrohanternih preloma u optimalnom vremenskom periodu, značajno smanjujući rizik od mogućih komplikacija lečenja.

Ključne reči:
kuk, prelomi; ortopedske procedure; fiksatori, unutrašnji; prelom, zarastanje; lečenje, ishod.

Introduction

The extension of life expectancy caused a growing number of patients with fractures of the proximal femur due to poor bone quality. For surgical treatment of these fractures different implants are still applied, which can be roughly divided into extramedullary and intramedullary implants. Complication rates arising during these fractures treatment using different surgical techniques and principles range in the literature from 10% to 20% [1–6]. Complications in the form of delayed healing, nonunion and subsequent breakage of implants can have serious consequences for the patients [7].

The first implants used for surgical treatment of intertrochanteric fractures were fixed-angle blade plates [8, 9]. After using this device more than a decade, many authors recommended them as the treatment of choices for intertrochanteric femur fractures, but the main complaint was that these implants did not allow controlled collapse and impaction at the fracture site without penetration of the femoral head [10].

In the early seventies of the last century dynamic hip screw (DHS) has begun to be used more and more for the treatment of intertrochanteric fractures. One of the main reasons for the growing popularity of the application of this method of fixation was the ability of the implant to resist penetration and screw threads to increase fixation in the proximal fragment providing controlled spontaneous dynamisation of proximal fragment to achieve fracture healing. But, lack of axial dynamisation of this extramedullar implant has led to complications such as pulling off of the side plate from the femoral shaft and dissociation of the sliding compression hip screw from the barrel [11].

In the mid-1980's developed the first intramedullary nails for fixation of intertrochanteric fractures, and the first clinical application of the so-called Gamma Nail first generation took place in 1988, evolving from the concepts of Gerhard Küntscher to treat trochanteric fractures [12]. The intramedullary position of Gamma Nail and other new intramedullary implants proximal femoral nail (PFN), proximal femoral nail anti-rotation (PFNA) considered to provide better biomechanical stability and still allowing controlled impaction of fracture [13]. Although considered to be mechanically stronger than extramedullary implants, because they are closer to the mechanical axis of the femur and hence has less bending moments on the implant, there is no consensus among surgeons which implant, extramedullary or intramedullary has a lower complication rate [14]. To avoid these complications, it is necessary to provide good fractures reduction, proper position of the lag screws, in order to achieve the most important biomechanical factors of the stability of fixation.

A very important biomechanical characteristic of each implant is the presence of dynamic components of the implant to provide secondary impaction of fracture to achieve union. If this biomechanical characteristics of the implant is not present, in situations with the delayed union of fracture there may occur implants brakage as the implant becomes the weakest part of the rigid fixation construct. The role of dynamization is well-known in the orthopedic profession as an essential component of healing fractures, especially of the lower extremities, as well as to promote fracture healing in an optimal timeframe. Fractures of the proximal femur, intertrochanteric fractures, require special attention during the treatment, not only because of its prevalence in the pathology of trauma, but also because of a high incidence of morbidity and mortality in injured patients. Therefore, there is still ongoing debate in the professional literature on the selection of the appropriate implant for the treatment of intertrochanteric fractures of femur.

The aim of this study was to examine the efficacy of application of selfdynamisable internal fixator (SIF) in the treatment of stable and unstable intertrochanteric fractures.

Methods

Between January 2000 and December 2009 in the Department of Orthopedics and Traumatology, University Hospital Niš, Niš, Serbia, a total of 247 patients underwent treatment of intertrochanteric fracture of the femur using the SIF metod of fixation. More than a half of the patients were older than 50 years. All fractures were classified according to the AO/OTA system of fracture clasification. Those with pathological intertrochanteric fractures were excluded from

the study. Our criterion for distinguishing stable from unstable intertrochanteric fractures was the absence of medial support that was considered the main criterion for fracture instability [15]. According to this criterion, 97 consecutive patients in our study sustained unstable intertrochanteric fracture of the hip (fracture types 31–A3.1-3,A2.2,A2.3) according to the AO classification.

All the patients had their operation within 72 h of admission, carried out in the operating room using an orthopaedic traction table and image intensifier. After closed reduction, the proximal femur was exposed through a lateral approach using 5–6-cm-long skin incision, beginning just distally from the trochanteric ridge, and a guide wire was passed into the femoral neck aiming at the centre of the femoral head using a 130 degree guide. The operation was carried out in a similar manner to a conventional DHS technique using a biological reduction concept without the attempt of open reduction or exposing fracture. Radiographs were taken postoperatively and at 6 to 8-week intervals during follow-up until clinical and radiological healing was achieved. During the first 6 weeks postoperatively, the patients were mobilised on partial to full weight bearing as far as were tolerated. Initial mobilisation was supervised by the physiotherapist. The mean hospital stay was 11.9 (range 7– 25) days, depending on patient mobility and social circumstances.

During the follow-up visits, the patients were assessed with respect to walking ability, hip movements and pain. Radiographs were taken to assess healing and the neck-shaft angle.

Implant characteristics

The main three characteristics of SIF developed by Mitković et al. [16, 17], are: possibility of spontaneous axial dynamisation, preservation of both periosteal and medullary bone blood circulation, and less invasive technique of application. This implant was approved by the National Drug and Medical Devices Agency. SIF has been investigated widely in the laboratories the Faculty of Mechanical Engineering and in terms of testing the maximum load to destruction of the implant and cyclic tests the effect of repetitive loading was also investigated experimentally in 60 animals during the preparation of PhD thesis [18].

The SIF is made of stainless steel (ASTM F 138-2). There are three different lengths of SIF for intertrochanteric fracture fixation, 100 mm, 150 mm, 200 mm, 250 mm. This variant consists of a trochanteric unit (for DHS), which extends distally as a bar. One or two clamps can be fixed to the bar. On the distal end, it has an anti-rotation dynamic unit, length 18 mm. On the trochanteric unit, there are three holes, but it is enough to introduce two screws (7 mm diameter) only into the neck and head of the femur, at 130 degrees (Figure 1).

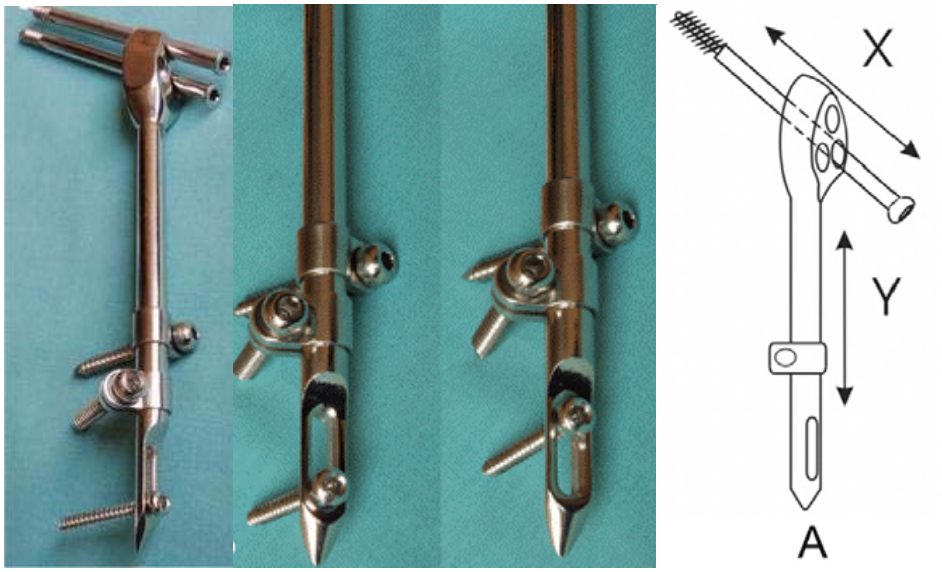
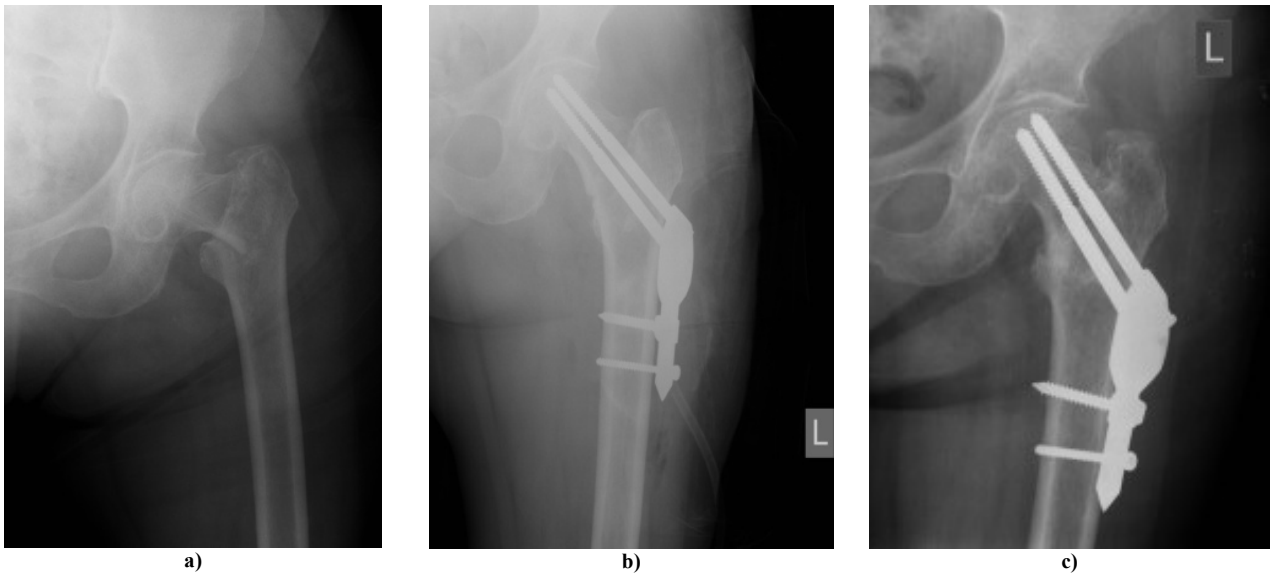


Fig. 1 – Concept of double dynamisation with selfdynamisable internal fixator implant.

Results

Of a total number of the treated patients, 134 were males and 113 females, aged 19 to 90 (average 49.6) years. More than a half of the patients were older than 50 years. The average duration of surgery was 47 min, the average blood loss 145 mL, and the average fluoroscopy time was 16 (8–97) sec. Of a total number of treated fractures (n = 247), 150 (60.7%) patients with intertrochanteric fractures were classified with stable fractures, while in 97 (39.3%) patients fractures were classified as unstable. Monitoring of the patients after the operation was carried out clinically and radiographically for a period of three to six months in all the patients, whereas in a two-year follow-up was conducted in a total of 176 (71.2%) patients. The average time for union was 3.7 (3–6.5) months. Double dynamisation (dynamisation along the neck and shaft of the femur) was observed in 85 (34.4%) patients, was on average 4.3 (1.5–10.5) mm. All fractures threated with dynamisation implants healed completely within no later than six months after the surgery.

Figure 2 showes radiographic findings during the effective treatment of the patient with stable intertrochanteric fracture.



a) b) c)

Fig. 2 – Radiographs of a 81-year-old female patient with stable osteoporotic intertrochanteric fractures (AO type 31-A1.2).

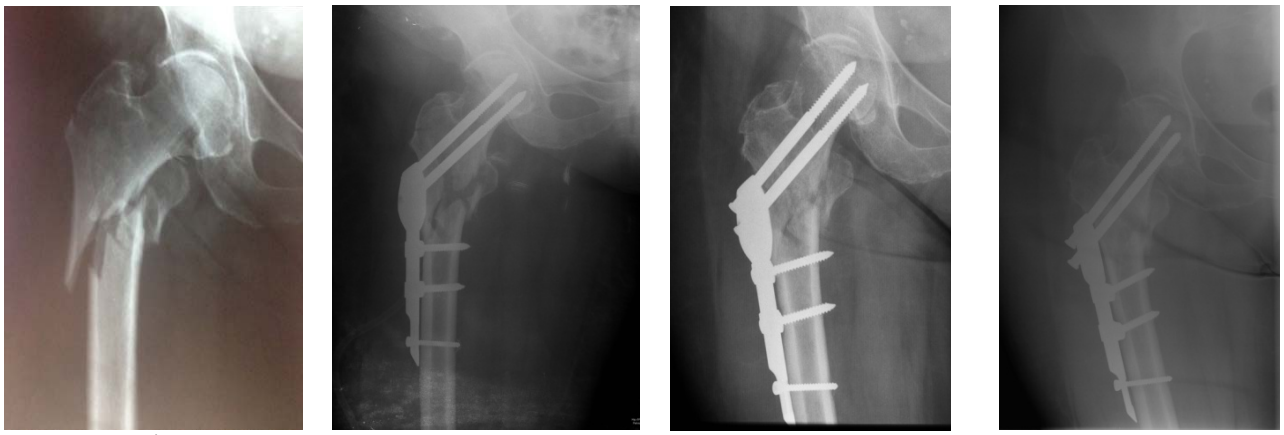
a) initial radiographic finding; b) postoperative radiography after osteosynthesis with a selfdynamisable internal fixator; c) radiography after 9 weeks-sufficient callus formation with double dynamisation of implant.

Cut-out phenomenon occurred in 17 (6.9%) cases, while in seven (2.8%) cases mechanical failure of implant was detected. These complications were detected within 3 to 6 weeks after the surgery. They were ultimately treated by removing the implants and intramedullary fixation with a long Gamma nail of third generation without bone grafting. All revised intertrochanteric fractures healed safely. During the study, there was no any case of thromboembolic complications, nor a single case of infection.

Figure 3 shows radiographs of the patient with unstable in tertrochanteric fracture.

For functional assessment we used the Salvati and Wilson score, which showed excellent results in 175 (70.1%) fractures, good results in 54 (21.9%) fractures and fair results in 18 (7.3%). Hardware removal was done in 157 patients, typically not before 12 months after fracture union.

A failure of DHS fixation due to the lack of axial dynamisation is shown in Figure 4.



a) b) c) d)

Fig. 3 – Radiographs of a 63-year-old-female patient with unstable, reverse obliquity, intertrochlanteric fracture (AO type 31-A3.3).

a) preoperatively; b) postoperatively, after osteosynthesis with selfdynamisable internal fixator; c) 6 weeks postoperatively, double dynamisation of implant has been achieved; d) after 12 weeks radiographic image showing fracture healing.

In cases of unstable intertrochanteric fractures, the average dynamisation was 6.3 (3.7–10.5) mm. Fracture consolidation had been achieved in the mean duration of 10.3 (7–19) weeks. The patients started weight bearing as soon as possible after the operation, with the recommendation to weight bearing in the first three weeks as tolerated as well, and after that all patients started to walk full weight bearing with crutches.

Figure 5 demonstrates a correct position of a lag-screws SIF implant in anterior-posterior and profile radiographic imaging to avoid cut-out complication.

Discussion

The use of dynamic implants with a sliding screw in the axis of the femoral neck is now the standard in internal fixation

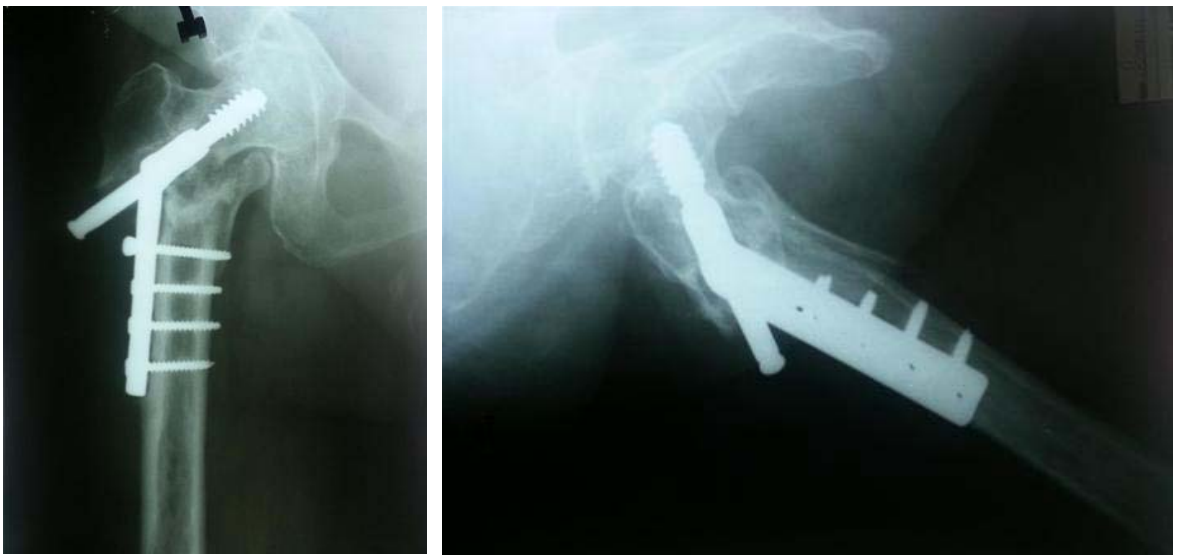


Fig. 4 – Failure of dynamic hip screw fixation due to the lack of axial dynamisation.

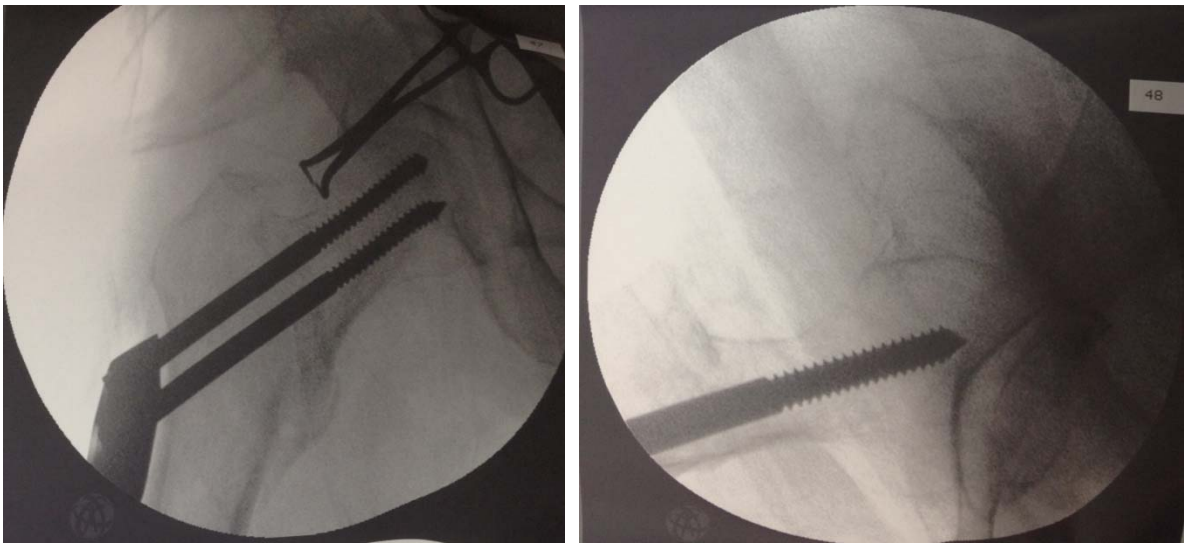


Fig. 5 – Correct position of lag-screws selfdynamisable internal fixator implant in anterior-posterior and profile radiographic imaging to avoid cut-out complications.

of intertrochanteric fractures. A sliding screw allows controlled collapse (dinamysation) and physiological compression of bone fragments at the fracture site, while preserving the neck-shaft femur axis. Weight bearing on the injured leg leading to the telescopic movement head-neck part of the femur together with the sliding screw, so that the possibility of fracture dynamisation directly depends on the ability of dynamisation of the sliding screw. While standing on the injured leg, the head of the femur is exposed to the force resulting from the effects of body weight and muscle contraction of the hip abductors [19–22]. This force acts downward and outward, and it tends to dynamization of head-neck part of the femur with sliding screw and simultaneously performs cross slide screw load, leading to the appearance of sliding friction between the screw and the main part of the implant. This friction occurs at the two points of the support sliding screws of the implants: the lower part of the medial and upper lateral foramen through which the sliding screw passes. Dynamisation occurs only when it overcomes this friction [23]. Failure of DHS fixation is especially present during fixation of unstable intertrochanteric fractures, with the failure rate from 11% to 56% [24, 25]. Despite this, a sliding hip screw remains the method of choice in surgical treatment of stable intertrochanteric femur fractures worldwide. In the largest published study to date on 1,024 patients with intertrochanteric fractures treated with DHS fixation, the authors obtained a low rate of fixation failure (3.2%), with the most common complications were cut-out of the lag screw from the femoral head with the incidence of 1.9% [26].

The analysis of our results after application of the SIF method of fixation in 247 patients, retrospectively monitored, treatment complications were found in a total of 24 (9.72%) patients.

The major complication was cut-out phenomen detected in 17 (6.9%) cases. Analysis of these cases showed incorrect lag-screws positioning into the femoral head leading to this

complication. The importance of lag screw placement was explained in detail in the study of Baumgaertner et al. [27]. They developed a simple method of measurement to describe the position of the screw. This measurement, the tip-apex distance, is the sum of the distance from the tip of the lag screw to the apex of the femoral head on anteroposterior radiograph and this distance on lateral radiograph, after controlling for magnification. To determine the value of this measurement in the prediction of so-called cut-out of the lag screw, 198 peritrochanteric fractures (193 patients) were studied by Baumgaertner et al [27]. Also, cut-out complications were detected in our study in the patients with surgery at the beginning of the study, so the learning curve plays an important role in the avoidance of technical errors of the SIF method.

As an alternative to this extramedullary sliding device we devoleped the SIF method of fixation for intertrochanteric femur fractures, in our institution (Clinic of Orthopeadic and Traumatology, University Hospital, Niš, Serbia), which has the possibility of dynamisation in two axes: axis of the femoral neck and the longitudinal femoral axis. After a period of biomechanical testing [23] of implants and experimental use of laboratory animals [18], clinical application of this method started in early 2000 [16, 17, 28].

The increased risk of intraoperative and late fracture of the femur and reoperation rate still remain a problematic aspect of proximal femoral nailing [29]. The most recent randomised comparative study whose objective was to compare the clinical outcome and the rate of complications in the application of new design proximal intramedullary implants, Gamma Nail and PFNA, showed that the risk for experiencing a postoperative complication after Gamma 3 nailing was 40% versus 45% after PFNA fixation, concluding that there is no significant difference in the overall clinical outcome and risk of complications between the PFNA and the Gamma 3 treated patients during the first postoperative year. These complications were principally the impaction of the fracture area, cut-out, and fracture of the femoral shaft at the tip of the implant [30]. All proximal femoral intramedullary nails of the new generation were designed to provide additional gliding mechanism of lag screw in the axis of the femoral neck, and dynamisation along the femoral shaft axis through oval holes in the distal part of the nail for dynamic locking in both stable and unstable intertrochanteric fractures. In some cases, dynamisation of intramedullary proximal nail can be blocked so that the implant becomes the weakest point of fixation, leading to nail breakage. Complications in the form of delayed union, nonunion, and (subsequent) implant breakage are less frequent, but may also have devastating consequences for the patient.

In this situation, Biber et al. [7] recommended the so-called lateral notching using a chisel they remove cortical bone right below the sleeve of the lag screw on the lateral side in order to achieve effective fracture impaction along the femoral shaft axis. Specifically, they noted in their series of 2,369 patients with intertrochanteric fractures surgically treated with proximal femoral Targon nail, some cases (14 patients, 0.6%) who were readmitted because of delayed union or nonunion. Normally distal dynamisation can be achieved either by removing the interlocking screws or by placing one screw into a long (dynamic) interlocking hole of the nail, but in these cases gliding of the nail was blocked by cortical support underneath the sleeve of the lag screw on the lateral side. In all of these cases healing was achieved 6 weeks later, after the additional new operation which included interlocking screw removal and lateral notching [7]. In our series of operated patients with the SIF method, dynamisation was observed in 85 (34.4%) patients, with no need for the new subsequent surgery to achieve further impaction of he fracture fragments.

Conclusion

According to our clinical data we can recommend self-dynamisable internal fixator as a safe extramedullary implant for fixation of both stable and unstable intertrochanteric fractures. It provides stable biological fixation of proximal femoral fractures, further adding impaction of the fragments along each axis (the axis of the femoral neck and the axis of the femoral shaft) whenever it is necessary to achieve the union.

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Validation and cross-cultural adaptation of the questionnaire ThyPRO in thyroid patients in Serbia

Primena upitnika ThyPRO kod bolesnika sa oboljenjem štitaste žlezde u Srbiji

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Abstract

Backgraund/Aim. The Thyroid Specific Patient Reported Outcome Measure (ThyPRO) questionnaire is self-administered and intended to measure quality of life of thyroid patients. The aim of this study was to investigate the validity and reliability of the translated new, ThyPRO questionnaire in Serbian patients with thyroid disease. **Methods.** The translation process followed an internationally accepted methodology. The questionnaire was validated in 100 consecutive thyroid patients hospitalized in a tertiary level hospital, between April and August 2012. Internal reliabilities of ThyPRO scales were assesessed using Cronbach's α coefficient. Association between age, gender, education, marital and employment status, place of living, diagnosis, current treatment, hormonal status and patient quality of life were determined using Pearson's (*r*) and Spearman's (*ϱ*) correlation coefficients. **Results.** Internal consistency and reliability for ThyPRO scales were satisfactory. Cronbach's α coefficients of 13 multi-item scales of the ThyPRO were > 0.83 (range 0.83–0.95). The scores, obtained by this questionnaire, correlated significantly with patients gender, employment status, diagnosis, current treatment and place of living. A highly significant inverse relationship was found between scores and hormonal status as well as between scores and disease duration. Patients' age, marital status and thyroid-stimulating hormone level did not influence any scale score. **Conclusion.** The ThyPRO may be useful in measuring health-related quality of life in patients with thyroid disease in Serbia.

Key words:
quality of life; questionnaires; thyroid diseases; hypothyroidism; hyperthyroidism.

Apstrakt

Uvod/Cilj. Upitnik za kvalitet života osoba sa oboljenjem štitaste žlezde *The Thyroid Specific Patient Reported Outcome Measure* (ThyPRO) je upitnik za samostalno popunjavanje i meri kvaliteta života tih bolesnika. Cilj ovog rada bio je da se ispita validnost i pouzdanost novog prevedenog specifičnog upitnika, ThyPRO kod bolesnika sa bolešću štitaste žlezde u Srbiji. **Metode.** Proces prevođenja je obavljen uz poštovanje internacionalno prihvaćene metode prevođenja upitnika. Upitnik je validiran na 100 uzastopnih bolesnika sa bolešću štitaste žlezde, koji su bili hospitalizovani od aprila do avgusta 2012. godine u instituciji tercijarnog nivoa zdravstvene zaštite. Interna pouzdanost skala ThyPRO upitnika je ispitana pomoću Cronbach-ovog α koeficijenta. Povezanost godina, pola, nivoa obrazovanja, bračnog statusa, zaposlenosti, mesta življenja, dijagnoze, trenutne terapije, hormonskog statusa i kvaliteta života bolesnika ispitana je korišćenjem Pearson-ovog (*r*) i Spearman-ovog (*ϱ*) koeficijenta korelacije. **Rezultati.** Interna konzistencija i pouzdanost skala ThyPRO upitnika je bila zadovoljavajuća. Cronbach-ov α koeficijent je bio veći od 0.83 (0.83–0.95) za 13 skala ThyPRO upitnika. Skorovi su statistički značajno korelirali sa polom bolesnika, zaposlenošću, dijagnozom, sadašnjom terapijom i mestom življenja. Visoko statistički zanačajan inverzni odnos je otkriven između skorova i hormonskog statusa i skorova i dužine trajanja bolesti. Godine starosti, bračni status i nivo tireostimulišućeg hormona nisu uticali na skorove ni jedne skale ovog upitnika. **Zaključak.** Upitnik ThyPRO se može koristiti u ispitivanju kvaliteta života povezanog sa zdravljem kod bolesnika sa bolešću štitaste žlezde u Srbiji.

Ključne reči:
kvalitet života; upitnici; tireoidna žlezda, bolesti; hipotireoidizam; hipertireoidizam.

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Introduction

The new thyroid-specific quality of life patient-reported outcome measure (ThyPRO) for benign thyroid disorders, is a specific quality of life questionnaire newly developed in Denmark, by Watt et al. [1–5]. It is the first specific questionnaire for patients with different thyroid diseases which was validated and standardized. The questionnaire includes 13 domains that cover physical and mental symptoms, well-being, function, and the impact of thyroid diseases on social and daily life and the overall health-related quality of life (HRQoL) of patients with thyroid diseases. The suffix PRO, in the name of the questionnaire, means 'patient report outcome', a term that is increasingly used instead of HRQoL. The questionnaire went through the four phases of the development: issue generation in which HRQoL issues of possible relevance for thyroid diseases were identified; operationalization in which relevant issues were selected and converted into items in a draft questionnaire; pre-testing in which a draft questionnaire was tested and revised based on problems observed within cognitive interviews; and quantitative scale validation to test internal consistency, reliability and validity of the constructed scale on a large sample of patients.

The questionnaire was validated and found to be useful in clinical studies. ThyPRO has been translated in 7 languages and culturally adapted [1–5].

The aim of this study was to investigate validity and reliability of the translated and culturally adapted ThyPRO questionnaire in a group of 100 patients with thyroid disease in Serbia.

Methods

The Serbian version of ThyPRO (ThyPROsr) questionnaire was conducted in 100 consecutive patients with thyroid diseases hospitalized in a tertiary level hospital for thyroid surgeries from April to August 2012. Inclusion criteria were: age over 16 years, benign thyroid disease and obtained written informed consent. Ethical approval for the study was received from the Ethics Committee of the Medical Faculty, University of Belgrade, Serbia. Exclusion criteria were major psychiatric disorders, proven malignancy and severe, chronic disease. All of the 102 patients admitted in this period fulfilled the inclusion criteria, but 2 of them had major psychiatric disorder and were excluded from this study. Since thyroid surgery is elective surgery, no patient had severe chronic disease that might influence HRQoL.

In this study HRQoL was assessed by ThyPROsr questionnaire. ThyPRO consists of 84 items, covering physical and mental symptoms, well-being and everyday life functioning and the impact of thyroid disease on participation, social and daily life. Items are grouped in 13 scales, goitre symptoms, hyperthyroid symptoms, hypothyroid symptoms, eye symptoms, tiredness, cognitive impairment, anxiety, depressivity, emotional susceptibility, impaired social life, impaired daily life, impaired sex life, cosmetic complaints, and 1 single item which measures general HRQoL. Each of the 13 ThyPRO scales is scored as a summary score and linearly transformed to the range 0–100 with increasing scores indicating decreasing HRQoL, meaning more symptoms or greater impact of disease. In addition, ThyPRO contains one item not included in any multi-item scale [5].

According to the internationally accepted methodology for translation and cultural adaptation of a HRQoL questionnaire, we followed the guidelines set up by the European Organisation for Research and Treatment of Cancer (EORTC) group [6] for the production of Serbian version of ThyPRO. This translation process involved 5 steps. Firstly, two independent translations of English version of ThyPRO questionnaire into Serbian were performed by two independent professional translators whose native language is Serbian with excellent knowledge of English language. Then, better of these two translations, was chosen as a "forward translation" by the coordinator. Then, "forward translation" was re-translated into English by professional whose native language is English with excellent knowledge of Serbian language. The developer of ThyPRO, Serbian coordinator, two qualified translators, clinicians and epidemiologist discussed controversial items to generate a version of the ThyPROsr which would be the most appropriate for the cultural environment of Serbia and acceptable for testing on thyroid patients. Then, in order to check the Serbian population's understanding and interpretation of the translated items, cognitive interviews were conducted within five thyroid patients, by the appropriate consultant recruited from the Health Research Associates (HRA). Finally, the results of these tests were discussed in the same group of experts. That stage led to the final Serbian version of ThyPRO. In order to assess patient's acceptability of ThyPROsr, the mean time required for completing the questionnaire was measured. The patients answered the questionnaire in the presence of a physician, who dealt with HRQoL assessment, so there was no missing data, nor reading and/or writing problems. Sociodemographic data and information about comorbidity were collected using demographic questionnaire. Other necessary data, laboratory results, previous and current treatment, exact diagnosis and duration of disease were collected from medical records.

ThyPROsr scale scores were calculated as a row summary scores, and then were linearly transformed to the range of 0–100. Lower values indicate better HRQoL, while higher values indicate worse HRQoL.

Internal reliabilities of ThyPROsr scales were assessed for multiple item scales using Cronbach's α coefficient. Cronbach's α coefficient ranges from 0–1, where 1 means perfect reliability.

Clinical validity was assessed comparing means of the summary scores with patient's age, gender, education, marital and employment status, place of living, clinical diagnosis, disease duration, current treatment and hormonal status. Pearson's (r) and Spearman's (q) correlation coefficients were used to investigate the relationship between the scores and the main clinical and demographic variables, as

suitable. A $p < 0.05$ was regarded significant. All the tests were formulated 2-tailed.

Results

The average age of the 100 consecutive patients included in the study was 48.77 years, and 88% of them were female, 12% male. All of them completed ThyPROsr questionnaire in the presence of the physician who dealt with HRQoL assessment and endocrine surgery. All the patients comprehend the questionnaire. There were no missing data. The average time to complete the questionnaire was 16 minutes.

Demographic and clinical characteristics of the patients with thyroid disease are shown in Table 1.

Table 1
Demographic and clinical characteristics of patients with thyroid disease

Characteristics	Values
Gender, n (%)	
female	88 (88)
male	12 (12)
Age (years), $\bar{x} \pm SD$	48.77 ± 13.13
Education (years), n (%)	
0–10	17 (17)
11–13	49 (49)
14–16	13 (13)
> 16	21 (21)
Marital status, n (%)	
single	12 (12)
married/unmarried couple	74 (74)
divorced	9 (9)
widow	5 (5)
Current employement status, n (%)	
unemployed	27 (27)
employed	45 (45)
retired	24 (24)
student	4 (4)
Diagnosis, n (%)	
non toxic goitre	51 (51)
toxic goitre	20 (20)
Graves' disease withTAO	19 (19)
Graves' disease without TAO	3 (3)
Hashimoto disease	7 (7)
Disease duration (years), $\bar{x} \pm SD$	7.23 ± 7.98
Current treatment, n (%)	
L-thyroxine	8 (8)
antithyroid drugs	30 (30)
other	0 (0)
none	62 (62)
Hormonal status, n (%)	
euthyroid	72 (72)
subclinical hypothyroid	3 (3)
subclinical hyperthyroid	25 (25)
hypothyroid	0 (0)
hyperthyroid	0 (0)

TAO – thyroid associated ophthalmopathy; $\bar{x}$ – mean value; SD – standard deviation.

Most of the patients accepted the questionnaire well, found it clear enough and easily understandable, while none of the items found to be unpleasant and embarrassing. Just one male patient, age of 68, found items concerning sexual function and satisfaction irrelevant, and one female patient, age 18, answered the questions concerning sexual function and satisfaction although she had no sexual experience yet.

The mean scale scores, internal consistencies (Cronbach's α) and reliabilities for these 13 scales ranged from 0.832 on hypothyroid symptom scale to 0.951 on cognitive problems scale (Table 2).

A significant relationship emerged between gender and cosmetic complaints scale ($\rho = -0.232$; $p < 0.05$), with higher scores in females indicating lower HRQoL in this domain. Also, we found a significant inverse relationship between education and goitre symptom scale ($\rho = -0.249$; $p < 0.05$) and education and eye symptom scale ($\rho = -0.222$; $p < 0.05$), with better HRQoL in the higher educated patients. Employment status significantly correlated with hyperthyroid symptom scale ($r = 0.203$; $p < 0.05$) and anxiety scale ($r = 0.198$; $p < 0.05$) with the employed patients scoring lower, indicating better HRQoL. Disease duration significantly correlates with goitre symptoms scale ($\rho = 0.221$; $p < 0.05$), cognitive problems scale ($\rho = 0.220$; $p < 0.05$) and impaired sex life scale ($\rho = 0.206$; $p < 0.05$), with shorter disease duration scoring lower, indicating better HRQoL. Significant inverse relationships emerged between hormonal level and some ThyPRO health items. Thyroxine (T4) level significantly correlated with impaired social life scale ($r = -0.276$; $p < 0.05$), hypothyroid symptoms scale ($r = -0.256$; $p < 0.05$) and eye symptoms scale ($r = -0.230$; $p < 0.05$). Triiodothyronine (T3) level significantly correlated with impaired social life scale ($r = -0.277$; $p < 0.05$). Higher T4 and T3 level scored lower scores on these ThyPRO health rating scales. Other factors significantly affecting symptom scale scores were: place of living, current treatment and diagnosis. We also found a significant correlation between current treatment and impaired daily life scale ($\rho = -0.272$; $p < 0.01$) and current treatment and cosmetic complaints scale ($\rho = -0.301$; $p < 0.01$), with lower scoring and better HRQoL in patients without current treatment. Domicile significantly correlated with impaired social life scale ($\rho = 0.198$; $p < 0.05$), showing that patients who lived in the capital, Belgrade, had better social life than patients who live in a country town or in a village. The diagnosis correlated with cosmetic complaints scale ($\rho = 0.323$; $p < 0.01$). The patients with non toxic and toxic goitre had less cosmetic complaints than those with Grave's disease-associated ophthalmopathy, thyreoid-associated ophthalmopathy (TAO), Grave's without TAO and Hashimoto disease. Patient's age, marital status and thyrotropine (TSH) level did not influence any scale score.

Discussion

ThyPROsr was found understandable and it was well accepted in Serbian patients with thyroid disease. The patients had physicians help and supervision during completion of the questionnaire, as it was the case in similar studies previously conducted in Serbian patients [7]. There was no

missing data. None of the items were found embarrassing by the patients. Just 2 patients commented items concerning sexual function and satisfaction as irrelevant.

In Serbian thyroid patients, internal consistency reliability for ThyPROsr scales ranged from 0.832 on hypothyroid symptom scale to 0.951 on cognitive problems scale. These reliability coefficients in Serbian patients with thyroid diseases indicate that the scales assessed by the ThyPROsr were appropriately measured.

Table 2

Descriptive statistics and reliability for the Serbian version of Thyroid Specific Patient Reported Outcome Measure (ThyPROsr)

Scales of measurement	Non toxic goitre (n = 51)	Toxic goitre (n = 20)	Graves' disease with TAO (n = 19)	Graves' disease without TAO (n = 3)	Hashimoto disease (n = 7)
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Goitre symptoms scale	25.22 ± 18.05	24.09 ± 18.54	22.37 ± 18.59	8.33 ± 8.60	33.44 ± 31.65
Hyperthyroid symptoms	28.49 ± 20.43	30.78 ± 19.59	39.97 ± 25.72	15.62 ± 14.32	31.25 ± 17.30
Hypothyroid symptoms scale	30.15 ± 25.08	26.62 ± 19.96	41.45 ± 28.35	37.50 ± 37.50	36.61 ± 37.05
Eye symptoms scale	16.73 ± 15.73	14.37 ± 15.62	37.01 ± 29.80	4.17 ± 7.22	11.61 ± 7.15
Tiredness scale	47.97 ± 24.64	53.04 ± 20.93	52.82 ± 28.71	51.19 ± 32.21	49.49 ± 22.34
Cognitive problems scale	22.79 ± 22.54	22.71 ± 23.28	18.42 ± 20.19	25.00 ± 43.30	8.93 ± 11.39
Anxiety scale	37.34 ± 26.80	30.00 ± 18.56	42.10 ± 28.53	34.72 ± 28.36	37.50 ± 20.41
Depressivity scale	38.30 ± 23.09	35.36 ± 16.95	41.16 ± 29.22	40.48 ± 48.49	38.26 ± 17.46
Emotional susceptibility	32.84 ± 23.91	29.17 ± 16.03	35.96 ± 25.18	42.59 ± 33.14	30.95 ± 24.08
Impaired social life scale	15.32 ± 18.51	14.37 ± 17.10	20.72 ± 30.12	27.08 ± 46.91	18.75 ± 35.90
Impaired daily life scale	21.32 ± 24.22	18.96 ± 24.72	30.92 ± 28.98	31.94 ± 35.92	31.55 ± 25.78
Impaired sex life scale	28.19 ± 34.26	27.50 ± 37.30	40.79 ± 32.50	33.33 ± 28.87	39.29 ± 42.35
Cosmetic complaints scale	14.79 ± 18.01	23.33 ± 21.52	29.60 ± 27.07	30.56 ± 31.27	36.31 ± 24.38

TAO – thyroid associated ophthalmopathy; $\bar{x}$ – mean value; SD – standard deviation.

Over the last few years there has been increasing focus on the HRQoL of the patients with thyroid cancer and patients with overt thyroid dysfunction. Limited reports are available on the HRQoL of patients with euthyroid or subclinical hyperthyroid or hypothyroid benign thyroid diseases [8–11]. We have identified several studies which evaluate the influence of different types of thyroid surgeries on HRQoL of the patients with benign thyroid diseases and low-risk, well-differentiated, thyroid carcinoma [12–14]. Studies which investigate factors that might influence HRQoL of patients with different benign thyroid disease, are lacking. To the best of our knowledge just two studies have investigated HRQoL in patients with different benign thyroid diseases, using ThyPRO. In those studies, none of the objective factors, including age, gender and type of thyroid dysfunction had a significant effect on patients' HRQoL [15,16].

We found highly significant inverse relationship between T4 and T3 level in euthyroid patients and some of HRQoL items, such as hypothyroid and eye symptoms and impaired social life. TSH did not influence any component of HRQoL. However, previous studies revealed somewhat different results. Most of the studies investigated HRQoL in thyroid cancers survivors. HRQoL is significantly better in patients under TSH-supressive doses of levothyroxine than in short-term hypothyroid patients, after 4 weeks of levothyroxine withdrawal [17]. It was also been shown that the HRQoL is worse in overt and subclinical hyperthyroid and hypothyroid patients than in healthy control group [18], but it normalizes upon achicving euthyroid state [19]. Some studies have shown that patients with lower TSH level have worse HRQoL [20–22], while other showed, similarly as our study, that TSH level does not influence HRQoL [22–24]. Patients with shorter lasting disease had significantly less goitre symptoms, cognitive problems and impaired sex life, indicating better HRQoL, than patients with longer lasting disease. Hoftijzer et al, [22] in their study reported the same findings. We have found less cosmetic complaints in male than in female patients. Significantly better HRQoL in males has been previously shown in several studies [25,26]. Higher educated patients had significantly better quality of life in some domains, goitre symptoms and eye symptom, than patients with lower education, quite similarly as demonstrated by Tan et al. [27] in their study. Being employed had a positive influence on some aspects of HRQoL, as it was presented by Tan et al. [27]. Patients with non toxic and toxic goitre had less cosmetic complaints and better quality of life than patients with autoimmune thyroid diseases, TAO, Graves without TAO and Hashimoto thyroiditis. Although, Miccoli et al. [28] had shown in their study that type of thyroid disease had no influence on HRQoL, recent studies have supported the hypothesis that thyroid autoimmunity *per se* affects the HRQoL regardless of hormonal status [16,29,30]. In our study, patient's age and marital status did not influence the HRQoL. Quite different results have been previously published. Miccoli et al. [28] also did not find significant difference in HRQoL depending on patient's age, but some other studies showed that HRQoL is better in younger patients as could be expected [25,27,31,32].

Conclusion

Serbian version of ThyPRO is a well accepted questionnaire. When administered with the help and supervision of the physician, it is easily filled-in, with no missing data. Reliability and validity of Serbian version of ThyPRO were good. Serbian version of ThyPRO questionnaire can be used for assessing health-related quality of life in Serbian patients with various benign thyroid disease.

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Incidence, predictors and prognostic implications of bleeding complicating primary percutaneous coronary intervention

Učestalost, prediktori i prognozni značaj krvarenja kao komplikacije primarne perkutane koronarne intervencije

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Abstract

Background/Aim. Data about bleeding complicating primary percutaneous coronary intervention (PCI) are more frequently obtained from randomized clinical trials on patients with acute coronary syndromes (ACS), but less frequently from surveys or registries on patients with ST-elevation myocardial infarction (STEMI). The aim of this study was to investigate the incidence, predictors and prognostic impact of in-hospital major bleeding in the population of unselected real-world patients with acute STEMI undergoing primary PCI. **Methods.** All consecutive patients presenting with STEMI who underwent primary PCI at a single large tertiary healthcare center between January 2005 and July 2009, were studied. Major bleeding was defined according to the Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) study criteria. We examined the association between in-hospital major bleeding and death or major adverse cardiac events (MACE) in patients treated with PCI. The primary outcomes were in-hospital and 6-month mortality and MACE. **Results.** Of the 770 STEMI patients treated with primary PCI, in-hospital major bleeding occurred in 32 (4.2%) patients. Independent predictors of major bleeding were advanced age (≥ 65 years), female gender, baseline anemia and elevated white blood cell (WBC) count and signs of congestive heart failure at admission (Killip class II-IV). In-hospital and 6-month mortality and MACE rates were more than 2.5-fold-higher in patients who developed major bleeding compared with those who did not. Major bleeding was a predictor of 6-month MACE, independent of a few risk factors (previous MI, previous PCI, diabetes mellitus and hypertension); (OR = 3.02; 95% CI for OR 1.20–7.61; $p = 0.019$), but was not a true independent predictor of MACE and mortality in the fully adjusted models. **Conclusion:** Patients of advanced age, female gender, with baseline anemia and elevated WBC count and those with Killip class II–IV at presentation are at particularly high risk of bleeding after primary PCI. Bleeding is associated with adverse outcome and may be an important marker of patient frailty, but it is not a true independent predictor of mortality/MACE.

Key words:
angioplasty, transluminal, percutaneous coronary; postoperative complications; hemorrhage; risk factors; prognosis; mortality; fibrinolytic agents.

Apstrakt

Uvod/Cilj. Podaci o krvarenju kao komplikaciji perkutanih koronarnih intervencija (PCI) češće se dobijaju putem randomizovanih kliničkih studija kod bolesnika sa akutnim koronarnim sindromima (ACS), a ređe putem popisa i registara bolesnika sa infarktom miokarda sa ST-elevacijom (STEMI). Cilj ove studije bio je da se ispita učestalost, prediktori i prognozni značaj velikog intrahospitalnog krvarenja kod populacije neselektovanih bolesnika sa STEMI lečenih metodom primarne PCI. **Metode.** U studiju su bili uključeni svi po redosledu primljeni bolesnici sa STEMI, podvrgnuti primarnoj PCI u velikom tercijarnom zdravstvenom centru u periodu između januara 2005. i jula 2009. godine. Veliko krvarenje definisano je prema kriterijumima studije *Global Use of Strategies to Open Occluded Coronary Arteries* (GUSTO). Ispitivali smo povezanost između velikog krvarenja nastalog tokom hospitalizacije i smrtnog ishoda, kao i glavnih neželjenih kardijalnih događaja (*major adverse cardiac events* – MACE) kod bolesnika lečenih metodom primarne PCI. Primarni ciljevi bili su bolnički i 6-mesečni mortalitet i glavni neželjeni kardijalni događaji. **Rezultati.** Od 770 bolesnika lečenih metodom primarne PCI,

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veliko krvarenje tokom hospitalizacije nastalo je kod 32 (4,2%) bolesnika. Nezavisni prediktori velikog krvarenja bili su odmaklo životno doba (≥ 65 godina), ženski pol, anemija i povećan broj leukocita na prijemu, kao i zastojna srčana insuficijencija klase Killip II–IV. Učestalost bolničke i 6-mesečne smrtnosti i MACE bila je više nego 2,5 puta veća kod bolesnika koji su imali veliko krvarenje nego kod bolesnika bez krvarenja. Veliko krvarenje bilo je prediktor 6-mesečnog MACE, nezavisno od nekoliko faktora rizika (prethodni MI, prethodni PCI, dijabetes melitus i hipertenzija), (OR 3,02; 95% CI 1,20 do 7,61; $p = 0,019$), ali nije bilo nezavisan prediktor MACE u modelu korigovanom za sve faktore rizika.

Zaključak. Bolesnici odmaklog životnog doba, ženskog pola, sa anemijom i povećanim brojem leukocita na prijemu kao i Killip klasom II–IV su u posebno povećanom riziku od krvarenja posle primarne PCI. Krvarenje je udruženo sa nepovoljnim ishodom i može biti značajan marker bolesnikovog nestabilnog stanja, ali nije u potpunosti nezavisan prediktor smrtnosti i MACE.

Ključne reči:
angioplastika, translumenska, perkutana, koronarna; postoperativne komplikacije; krvarenje; faktori rizika; prognoza; mortalitet; fibrinolitici.

Introduction

The widespread use of potent antithrombotic and fibrinolytic drugs for treatment of patients with acute coronary syndromes (ACS), coupled with the use of invasive procedures, has considerably reduced rates of recurrent ischemic events and death. However, the uses of multiple antiplatelet agents and anticoagulants have increased the risk of bleeding complications. Rates of bleeding in ACS and primary percutaneous coronary intrerventions (PCI) trials and registries have been reported to occur in up to 30% of patients [1, 2]. The incidence of major bleeding in recent large randomized trials ranged from as low as 0.2% to as high as 9.1% [3].

Most of data refer to bleeding complications among patients presenting with non-ST-elevation ACS (NSTE-ACS) and those undergoing elective PCI. Much less data reported about bleeding complicating primary PCI [4–6]. In addition, data about bleeding complicating primary PCI are mostly obtained from randomized clinical studies of specific patients populations; however, limited data obtained from real-world patients with ST-elevation myocardial infarction (STEMI). Both data, from registries and randomized trials, have indicated that bleeding is associated with worse clinical outcomes [1, 7, 8].

The aim of this study was to investigate the incidence, predictors and prognostic impact of periprocedural major bleeding in the population of unselected, consecutive patients undergoing contemporary primary PCI for STEMI in a single high-volume healthcare center in Serbia.

Methods

Study population

We analyzed 770 consecutive STEMI patients who underwent primary PCI between January 1, 2005 and July 30, 2009 at the Cardiology Clinic, Clinical Center of Serbia, Belgrade. Data was obtained from the computerized registry format of the ACS patients admitted to the Coronary Care Unit A, Emergency Center of Belgrade, Serbia.

Study definitions

Acute STEMI definition was based on the history of chest pain/discomfort lasting for at least 20 min attributed to myocardial ischemia, accompanied by persistent ST-segment elevation of ≥ 1 mm in ≥ 2 contiguous limb leads or ≥ 2 mm in precordial leads; or presumable new left bundle branch block; or true posterior MI with ST depression of ≥ 1 mm in ≥ 2 contiguous anterior leads [9].

In this study, major bleeding definition was modified from the Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) study criteria to include both severe (life-threatening) and moderate GUSTO bleeding categories [3, 10]. The GUSTO system defines moderate bleeding as the loss of blood requiring blood transfusion and defines severe or life-threatening bleeding as intracerebral bleeding or bleeding resulting in substantial hemodynamic compromise requiring treatment [10].

Re-myocardial infarction (re–MI) was defined using the standard criteria for Q-wave and non-Q-wave myocardial infarction [11].

Cardiogenic shock was defined as systolic blood pressure of < 90 mmHg for at least 30 min or when inotropic support was needed to maintain systolic blood pressure over 90 mmHg [9, 12].

Percutaneous coronary intervention procedure and subsequent antithrombotic medications

All the patients underwent coronary angioplasty and intracoronary stent implantation using standard percutaneous techniques only via the femoral artery. None of the patients had a non-femoral access. Unfractionated heparin (UFH) was administered as an intravenous bolus of 100 IU *per* kilogram of body weight or 50–60 IU/kg, if glycoprotein IIb/IIIa inhibitor (GPI) had given [12, 13]. Aspirin (300 mg orally) was preloaded in all the patients, after which 100–300 mg was given orally every day during the first 30 days and 100 mg every day thereafter indefinitely. Clopidogrel was given as a loading dose of 600 mg before insertion of the catheter, and 75 mg orally every day for 1 year [12, 13]. The GPI was administered based on operator discretion. The only GPI used in our study was tirofiban, given as an intravenous bolus of 10 μg *per* kilogram followed by an infusion of 0.15 μg *per* kilogram *per* minute, adjusted for renal impairment according to the label, and was continued for 24 hours. After removal of the shit, hemostasis was secured with manual compression.

End points and follow-up data

The primary composite end point included in-hospital and 6-month mortality from any cause, and in-hospital and 6-

month major adverse cardiac events (MACE). MACE were defined as a composite of death, re-MI, and repeated target vessel vascularization (TVR) [14]. Out-of-hospital clinical outcomes were obtained by telephone interviews conducted by educated medical doctors or in the outpatient clinic at 6-month follow-up visit. Follow-up data were available for 89% of the patients at 6 months.

Statistical analysis

Descriptive statistics was computed as mean values and standard deviation (SD) for continuous variables (or median values and interquartile range – IQR if skewed) and as absolute frequencies and percent values for categorical variables. Analysis of normality of the continuous variables was performed with the Kolmogorov–Smirnov test.

The patients were divided into two groups: those with in-hospital major bleeding and those without major bleeding, and also those with in hospital and 6-month death/MACE and those without it. Two group comparisons were performed using independent Student's t-test or Mann-Whitney *U*-test for continuous variables and the $\chi 2$-test for categorical variables. Potential collinearity between variables was assessed using Pearson's correlations.

Multivariate logistic regression analysis was performed to determine the independent predictors of in-hospital major bleeding, as well as in hospital and 6-month death and MACE. The criterion for the entry and removal of variables was set at 0.05 and 0.20, respectively. Only the noncollinear variables that were significant at the 20% level at univariate analysis were included in the multivariate models.

The variables included in the prediction of major bleeding were age, gender and weight; history of myocardial infarction, history of PCI, diabetes mellitus, systemic hypertension, smoking and chronic renal failure; hemoglobin baseline and white blood cell count; Killip class II-IV; use of GPI.

In order to better assess and analyze different groups of possible predictors of mortality and MACE, besides bleeding, three models of logistic regression analysis were developed. The first model was adjusted for demographic variables (age and gender), the second model was adjusted for risk factors (previous MI, previous PCI, diabetes mellitus and hypertension). The third model, termed fully adjusted, was adjusted for any of the additionally important potential confounding variables as age, gender, history of MI, history of PCI, diabetes mellitus and hypertension.

The performance of the multivariate models was studied with respect to discrimination and calibration. Model discrimination was assessed with the c-statistic, and model calibration was assessed with the Hosmer–Lemeshow statistic. To test the stability of the stepwise selection process of the regression models and assess the robustness of the variables, the bootstrap resampling procedure (1000 bootstrap samples) was used.

A two-sided *p*-value of 0.05 was considered statistically significant. All analyses were performed using Statistical Package for Social Sciences for Windows, release 18.0.2 (SPSS Inc., Chicago, IL).

Results

Patients' characteristics

Of 770 patients treated with primary PCI because of STEMI, 32 (4.2%) patients developed major bleeding. The patients with major bleeding were older, more frequently female gender and of lower body weight. They also had higher rate of systemic hypertension and chronic renal failure, more likely to have lower level of baseline hemoglobin, and less likely to be current smokers. The patients with major bleeding more often presented with heart failure estimated as Killip class II–IV and elevated white blood cell count compared with those without major bleeding (Table 1).

Table 1

Baseline characteristics of the patients with and without major bleeding

Patients' characteristics	Major bleeding (n = 32)	No major bleeding (n = 738)	*p*
Mean age (years), $\bar{x} \pm SD$	67.8 ± 11.3	58.6 ± 11.2	< 0.0001
Female gender (%)	56.3	24.1	< 0.0001
Previous MI (%)	18.8	12.9	0.337
Previous PCI (%)	3.1	3.4	0.936
Previous CABG (%)	0.0	2.0	0.415
Diabetes mellitus (%)	21.9	15.6	0.340
Systemic hypertension (%)	78.1	61.4	0.05
Current smoking (%)	40.6	62.5	0.01
Chronic renal failure* (%)	60.0	27.3	< 0.0001
Weight (kg), [median (IQR)]	75.0 (68.0, 80.0)	81.0 (73.0, 90.5)	0.002
Hemoglobin at admission (g/dL), [median (IQR)]	13.6 (12.3, 14.4)	14.6 (13.5, 15.5)	< 0.0001
Anemia at admission† (%)	28.1	10.9	0.003
White blood cell count ($1{,}000/mm^3$), [median (IQR)]	11.8 (10.2, 15.9)	11.4 (9.2, 13.9)	0.130
Killip class II-IV (%)	25.0	5.4	< 0.0001
Cardiogenic shock (%)	9.4	2.7	0.03

***Chronic renal failure defined as creatinine clearance of < 60 mL/min estimated by the Cockcroft-Gault formula;**
†Anemia was defined as hemoglobin levels of < 120g/l for women and < 130 g/L for men; IQR – interquartile range.
MI – myocardial infarction; PCI – percutaneous coronary intervention; CABG – coronary artery bypass grafting.

Angiographic and procedural results

At angiography, patients with major bleeding had similar proportion of right, left and circumflex coronary artery as a target coronary artery, compared with those without major bleeding (Table 2). In the present study, the application of GPI was infrequent and there was no difference in the use of this agent between patients with and without major bleeding.

Predictors of major bleeding

In multivariate logistic regression analysis, predictors of major bleeding in patients undergoing primary PCI were: age > 65 years, female gender, Killip class II–IV, anemia and elevated white blood cell count at admission. The strongest predictor of bleeding was heart failure estimated as Killip class II–IV (Table 3). Chronic renal failure and lower body weight were found to be predictors of major bleeding by univariate but not by multivariate analysis.

Table 2

Angiographic results and additional therapy

Variable	Major bleeding (n = 32)	No major bleeding (n = 738)	*p*
Target coronary artery (%)			0.642
right coronary artery	50.0	41.7	
left anterior descendent	43.8	45.1	
left circumflex	12.7	6.3	
by-pass graft	0.0	0.4	
Additional therapy (%)			
GP IIb/IIIa inhibitors	21.9	20.6	0.861

Table 3

Predictors of major bleeding

Variable	Univariate logistic regression analysis		Multivariate logistic regression analysis	
(Major bleeding)	OR (95%CI)	*p*	OR (95%CI)	*p*
Age ≥ 65 years	4.83 (2.17–10.72)	0.000	2.72 (1.01–7.33)	0.049
Female gender	3.99 (1.86–8.54)	0.000	3.08 (1.20–7.94)	0.020
Current smoking	0.41 (0.19–0.87)	0.021		
Chronic renal failure	3.99 (1.79–8.86)	0.001		
Anemia at admission	3.18 (1.39–7.29)	0.006	3.68 (1.47–9.18)	0.005
WBC *per* 1000/mm^3 increase	1.06 (1.02–1.11)	0.008	1.10 (1.03–1.18)	0.003
Body weight *per* 1 kg increase	0.96 (0.93–0.99)	0.004		
Systemic hypertension	2.25 (0.86–5.89)	0.100		
Killip class II–IV, %	5.82 (2.46–13.76)	0.000	5.11 (1.74–15.03)	0.003

WBC – white blood cell count.

Clinical bleeding characteristics

In all the patients with major bleeding, clinical bleeding was identified. According to the bleeding localization, 11 (34.4%) patients had hematemesis, 3 (9.4%) patients developed melena, 7 (21.9%) experienced hematuria, 2 (6.2%) had retroperitoneal bleeding, and 9 (28.1%) had large access site hematoma. Two patients who experienced gastrointestinal bleeding had prior gastritis, but none of them had prior gastric ulcer. Immediately gastroscopy was performed in only one patient showing multiple gastric erosions. The patients with gastrointestinal bleeding had discontinuation of dual antiplatelet therapy and were treated with proton pump inhibitors. Three patients with gastrointestinal bleeding experienced subacute stent thrombosis that occurred after discontinuation of dual antiplatelet therapy. Reintervention was done successfully and all three patients survived the next 6 months. The patients with retroperitoneal hematomas had temporary discontinuation of dual antiplatelet therapy. Among patients with hematuria, discontinuation of dual antiplatelet therapy was evidenced in 1 patient and of clopidogrel in 1 patient, too. Two patients with site hematomas had discontinuation of dual antiplatelet therapy and one patient had discontinuation of clopidogrel only.

Major bleeding and outcomes

The patients with *versus* without major bleeding had 3-fold higher rates of in-hospital mortality [9.4 % (cardiac 6.3%, non-cardiac 3.1%) *vs* 2.8%; $p = 0.03$] and 2.5-fold higher rates of 6-month mortality [15.6% (cardiac 9.4%, non-cardiac 6.2%) *vs* 5.8%, $p = 0.03$]. The patients with major bleeding had higher rates of in-hospital and 6-month MACE (15.6% *vs* 6.0%; $p = 0.03$ and 28.1% *vs* 10.3%; $p = 0.002$, respectively) versus those without major bleeding. All bleeding patients died during the first 6 months of gastrointestinal bleeding.

The association of in-hospital bleeding with in-hospital and 6-month mortality and MACE was evaluated using multivariate regression analysis (Table 4). Three models were used for multivariate analysis. The c-statistics was significant among most of the logistic regression models. The Hosmer-Lemeshow statistics was non significant for all models indicating good model fit, except for risk factors adjusted in-hospital and 6-month mortality. Major bleeding was found to be an important predictor of MACE after six months, inde-

Table 4

Association of in-hospital bleeding with all-cause mortality and MACE during follow up

Parameters	In-hospital		6- month	
	No bleeding	Major bleeding	No bleeding	Major bleeding
All cause mortality events/number at risk	21/717	3/29	38/653	5/32
	Reference	OR (95% CI)	Reference	OR (95% CI)
Unadjusted	1.00	3.53 (1.02–12.22)	1.00	3.00 (0.96-9.36)
Demographic adjusted*	1.00	1.57 (0.39–6.35)	1.00	1.46 (0.40–5.31)
Risk factors adjusted†	1.00	2.75 (0.76–9.98)	1.00	2.50 (0.76–8.25)
Fully adjusted‡	1.00	1.36 (0.30–6.06)	1.00	1.34 (0.35–5.21)
MACE events/number at risk	44/738	5/32	67/653	9/32
	Reference	OR (95% CI)	Reference	OR (95% CI)
Unadjusted	1.00	2.92 (0.93–9.13)	1.00	3.42 (1.40–8.39)
Demographic adjusted*	1.00	1.68 (0.46–6.17)	1.00	2.09 (0.73–5.95)
Risk factors adjusted†	1.00	2.50 (0.82–7.66)	1.00	3.02 (1.20–7.61)
Fully adjusted‡	1.00	1.50 (0.40–5.63)	1.00	1.95 (0.67–5.67)

*** Adjusted for age and gender;**
† Adjusted for previous myocardial infarction, previous percutaneous coronary intervention, diabetes mellitus and hypertension;
‡ Adjusted for age, gender, previous myocardial infarction, previous percutaneous coronary intervention, diabetes mellitus, hypertension;
MACE – major adverse cardiac events.

pendent of a few risk factors (previous MI, previous PCI, diabetes mellitus and systemic hypertension); (OR 3.02, 95% CI 1.20 – 7.61; $p = 0.019$). However, in the fully adjusted model, bleeding was not a predictor of either MACE or mortality.

Discussion

The main findings of our study show that major bleeding occured in 4.2% of 770 unselected consecutive STEMI patients who underwent primary PCI. The age ≥ 65 years, female gender, anemia and elevated white blood cell count at admission, as well as heart failure (Killip class II-IV) independently predicted major bleeding. Major bleeding was associated with 3-fold-higher in-hospital and 6-months mortality and 2.5-fold-higher MACE rates. In addition, chronic renal failure and lower body weight were found to be predictors of major bleeding in our univariate analysis.

The rates of major bleeding complications are highly variable, generally higher in registries than those of clinical trials [1, 14]. The incidence of bleeding depends mainly on the clinical setting and on the definition of bleeding events [2]. Fuchs et al. [6] reported major bleeding in 3.5% of 831 consecutive patients underwent primary PCI for STEMI. Kinnaird et al. [15] found major bleeding in 5.4% of 10 974 unselected patient underwent PCI using TIMI classification of bleeding.

In our study, the patients of ≥ 65 years old were at almost 3-fold increased risk of major bleeding compared with younger patients. Manoukian and al. [8] found that elderly patients (≥ 75 years old) of Acute Catheterization and Urgent Intervention Triage strategy (ACUITY) trial population were at 1.64-fold increased risk of major bleeding. The reasons for the higher bleeding risk in the elderly are likely multifactorial, including reduced renal function, greater sensitivity to anticoagulant agents as well as concomitant peripheral vascular disease with more frequent access site bleeding [16].

In the current study the patients with anemia at admission were at 3.68-fold increased risk of major bleeding. In trials Randomized Evaluation in Percutaneous Coronary Intervention Linking Angiomax to Reduced Clinical Events-2 (REPLACE) and both ACUITY and Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction (HORIZONS-AMI), the risk of major bleeding was doubled in patients presenting with baseline anemia [16, 17]. Therefore, more common major bleeding, especially gastrointestinal in anemic patients, emphasizes the importance of a thorough search for predisposing bleeding sites and hemorrhagic diathesis.

Our results show that elevated white blood cell count was strongly associated with in-hospital bleeding, independent of other risk factors. With such increase in white blood cell count *per* 1000 cells/mm^3 the patients were in 1.1 higher risk of major bleeding. In both ACUITY and HORIZONS-AMI trials, Mehran et al. [17] presented that higher white blood cell count in patients with STEMI and high-risk ACS treated with PCI predicts in-hospital major bleeding [17]. Recent analysis of the HORIZONS-AMI patient population confirmed that elevated baseline white blood cell count (> 11,000 *per* 1 mm^3) drawn at the time of presentation with STEMI was an independent predictor of infarction size as assessed by peak creatinine phosphokinase level, and of 1-year cardiac mortality, noncardiac mortality, and major bleeding [18]. Palmerini et al. [18] discussed that these data suggest that a high level of systemic inflammation in the early phase of STEMI (as reflected by the white blood cell count) is strongly associated with 1-year mortality. Leukocytes may have prothrombotic effects and may also result in release of proinflamatory and vasculotoxic factors which effects may contribute to reperfusion injury and subsequent extension of myocardial necrosis. However, the mechanisms linking high white blood cell count with major bleeding are unknown and warrant further study [18].

Female gender maybe associated with an increased risk for mayor bleeding compared with male gender, bud data are inconsistent [6, 7, 19]. The present study provides evidence that women are at 3-fold higher risk of major bleeding after

primary PCI by multivariate analysis. Mehran et al. [17] combined databases of ACUITY (patients with unstable angina or NSTEMI) and HORIZONS-AMI trials and found that female gender was an independent predictor of 2.32-fold increase in major bleeding. The exact mechanisms for the hemorrhagic risk in women are unknown. These findings may be related to smaller vessels size and therefore higher incidence of vascular access-site-related complications. In addition, because of smaller body mass, there is the tendency to over-anticoagulation in women [16].

In the present study renal impairment was associated with major bleeding, but was not found to be an independent predictor of this complication. The reason for this finding could be a small number of participants in our study. In registries and trials which include several thousand patients [Global Registry of Acute Coronary Events (GRACE), REPLACE-2, ACUITY], renal impairment is consistently associated with a high risk of bleeding [8, 19, 20].

In this study congestive heart failure at admission estimated as Killip class II-IV was strongly associated with major bleeding. In a randomized clinical study HORIZONS-AMI patients with Killip class II–IV were at 1.78 higher risk of in-hospital major bleeding than patients without congestive heart failure [21]. In the GRACE the risk of bleeding in patients with STEMI and Killip class IV was 1.73-fold higher than in patients with STEMI but without Killip class IV. Hypotension with subsequent tissue hypoperfusion may cause gastritis or ulceration and increase the likelihood of gastrointestinal bleeding. Affecting renal and liver function, hypoperfusion adversely affects the coagulation system and platelet function [15]. In our study cardiogenic shock was significantly associated with major bleeding, but was not the independent predictor of major bleeding.

Several scoring systems have been developed to predict major bleeding in patients treated with PCI. Mehran et al. [17] developed a practical integer risk score for NSTEMI and STEMI patients undergoing PCI to predict the risk and implications of major bleeding in ACS. This risk score consists of 7 variables estimated as the independent predictors of major bleeding within 30 days: female sex, advanced age, elevated serum creatinine and white blood cell count, anemia, presentation (STEMI or NSEMI) and treatment with GPI. Mrdović et al. [22] developed a simple and accurate risk model for predicting the risk of 30-day bleeding after primary PCI. The model included 5 independent predictors of bleeding: female gender, history of peptic ulcer, creatinine clearance at admission (< 60 mL/min), hemoglobin at presentation (< 125g/dL), and Killip class II-IV at admission [22].

Although several concordant reports have shown that bleeding complicating PCI is associated with increased mortality rates, the association between bleeding and mortality or MACE are as yet poorly understood [5, 6, 8, 15]. In the combined ACUITY/HORIZONS-AMI data-base, major bleeding was an independent predictor of a 3.2-fold increase in 1-year mortality. The negative impact of bleeding in many patients who survive the bleeding event itself develops overtime, and is clearly visible at 30 days, but expands to 6 months and beyond [8, 17]. We observed 3-fold increase in in-hospital and 6-month mortality rates and 2.5-fold higher rates of MACE among patients with major bleeding.

In our study, using multivariate regression analysis, major bleeding was an important predictor of MACE at 6-month follow-up, independent of risk factors such as previous MI, previous PCI, diabetes mellitus and systemic hypertension. However, the important fact is that bleeding was not a true independent predictor of MACE or mortality in the fully adjusted models. What this means it that bleeding is a very important marker of future MACE/mortality, but not the independent predictor and that it is unlikely to be a direct cause. The real possibility is that bleeding is a marker for the frailty of patient and their clinical likelihood of suffering from a poor outcome for other reasons. Indeed, in multivariable analysis, we identified older age, female gender, anemia, elevated white cell count and Killip class II-IV at admission as independent predictors of bleeding. These are all markers of frail, sick patients that are intrinsically more likely to suffer adverse outcome such as MACE or mortality.

Study limitation

Our study had some limitations. First, these study observations were derived from a retrospective analysis, thus had carry the inherent limitations of such mode of evaluation. Second, in anemic patients, a relative lesser drop in hemoglobin would trigger blood transfusion (moderate bleeding according to the GUSTO criteria), but might not actually represent "major bleeding". Third, in our primary PCI procedure, the access was exclusively trans-femoral, and the use of radial access may have changed the finding of this study.

Conclusion

Major bleeding in STEMI patients treated with primary percutaneous coronary intervention is associated with 3-fold-higher in-hospital and 6-month mortality/major adverse cardiac events rates. Patients of advanced age and female gender, those with anemia, elevated white blood cell count and heart failure on admission are at particularly high risk of bleeding. Although major bleeding is the predictor of 6-month major adverse cardiac events independent of some risk factors (previous MI, previous PCI, diabetes mellitus and hypertension), it is not a true independent predictor of major adverse cardiac events or mortality, but may be an important marker for the frailty of patients that are more likely to suffer an adverse outcome.

Conflict of interest

None declared.

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The clinical course of non-muscle invasive bladder cancer after transurethral resection of the tumor with or without subsequent intravesical application of bacillus Calmette-Guérin: The influence of patients gender and age

Klinički tok karcinoma mokraćne bešike bez zahvatanja mišićnog sloja njenog zida posle transuretralne resekcije tumora sa ili bez naknadne intravezikalne aplikacije bacila *Calmette-Guérin*: uticaj pola i godina života bolesnika

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Abstract

Bacground/Aim. The therapy with intravesical instillation of bacillus Calmette-Guérin (BCG) after transurethral resection (TUR) of tumor is the gold standard of treatment of non-muscle invasive bladder cancer (NMIBC). The role and importance of BCG intravesical therapy in various shape of tumors, were confirmed by our previous investigation. The aim of this study was to examine whether incidence of recurrence and tumor regression differs depending on sex and age of patients. **Methods.** This study included a total of 899 patients suffering from NIMBC, treated at our institution from January 1, 2007 to March 1, 2013. Two groups of patients were formed: patients underwent TUR + BCG therapy (the group I) and the group II with patients in whom TUR was performed as only therapy. These two groups of patients were divided into subgroups of respondents male and female, age 60 years or younger and older than 60 years. Statistical analysis was performed using χ^2 test and the Kolmogorov-Smirnov test. **Results.** This research suggests that if the frequency of recurrence is seen as the only parameter, considering all the subjects, the lowest recurrence rate was determined in the male subjects, aged 60 years and younger who had received BCG after TUR. A high statistical significance was found in the incidence of recurrence in patients younger than 60 years, depending on the response to the therapy, while in those older than 60 years, the difference was at the level of statistical significance. This can be attributed to a certain degree of infravesical obstruction in older men. **Conclusions.** Sex and age of patients may have a significant influence on the course and outcome of NMIBC. The disease has the most malignant and most aggressive behavior when present in males older than 60 years.

Key words:
urinary bladder neoplasms; recurrence; sex; age factors; risk factors; immunotherapy; urological surgical procedures.

Apstrakt

Uvod/Cilj. Intravezikalna imunoterapija bacilom Calmette-Guérin (BCG) smatra se zlatnim standardom u lečenju tumora mokraćne bešike bez zahvatanja mišićnog sloja [*non-muscle invasive bladder cancer* (NMIBC)] nakon transuretralne resekcije (TUR) tumora. Uloga i značaj BCG intravezikalne terapije za ishod lečenja, zavisno od oblika i veličine NMIBC, utvrđeni su našim prethodnim istraživanjem. Cilj ovog rada bio je da se utvrdi incidencija recidiviranja i progresije bolesti u zavisnosti od pola i životnog doba bolesnika. **Metode.** Ispitivanjem je obuhvaćeno 899 bolesnika sa NIMBC, lečenih u našoj instituciji od 1.1.2007 do 1.3.2013. Formirane su dve grupe bolesnika: bolesnici lečeni kombinacijom terapija TUR i BCG (grupa I) i bolesnici podvrgnuti samo terapiji TUR (grupa II). Ove dve grupe bolesnika podeljene su, zatim, u podgrupe: ispitanici muškog i ženskog pola, stari 60 godina ili mlađi, i stariji od 60 godina. Statistička analiza obavljena je primenom χ^2 testa i Kolmogorov-Smirnov testa. **Rezultati.** Posmatrajući učestalost recidiviranja kao jedini parametar, uzevši u obzir sve ispitanike, najniža stopa recidiva nađena je kod ispitanika muškog pola, starih 60 godina ili mlađih, koji su primali BCG nakon TUR-a. Visoka statistička značajnost u učestalosti recidiviranja nađena je kod mlađih od 60 godina, zavisno od primenjene terapije, dok je kod starijih od 60 godina ova razlika bila na nivou statističke značajnosti. Ovo se može pripisati određenom stepenu infravezikalne opstrukcije kod starijih muškaraca. **Zaključak.** Pol i starost bolesnika mogu imati značajan uticaj na tok i ishod NMIBC. Bolest se najmalignije i najagresivnije ponaša kada je prisutna kod osoba muškog pola, starijih od 60 godina.

Ključne reči:
mokraćna bešika, neoplazme; recidiv; pol; životno doba, faktori; faktori rizika; imunoterapija; hirurgija, urološka, procedure.

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Introduction

Urinary bladder cancer, transitional cell carcinoma (TCC) is one of the most common malignancies in the USA and Europe. Most bladder tumors (75–85%) are non-muscle invasive tumors (NMIBC) at the moment when they were diagnosed [1, 2]. After more than 30 years of research, intravesical instillation of bacillus Calmette-Guérin (BCG) after the transurethral resection (TUR) of bladder tumor (TUR BT) remains the most effective intravesical treatment in NMIBC, but there is still room for improvement [3–5]. BCG has currently become the most commonly used intravesical agent and is known to be superior to other intravesical agents for prevention of tumor recurrence [2, 6–9]. The standard BCG induction treatment consists of six weekly bladder instillations. Many institutions give 3–21 additional instillations during the first three years to improve results [10]. Although this therapy has been proven to significantly reduce the incidence of stage progression and recurrence in NMIBC [11, 12] it was also registered that it has minor side effects occurring in 35–71% of patients and significant morbidity in 5–23% of patients due to systemic sepsis [13].

TCC is the fourth most frequent malignancy diagnosed in men and the eighth most frequent among women in the USA. The influence of factors such as gender and age on the incidence, prognosis and survival has not yet been elucidated sufficiently [14]. It is believed that the binding of BCG to the surface of urothelium is the most important step in the BCG anti-tumor activity and studies have shown that the hormonal milieu in the organism of patient may have a significant role in the establishment of this link [15]. Previous studies, which have dealt with the evaluation of the impact of gender on the outcome of BCG therapy, showed different results starting from the fact that there is no connection between sex and treatment outcomes [1, 16, 17] up to, data that the shorter period without disease recurrence in women was found after BCG therapy [18]. A literature review that talked about the influence of gender and age on treatment outcome of TCC was published by Shariat et al. [19] in their work during 2009. Marsit et al. [20] in their study on 331 patients with TCC described the importance of male sex and age in the prediction of outcome of disease in patients suffering from NIMBC with a high grade of disease. In our institution in compliance with international standards this therapy was applied. Regarding recurrence within one year of monitoring, the frequency was consistent with published data – 15% to 20%, depending on the period of follow-up. The frequency and severity of adverse effects of treatment were also in line with literature data. According to our experience, the most common side effects were chills, fever, micro- and macrohematuria. Significantly less common were the severe complications such as the development of tuberculosis (TBC) of urinary tract, miliary TBC of lung, bladder contracture, reduced bladder capacity, urethral stenosis. Most rare were complications such as TBC encephalitis and hepatitis. This therapy was applied in our Institution regularly until the start of 2012 year and after that due to the discontinuance of production of this medication (ImmuCyst®, Sanofi Aventis) and as no similar product has been registered, so far, for the Serbian market, TUR BT has been the only treatment for patients suffering from NMIBC. By our previous investigation [21], the role and importance of BCG intravesical therapy at various shape of tumors, has been confirmed. The aim of this study was to examine whether the incidence of recurrence and tumor progression differ depending on sex and age of patients.

Methods

The study included patients with NMIBC, treated and controlled in our Institution in the period from January 1, 2007 to March 1, 2013. The study included a total of 899 respondents of both sexes, [male 660 (73.4%), female 239 (26.6%)], various ages (average 61.05 ± 10.52 years), and different occupations. Whether respondents belong to the risk group of developing bladder cancer and recurrence of the disease did not affect the possibility that respondents were included in the study. Respondents, depending on the applied treatment, were divided into two groups: patients who underwent BCG intravesical therapy after TUR of the tumor (TUR + BCG) – the group I, 674 subjects, and the group in which TUR of the tumor was the only treatment – the group II, 225 subjects. The patients with intravesical BCG therapy, received a single dose *per* week following the therapy, a total of six weeks. Given that our goal was to determine whether the frequency of recurrence varies depending on sex and age of patients, the two groups of patients were further divided according to sex and age – the male up to 60 years of age and older, and the women up to 60 years of age and older. Out of 660 male subjects, 487 belonged to the group I, and 173 to the group II. The group I included 222 patients to 60 years of age and 265 subjects older than 60 years, and the group II 77 patients up up to 60 years of age and 96 patients older than 60 years. Of 239 female subjects in the group I were 187 respondents, and in the group II 52 subjects. In the group I there were 89 patients up to 60 years of age and 98 patients older than 60 years, and in the group II, 28 patients up to 60 years of age and 24 patients older than 60 years.

After the therapy had been conducted, all of the respondents were in regular quarterly controls that involved basic laboratory tests, ultrasonic examination and uretrocystoscopy. Based on the results obtained in the controls, it was established if and when there was a recurrence of the disease, depending on the applied therapy, whether patients with the developed recurrence, progressed in the grade and stage of the disease.

All the results in the text and tables are presented as the mean value ± standard deviation (SD). The significance in the differences in frequencies distributions of individual parameters was checked using the χ^2 test or Kolmogorov-Smirnov test in cases when the frequencies were less than 5. The correlation of various parameters was investigated using parametric or nonparametric correlation analysis (Pearson). The three levels of statistical significance were determined: $p < 0.05$; $p < 0.01$ and $p < 0.001$. Data processing was performed using the commercial statistical software for PCs (Stat for Windows, R.4.5, Stat Soft, Inc., USA, 1993).

Results

The frequency of recurrence of NMIBC depending on the age group and response to the therapy concerning all the subjects, is shown in Table 1.

The frequency of recurrence of NMIBC depending on age and the applied therapy in the females is shown in Table 3 it is shown that there was no statistically significant difference in the incidence of recurrence in female subjects nor in relation to the applied therapy and age, despite the fact that

Table 1

Frequency of non-muscle invasive bladder cancer recurrence depending on the age group and response to the therapy concerning all the subjects

Therapy	Age groups (years)				*p*
	≤ 60		> 60		(≤ 60: > 60)
	Patients (n)	Recurrences, [n (%)]	Patients (n)	Recurrences, [n (%)]	
TUR + BCG	311	37 (11.9)	363	96 (26.4)	$\chi^2 = 21.47$; $p < 0.001$
TUR	105	32 (30.5)	120	43 (35.8)	n.s.
p (TUR + BCG : TUR)		$\chi^2 = 18.26$; $p < 0.001$		$\chi^2 = 3.43$; $p = 0.064$	

TUR – transurethral resection; BCG – bacillus Calmette-Guérin.

The results in Table 1, show that in the patients up to 60 years of age there was a statistically significant difference in the frequency of recurrence between the group I and the group II, whereas this difference was not present in the patients over 60 years. At the same time, in the group I there was a highly statistically significant difference in the frequency of recurrence depending on age, with a statistically higher incidence of recurrence in the patients older than 60 years. In the group II this significance was not noticed.

Results presented in Table 2 show that regarding males there was a statistically significant difference in the frequency of recurrence between the groups I and II, and in those up to 60 years this difference was even highly statistically significant. It is also shown that in the group I there was a highly statistically significant difference according to age, while it was not registered in the group II, although the recurrence rate was the highest among those older than 60 years who did not receive BCG therapy after TUR (Table 2).

the percentage of recurrence between the groups I and II, in the patients up to 60 years of age, differsed by as much as 15%. It raises the question of the relevance of comparison of the small absolute numbers of recurrences.

The incidence of disease progression concerning grade (G) and stage of disease (T) at recurrences, registered in all the male respondents, depending on the applied therapy is shown in Table 4.

The results shown in Table 4 indicate that there was a statistically significant difference in the incidence of disease progression in recurrences, so the progression of the disease was significantly more frequent in males, who did not receive BCG therapy after TUR.

Table 2

Frequency of recurrence of non-muscle invasive bladder cancer depending on the age group and the applied therapy observed only in the males

Therapy	Age groups (years)				*p*
	≤ 60		> 60		(≤ 60: > 60)
	Patients (n)	Recurrences [n (%)]	Patients (n)	Recurrences [n (%)]	
TUR + BCG	222	25 (11.3)	265	80 (30.2)	$\chi^2 = 24.48$; $p < 0.001$
TUR	77	24 (31.2)	96	41 (42.7)	n.s.
p – TUR + BCG : TUR		$\chi^2 = 15.11$; $p < 0.001$		χ^2=4.41; $p < 0.05$	

For abbreviations, see under Table 1.

Table 3

Frequency of recurrence of non-muscle invasive bladder cancer depending on age and the applied therapy observed only in the females

Therapy	Age groups (years)				*p*
	≤ 60 years		> 60 years		(≤ 60: > 60)
	Patients (n)	Recurrences [n (%)]	Patients (n)	Recurrences [n (%)]	
TUR + BCG	89	12 (13.5)	98	16 (16.3)	n.s.
TUR	28	8 (28.6)	24	3 (12.5)	n.s.
p – TUR + BCG : TUR		n.s.		n.s.	

For abbreviations, see under Table 1.

The incidence of the disease progression to grades (G) and stage of the disease (T) in recurrences, registered in all the female subjects, depending on the applied therapy is shown in Table 5.

The results in Table 5 shows that there was no significant difference in the incidence of the disease progression at

Table 4

Incidence of disease progression concerning grade (G) and stage of disease (T) at recurrences, registered in all the male respondents, depending on the applied therapy.

Therapy	Treated patients (n)	Recurrences with progression [n (%)]	
		Progression G	Progression G + T
TUR + BCG	487	47 (9.6)	41 (8.4)
TUR	173	34 (19.6)	25 (14.4)
p (TUR + BCG : TUR)		$\chi^2 = 10.95$; $p < 0.001$	$\chi^2 = 4.51$; $p < 0.05$

For abbreviations, see under Table 1.

Table 5

Incidence of disease progression to grades (G) and stage of the disease (T) in recurrences, registered in all the female subjects, depending on the applied therapy

Therapy	Treated patients (n)	Recurrences with progression [n (%)]	
		Progression G	Progression G + T
TUR + BCG	187	3 (1.6)	3 (1.6)
TUR	52	4 (7.7)	3 (5.8)
p (TUR + BCG : TUR)		n.s.	n.s.

For abbreviations, see under Table 1.

recurrences registered in the females, depending on the applied therapy, although it remains an open question whether these findings can be considered relevant, given the low absolute number of relapses with progression.

The frequency of disease progression to grades (G) and stage of the disease (T) in recurrences, depending on the applied therapy registered in the male respondents aged 60 years or younger, is shown in Table 6.

The results in Table 6 indicates that there was a statistically highly significant difference in the incidence of disease progression at recurrences in the males aged 60 years and younger, according to the applied therapy.

The frequency of disease progression to grades (G) and stage of the disease (T) in recurrences, registered in the male subjects, older than 60 years, depending on the applied therapy is shown in Table 7.

The results in Table 7 show that there was no statistically significant difference in the incidence of progression of the disease in recurrences, registered in the male subjects older than 60 years, depending on the applied therapy.

Discussion

The role and significance of intravesical BCG immunotherapy after TUR BT in reducing the rate of recurrence was confirmed by numerous publications, e.g. by Gontero et al. [12]. In their survey, they concluded that intravesical BCG therapy should be considered as the most effective form of intravesical therapy, but the role of this therapy in the progression of the disease in papillary tumors remains to be elucidated.

Our research, clearly shows that the frequency of recurrence in patients with no BCG therapy after TUR was statistically significantly higher than among patients in whom the therapy was applied, which is in line with the results obtained by Brandau and Suttmann [3] and Herr and Morales [11].

In addition to this, based on the established knowledge our aim was also to determine a possible impact, of age and sex of patients subjected to different forms of treatment on the frequency of recurrence and progression of the disease.

Table 6

Frequency of disease progression to grades (G) and stage of the disease (T) in recurrences, registered in the male respondents aged 60 years or younger, depending on the applied therapy

Therapy	Treated patients (n)	Recurrences with progression [n (%)]	
		Progression G	Progression G + T
TUR + BCG	222	8 (3.6)	6 (2.7)
TUR	77	13 (16.9)	11 (14.3)
p (TUR + BCG : TUR)		$\chi^2 = 13.47$; $p < 0.001$	$\chi^2 = 12.22$; $p < 0.001$

For abbreviations, see under Table 1.

Table 7

Frequency of disease progression to grades (G) and stage of the disease (T) in recurrences, registered in the male subjects, older than 60 years, depending on the applied therapy

Therapy	Treated patients (n)	Recurrences with progression [n (%)]	
		Progression G	Progression G + T
TUR + BCG	265	39 (14.7)	35 (13.2)
TUR	96	21 (21.9)	14 (14.6)
p (TUR + BCG : TUR)		n.s.	n.s.

For abbreviations, see under Table 1.

There are still present controversies on this topics, even in studies that included a representative number of respondents, as that of Madeb and Messing [14] in 2004.

Our results showed that in the patients up to 60 years of age there was a statistically significant difference in the frequency of recurrence between the group of patients with BCG after TUR and the group of patients with TUR as the only treatment, whereas this difference was not present in the patients above 60 years of age. At the same time, in the group of patients with BCG after TUR there was a statistically significant difference in the frequency of recurrence depending on age, with the incidence of recurrence statistically higher in the patients older than 60 years, while in the group of patients with TUR as the only treatment this significance was not noticed. Thus, it follows that if the frequency of recurrence is seen as the only parameter, considering all the subjects, the lowest recurrence rate was in the patients with BCG after TUR, up to 60 years, that is partly consistent with the results published by Sylvester et al. [1], Babjukand et al. [2] and Brandau and Suttmann [3].

Considering only male subjects, there was a high statistical significance of the difference in the frequency of recurrence in younger than 60 years, depending on the applied therapy, and in those older than 60 years this difference was on the level of statistical significance. This difference can be attributed to a certain degree of infravesical obstruction, that is present in older men. In patients with no BCG there was no statistically significant difference in frequency of recurrence.

Further, in the females there was no statistically significant difference in the incidence of recurrence, although in the women younger than 60 years, the incidence of recurrence if they did not receive BCG increased by as much as 15%. However, it should be considered that, regarding only females, when the population is divided into groups and subgroups, the absolute number of recurrences was small, so the representativeness of the results remains an open question. These results suggest that in addition to the forms of the therapy, sex and age may have an impact on the incidence of recurrence rate of NMIBC. These results are partly in line with the results published by Madeb and Messing [14], Chen et al. [15] and Shariat et al. [19].

If we look at the progression of the disease from the point of our results it can be seen that in the men with no BCG treatment the incidence of disease progression is statistically more frequent, while this significance was not registered in the females. As we already mentioned, the fact of a small absolute number of recurrences with disease progression in women should not be overlooked. Our results are not consistent with the results published by Takenaka et al. [16] and Lerner et al. [17]. Our findings, however, show a considerable degree of agreement with the results published by Chen et al. [15].

Further analysis of the obtained results show to what extent could age of men, in addition to forms of the applied therapy impact the incidence of disease progression at recurrences. In the males aged 60 years and younger, the incidence of disease progression was statistically more frequent depending on whether they received BCG therapy or not, while this significance was not observed in the subjects older than 60 years, which is consistent with the results published by Chen et al. [15], and partly in compliance with the results published by Marsit et al. [20].

The results of our study indicate that the use of BCG therapy in patients with NMIBC has a definite significance, as confirmed also by the results of other authors. In our research, however, there are the results that are not fully in accordence with the results of other researchers, pertaining to the influence of gender and age of the respondents on the frequency of recurrence and progression of the grade and stage of the disease.

Conclusion

Sex and age of a patient may have a significant influence on the course and outcome of non-muscle invasive bladder cancer. Also, non-muscle invasive bladder cancer has the most malignant and most aggressive behavior when present in males older than 60 years.

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Reliability and validity of the Serbian version of Children's Dental Fear Questionnaire

Pouzdanost i punopravnost srpske verzije upitnika za procenu straha od stomatologa kod dece

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Abstract

Background/Aim. Dental anxiety leads to avoidance of dental treatment and could lead to impaired oral health. The aim of this study was to determine the reliability of the Serbian version of Children's Fear Survey Schedule Dental Subscale (CFSS-DS) and the relations between dental anxiety and oral health status in a sample of Serbian schoolchildren. **Methods.** The CFSS-DS scale was translated into Serbian and administered to 231 (12-year old) patients of the Pediatric Dental Department, Public Health Center Čukarica, Belgrade. The number of healthy, decayed, missing and filled teeth (DMFT score) in children was determined by a clinical exam. **Results.** The average CFSS-DS score was 26.47 ± 10.33. The girls reported higher anxiety than the boys ($p < 0.05$). Most common fears were drilling, choking, going to the hospital and anesthesia. Lower CFSS-DS scores were recorded in children with all healthy teeth ($p < 0.05$). Children with higher CFSS-DS scores mostly visit the dentist due to pain or parental insistence, and those with lower anxiety scores more often visited dentist due to regular check-ups or non-invasive treatments ($p < 0.01$). A high value of the Cronbach's coefficient of internal consistency ($\alpha = 0.88$) was found in the entire scale. **Conclusion.** The Serbian version of CFSS-DS questionnaire is reliable and valid psychometric instrument for evaluation of dental fear in Serbian children. Dental anxiety negatively affects dental attendance and oral health of the examined schoolchildren.

Key words:
child; dental anxiety; questionnaires; serbia.

Apstrakt

Uvod/Cilj. Strah od stomatologa dovodi do izbegavanja stomatološkog lečenja što može narušiti oralno zdravlje. Cilj istraživanja bio je da se utvrdi pouzdanost Srpske verzije upitnika za procenu straha od stomatologa kod dece (CFSS-DS) i uticaj straha od stomatologa na oralno zdravlje u grupi školske dece iz Srbije. **Metode.** CFSS-DS upitnik je preveden na srpski i testiran na uzorku od 231 ispitanika (uzrasta 12 godina) u Odeljenju za dečju stomatologiju Doma zdravlja Čukarica u Beogradu. Kliničkim pregledom utvrđen je broj zdravih, karijesnih, ekstrahiranih i plombiranih zuba (KEP indeks). **Rezultati.** Prosečan CFSS-DS skor bio je 26,47 ± 10,33. Utvrđen je viši stepen anksioznosti kod devojčica nego kod dečaka ($p < 0.05$). Najčešći strahovi odnosili su se na bušenje, davljenje, odlazak u bolnicu i anesteziju. Niži CFSS-DS skor imala su deca sa svim zdravim zubima ($p < 0.05$). Deca sa višim CFSS-DS skorom najčešće su odlazila stomatologu zbog bola ili insistiranja roditelja, dok su deca sa nižim stepenom anksioznosti posećivala stomatologa radi redovne kontrole i neinvazivnog lečenja ($p < 0,01$). Visoka vrednost Kronbahovog koeficijenta interne konzistencije ($\alpha = 0.88$) utvrđena je na nivou celokupnog upitnika. **Zaključak.** Srpska verzija CFSS-DS upitnika pouzdan je i validan psihometrijski instrument za procenu straha od stomatologa kod dece. Strah od stomatologa negativno utiče na odlazak stomatologu i oralno zdravlje ispitanika.

Ključne reči:
deca; anksioznost, stomatološka; upitnici; srbija.

Introduction

Dental fear is a phenomenon frequently encountered in dental practice. Anxiety may lead to avoidance of dental treatment or disruptive behavior during treatment [1, 2], which is stressful both for the patient and the dental team and negatively affects treatment outcomes [3]. People with high dental fear are more likely to delay treatment, leading to more extensive dental problems and symptomatic visiting patterns which feeds back into the maintenance or exacerbation of existing dental fear [4].

Dental fear (DF) and dental anxiety (DA) in children are proven to be of a multifactor etiology. The experience of pain and trauma during dental treatment has been suggested

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to play an important role in the onset of dental fear [5, 6]. Other factors such as the child psychological development, age, gender and social background are also important [7, 8]. No straight cause-consequence model in explanation of children's dental anxiety has been found, because its multifactorial etiology accounts for interaction of different dispositional and situational concomitant factors [9].

In order to assess a child's anxiety from a more complex view prospective, various measurements are used, since different questionnaires might measure the different aspects of dental anxiety. One psychometric scale that is widely used in pediatric dentistry research is the Children's Fear Survey Schedule Dental Subscale (CFSS-DS), initially presented by Cuthbert and Melamed [10]. The CFSS-DS is a self-report or parental report 15-item questionnaire intended to measure DF in children. Satisfactory reliability and validity of the scale has been reported [11, 12]. CFSS-DS is commonly used in studies examining prevalence and possible predictors and concomitants of DF in children, and also of correlations between DF and dental behavior management problems [13].

There is a lack of data on dental anxiety problems in Serbian children. Since the Serbian version of CFSS-DS has not been applied before, our aim was to asses dental anxiety in a sample of children using CFSS-DS, to explore its psychometric properties (the reliability and validity) and to assess the relationship between DF, oral health related behavior and dental status of children.

Methods

Subjects

The convenience sample consisted of 231 12-year old children (110 boys and 121 girls), who attended the Pediatric Dental Department, Public Health Center Čukarica, Belgrade. All the patients of this age, regardless their dental status, number of visits or types of treatment previously received, were invited to participate. Patients with symptoms of acute toothache or any other dental emergency (bleeding, swelling, dental trauma) were excluded from the sample. Children with systemic diseases or/and handicap was also excluded.

The parents of the children who participated signed an inform consent form. This research was in accordance with the Declaration of Helsinki and approved by the Ethical Committee of the Faculty of Stomatology in Pančevo, University Business Academy, Novi Sad, Serbia.

Instruments

We used two questionnaires in our study. The first one consisted of 14 questions which pertained to the socio-demographic characteristics of child (gender, family structure and child's grades in the previous academic year), oral hygiene habits (frequency and duration of brushing, use of fluoride supplements), sugar consumption pattern and dental visits behavior (frequency and reasons of dental visits, avoiding treatment, self-perceived treatment needs).

To assess dental anxiety and fear in children, we used the CFSS-DS. English version was translated into Serbian language (Addendum) and back translated by the two bilingual dentists (translation is available from the first author). We used self-report version of a scale (children were filling the data, which is opposite to parental-report questionnaire, where parents answer to the same questions in behalf of their children). CFSS-DS consists of 15 items related to various aspects of dental treatment. Each item can be scored on a five-grade scale, from 1 (not afraid) to 5 (very afraid). The responses sums ranged from 15 to 75. The scores of 38 and over are used to be indicative of DF in children [14], and scores of 32 and above of a risk range [15]. Accordingly, we classified the subjects into three groups (Table 1): children with low anxiety levels (with CFSS-DS score < 32), moderately anxious children (≥ 32 score ≤ 38), and anxious children (score > 38).

The children filled-in the questionnaires while sitting in the waiting room prior to receiving the treatment.

Table 1
Distribution of subjects according to the Childrens Fear Survey Schedule-Dental Subscale (CFSS-DS) score range, in relating to gender

Anxiety levels	Boys	Girls	Total
	n (%)	n (%)	n (%)
Low (score < 32)	91 (82.0)	87 (72.5)	178 (77.1)
Medium (≥ 32 score ≤ 38)	7 (6.3)	13 (10.8)	20 (8.7)
High (score > 38)	13 (11.7)	20 (16.7)	33 (14.3)

$\chi^2 = 3.03$; df = 2; $p = 0.220$.

Clinical data

A single trained dentist recorded dental status of children (number of healthy, decayed, missing and filled teeth – DMFT score) under clinical conditions, using a dental mirror and probe, according to the World Health Organization (WHO) criteria.

Statistical analysis

We used the SPSS statistical software (SPSS for Windows, release 17.0, SPSS, Chicago, IL) for analysis. The data were analyzed regarding the questionnaire variables according to the age and gender and tested with the χ^2 test, Student's *t*-test and one-way ANOVA. Pearson's correlation coefficients were calculated among variables in the total group. The predetermined significance levels were set at 0.05. Cronbach's alpha was used to analyze internal consistency reliability. The exploratory factor analysis was carried out and rotated (Varimax rotation with Kaiser's normalization) to establish the statistical separation of the CFSS-DS items into factors. The decision on the final number of factors was based on the Kaiser's criterion (eigen value > 1).

Results

According to the CFSS-DS score, the majority of children expressed low to moderate dental anxiety (total average score = 26.47 ± 10.33). Only 33 (14.3%) subjects expressed

high dental anxiety with CFSS-DS score above 38 (Table 1). The girls had significantly higher (t = -2.35; df = 229; p = 0.019) mean anxiety score (27.98 ± 10.55) than boys (24.81 ± 9.86).

The mean DMFT score was 3.42 ± 2.52 (with 1.54 ± 1.90 number of decayed, 0.27 ± 0.71 of missing and 1.60 ± 1.72 of filled teeth). The children with all healthy teeth had significantly lower total mean anxiety score (24.36 ± 8.10) than those with DMFT > 0 (mean anxiety score = 27.23 ± 10.91; p = 0.024).

Almost two thirds of the children (73.48%) were satisfied with their oral health at the moment of testing. Mean anxiety scores were higher in children who rated their oral health as "poor" or "very poor" than "good" or "excellent" (df = 3; F = 5.013; p = 0.002).

The majority of the children (79.48%) reported dental visit within last year. The children who reported dental visits within past 12 months had a lower mean anxiety score (25.36 ± 8.80) than those who did not visit the dentist for this period (29.08 ± 11.62) or could not remember (31.24 ± 15.43; df = 3; F = 3.435; p = 0.018). Most often, the children visited the dentist due to regular check-up (43.23%); 28.38% stated dental pain as main reason; 9.17% reported visits due to parental insistence and 19.21% of the subjects stated "other reasons" of visits. Higher CFSS-DS scores were obtained in the group of children who mostly visited a dentist due to pain (29.11 ± 11.06) or parental insistence (29.76 ± 13.43), compared with the children who went for regular check-ups (23.86 ± 7.50) or other non pain-related treatments (26.16 ± 10.43; df = 3; F = 4.697; p = 0.003).

Table 2 shows the arithmetic means and standard deviations of the results of CFSS-DS components in the sample. The following CFSS-DS items had the highest mean values in our sample: 8) the dentist drilling, 12) choking, 13) having to go to the hospital and 3) anesthesia (injections), while the lowest mean value was recorded for item 4) having someone examines your mouth. The girls expressed significantly higher dental anxiety than the boys in relation to anesthesia ($p < 0.01$), strangers ($p < 0.001$), sound of a drill ($p < 0.05$) and choking ($p < 0.05$).

Reliability measures

Cronbach's alpha coefficient of internal consistency in the entire CFSS-DS scale was 0.88. The corrected values of item-total correlations are shown in Table 3. The lowest val-

Table 2

Mean values and standard deviations of Childrens Fear Survey Schedule-Dental Subscale (CFSS-DS) items

Item n = 231	Total mean (SD)	Boys mean (SD)	Girls mean (SD)	p *
1. Dentist	1.70 (0.98)	1.58 (0.93)	1.80 (1.02)	0.092
2. Doctor	1.34 (0.80)	1.24 (0.70)	1.43 (0.87)	0.061
3. Anesthesia (injections)	2.13 (1.20)	1.92 (0.20)	2.33 (1.17)	0.009
4. Having someone examine your mouth	1.21 (0.52)	1.20 (0.51)	1.21 (0.53)	0.925
5. Having to open your mouth	1.24 (0.712)	1.27 (0.78)	1.21 (0.65)	0.525
6. Having a stranger touch you	1.89 (1.10)	1.64 (1.03)	2.12 (1.12)	0.001
7. Having someone look at you	1.33 (0.69)	1.28 (0.68)	1.38 (0.70)	0.323
8. The dentist drilling	2.47 (1.38)	2.29 (1.40)	2.63 (1.34)	0.066
9. The sight of the dentist drill	2.05 (1.31)	1.89 (1.21)	2.20 (1.38)	0.074
10. The sound of the dentist drill	2.04 (1.33)	1.82 (1.24)	2.24 (1.37)	0.015
11. Having dentist put instruments in your mouth	1.84 (1.14)	1.71 (1.03)	1.96 (1.23)	0.097
12. Choking	2.44 (1.42)	2.19 (1.36)	2.67 (1.43)	0.011
13. Having to go to the hospital	2.35 (1.36)	2.23 (1.41)	2.46 (1.30)	0.197
14. People in white uniforms	1.28 (0.78)	1.17 (0.77)	1.28 (0.80)	0.868
15. Having the dentist clean your teeth	1.37 (0.89)	1.41 (0.95)	1.33 (0.82)	0.458

***p* value of independent samples (*t*-test was used to compare means between boys and girls).**

Table 3

Corrected values of item-total correlations

Item	$R_{Item-Total}$	Cronbach's Alpha if Item Deleted
1	0.616	0.865
2	0.507	0.870
3	0.508	0.870
4	0.284	0.877
5	0.569	0.869
6	0.501	0.869
7	0.408	0.874
8	0.661	0.861
9	0.734	0.857
10	0.709	0.858
11	0.658	0.862
12	0.484	0.872
13	0.454	0.874
14	0.476	0.871
15	0.556	0.868

ues were found for item 4 ("someone examines your mouth") and item 7 ("someone is looking at you"). In factor analysis (principal component analysis and Varimax rotation with Kaiser normalization) the 3 groups of factors were extracted with eigen values above 1, which explained 59.11% of variance. The results of analysis are shown in Table 4. The first factor explain 22.91% of variance and a high correlation with CFSS-DS item related to the use of dental drill. The second factor explain 21.23% of variance and correlation with fear of doctors, opening the mouth, being watched by strangers, people in white uniforms. The third factor explain 14.97% of variance. It was related to the CFSS-DS items pertaining to the choking, being touched by the strangers and going to the hospital.

Factor analysis on CFSS-DS in studies of DF in children has been reported in the literature [19]. In samples not selected for high DF, three factors of DF have been indicated: fear of highly invasive dental procedures, fear of less invasive aspects of treatment, and fear of medical aspects and strangers [18, 20, 21]. In a study of highly DF children, a stronger four-factor pattern explaining 60% of the variance was found: fear of general, less invasive aspects of dental treatment, fear of medical aspects, fear of drilling, and fear of strangers (including choking) [22].

The factor structure of Serbian version of CFSS-DS scale revealed 3 groups of factors that explained 59.11% of variance. Not a single factor precedes in explaining the total variance of results, which is similar to the study from Japan [18]. The first factor was related to the usual dental situations (the sight and sound of a drill, and drilling) and explained 22.91% of variance. The factor II explained 21.23% of variance in our study and it correlated with the fear of doctors. This factor was also related to the non-invasive dental procedures and being looked at. The third factor explained 14.97% of variance and it was related to choking, strangers and going to the hospital. The similar three factors have been reported in other populations (the Netherlands [20], Finland [21]). The factor four was reported in the study on Chinese immigrants in Canada, and the additional factor was related to the fear of being looked at or touched [23]. In a Bosnian version of CFSS-DS the fourth factor was also related to unusual situations that did not belong to usual experiences in dental office or hospital surrounding [16].

Table 4

Rotated factorial matrix

Components	Factors 1	2	3
1. Dentist	0.471	0.521	0.170
2. Doctor	0.184	0.762	0.083
3. Anesthesia/injections	0.363	0.377	0.260
4. Having somebody examine your mouth	0.190	0.499	-0.143
5. Having to open your mouth	0.186	0.723	0.259
6. Having a stranger touch you	0.167	0.160	0.762
7. Having somebody look at you	-0.135	0.655	0.429
8. The dentist drilling	0.882	0.150	0.111
9. The sight of the dentist drilling	0.881	0.222	0.149
10. The noise of the dentist drilling	0.854	0.144	0.240
11. Having somebody put instruments in Your mouth	0.644	0.235	0.331
12. Choking	0.224	0.068	0.774
13. Having to go to the hospital	0.230	0.142	0.603
14. People in white uniforms	0.077	0.595	0.345
15. Having the dentist clean your teeth	0.354	0.661	0.067
(%) of variance explained	22.91	21.23	14.97
Eigen value	5.872	1.703	1.292

Correlations

The Pearson's correlation coefficients showed a significant correlation between the CFSS-DS score and gender ($r = 0.163$, $p = 0.013$), daily frequencies of sugar intake ($r = 0.200$, $p = 0.003$), frequency of dental attendance ($r = 0.162$, $p = 0.014$), self-rated oral health ($r = 0.209$, $p = 0.001$) and DMFT ($r = 0.185$, $p = 0.005$).

Discussion

Although the topic clearly deserves close attention as the crucial dental public health issue [13], little is known about dental fear among children in Serbia. As cultural and social norms of behavior can affect the development and expression of children's fear, and as dental care systems can vary considerably across cultures, normative data in each culture are needed. The Serbian version of CFSS-DS had high Cronbach's coefficient of internal consistency ($\alpha = 0.88$), which was in accordance with the findings of the authors from Croatia ($\alpha = 0.83$) [12], Bosnia ($\alpha = 0.86$) [16], Greece ($\alpha = 0.85$) [17], Japan ($\alpha = 0.91$) [18] and Taiwan ($\alpha = 0.94$) [13].

In our study the girls had significantly higher anxiety scores than boys, which is similar to the findings of Majstorovic et al. [24] and Nakai et al. [18]. Children with irregular dental attendance pattern or those who needed dental treatment due to decay also expressed higher DF. In a study of Milsom et al. [25] DF was closely associated with asymptomatic, irregular attendance pattern, a history of extraction and having a dentally anxious parent.

We used a self-report version of CFSS-DS questionnaire in children aged 12, although the parental reports are more often

used with children under 13 years of age [7]. However, all the subjects were able to answer the questions, indicating that the questions were understandable and clear. The limitation of this study is the use of the suitable sample. Therefore, this study should be considered a prospective one, and the results regarding prevalence of high dental fear and DMFT score values could not be generalized to the entire population of Serbian twelve years old schoolchildren. On the other hand, the reliability and validity of Serbian version of CFSS-DS questionnaire could be considered satisfying. The cause and effect dynamics of relationships found between dental anxiety, dental status and visiting patterns need to be further investigated.

Conclusion

Dental health professionals need to understand the dynamic nature of child dental anxiety in order to appreciate hidden feelings and underlying complexity associated with anxious child patients. Anxious children tend to avoid regular dental check-up, more frequently visit dentist due to pain or parental insistence and have more impaired teeth.

The Children Fear Survey Schedule Dental Subscale questionnaire is a reliable and valid psychometric instrument for dental fear evaluation in Serbian children, due to its further application for research of dental fear and numerous associated factors.

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Addendum

Serbian version of CFSS-DS questionnaire

Zaokruži samo jedan broj (1, 2, 3, 4 ili 5) koji označava koliko se plašiš navedenih osoba ili situacija:

1. Koliko se plašiš stomatologa?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
2. Koliko se plašiš lekara?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
3. Koliko se plašiš injekcija / anestezije?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
4. Koliko se plašiš kada ti neko pregleda zube?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
5. Koliko se plašiš kada treba da otvoriš usta kod stomatologa?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
6. Koliko se plašiš kada te dodirne neko nepoznat?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
7. Koliko se plašiš kada neko neprestano gleda u tebe?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
8. Koliko se plašiš kada stomatolog radi sa bušilicom u tvojim ustima?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
9. Koliko se plašiš kada vidiš stomatološku bušilicu?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
10. Koliko se plašiš kada čuješ zvuk stomatološke bušilice?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
11. Koliko se plašiš kada stomatolog unese instrumente u tvoja usta?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
12. Koliko se plašiš gušenja (davljenja)?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
13. Koliko se plašiš kada treba da ideš u bolnicu?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
14. Koliko se plašiš kada vidiš ljude u belim mantilima?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim
15. Koliko se plašiš kada ti stomatolog mašinski pere zube?	1 – ne bojim se	2 – malo se bojim	3 – bojim se prilično	4 – mnogo se bojim	5 – veoma mnogo se bojim

ORIGINAL ARTICLE

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Clinical significance of soluble Fas plasma levels in patients with sepsis

Klinički značaj nivoa rastvorljivog Fas u plazmi kod bolesnika sa sepsom

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Abstract

Background/Aim. The goal of modern clinical and experimental researches in the field of sepsis is to find one or more sensitive parameters that could predict the severity of sepsis and its outcome. In this study we investigated and compared the relationship of initial soluble Fas (sFas) plasma levels as well as Acute Physiology, Age and Chronic Health Evaluation II (APACHE II) score in 58 septic patients with severity and outcome of sepsis. **Methods.** The diagnosis and assessment of disease severity was performed on the same day, based on clinical and laboratory parameters. The blood samples were used for monitoring of laboratory standard parameters necessary for the diagnosis of sepsis, organ dysfunction and assessment of disease severity, as well as for determination of levels of sFas. According to consensus criteria, patients were divided into those with sepsis (n = 16), severe sepsis (n = 30) or septic shock (n = 12), those with (n = 26) and without (n = 32) multiple organ dysfunction syndrome (MODS), and survivors (n = 45) and non-survivors (n = 13). **Results.** Plasma sFas level (9.7 ± 10.1; 0–44.2 U/mL) was elevated in 54.4% of patients. All the patients with septic shock, 76.9% of the patients with MODS and 84.6% patients who died had elevated sFas level. We observed a strong positive correlation between sFas and APACHE II score ($p < 0.001$). The level of sFas was significantly higher in patients with septic shock compared to normotensive patients ($p < 0.001$), patients with MODS compared to those without MODS ($p < 0.001$) and survivors compared to nonsurvivors ($p < 0.01$). **Conclusions.** Our results suggest that initial sFas plasma levels in patients with sepsis correlated with the values of APACHE II score and separated very well the patients with septic shock *versus* the normotensive patients, the patients with and without MODS, and survivors *versus* non-survivors.

Key words:
Sepsis; antigens, cd95; plasma; prognosis.

Apstrakt

Uvod/Cilj. Cilj savremenih kliničkih i eksperimentalnih istraživanja u oblasti sepse je da se pronađe jedan ili više osetljivih parametara koji bi mogli da predvide težinu sepse i njen ishod. Cilj ovog rada bio je ispitivanje i upoređivanje odnosa početnih nivoa rastvorljivog Fas (sFas) u plazmi, kao i *Acute Physiology, Age and Chronic Health Evaluation* (APACHE) II skora sa težinom i ishodom sepse kod 58 bolesnika sa sepsom. **Metode.** Na osnovu kliničkih i laboratorijskih parametara istog dana postavljana je dijagnoza i vršena je procena težine bolesti. Iz uzoraka krvi određivani su standardni laboratorijski parametari potrebni za postavljanje dijagnoze sepse, disfunkcije organa i procenu težine bolesti, a, takođe, izmeren je i nivo sFas u plazmi. Prema konsenzus kriterijumima, bolesnici su podeljeni u grupe sa sepsom (n = 16), teškom sepsom (n = 30) ili septičkim šokom (n = 12), grupe sa (n = 26) i bez (n = 32) sindroma multiorganske disfunkcije (*multiple organ dysfunction syndrome* – MODS), i na preživele (n = 45) i bolesnike sa smrtnim ishodom (n = 13). **Rezultati.** Povišene nivoe sFas u plazmi (9,7 ± 10,1; 0–44.2 U/mL) imalo je 54,4% bolesnika, i to svi bolesnici sa septičkim šokom, 76,9% bolesnika sa MODS i 84,6% bolesnika sa smrtnim ishodom. Utvrđena je značajna pozitivna korelacija nivoa sFas u plazmi i APACHE II skora ($p < 0,001$). Bolesnici sa septičkim šokom imali su značajno više prosečne nivoe sFas u odnosu na normotenzivne bolesnike ($p < 0.001$). Značajno viši nivoi sFas utvrđeni su kod bolesnika sa MODS nego kod bolesnika bez MODS ($p < 0.001$), a značajno niži kod preživelih nego kod bolesnika sa smrtnim ishodom ($p < 0.01$). **Zaključak.** Početni nivoi sFas u plazmi kod bolesnika sa sepsom pozitivno korelišu sa vrednostima APACHE II skora i međusobno se razlikuju između bolesnika sa septičkim šokom i normotenzivnih bolesnika, bolesnika sa i bez MODS, kao i između preživelih i bolesnika sa smrtnim ishodom.

Ključne reči:
sepsa; antigeni, cd95; plazma; prognoza.

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Introduction

Sepsis and its complications, septic shock and multiple organ dysfunction syndrome (MODS) despite the great advances in medical science, is still a very difficult clinical problem. It is unknown whether progress has been made in decreasing their mortality rate [1–4]. The goal of modern clinical and experimental research in the field of sepsis is to find one or more of sensitive parameters that could predict the severity of sepsis and its outcome. For the clinicians it is particularly important that these indicators are defined at the time of hospitalization, and the diagnosis of sepsis, in order to choose the most appropriate method of treatment [5–7]. Although some mediators of immune-inflammatory processes, especially those in the network of cytokines, may be important markers that reflect the degree of severity of sepsis, none of them is absolutely reliable indicator of the outcome of sepsis [8, 9].

Intensive studies of the pathophysiology of sepsis in recent twenty years have resulted in the knowledge that apoptosis is an important mechanism of cell death in animal models of sepsis and endotoxemia. Hypoperfusion and ischemia in experimental sepsis promote apoptosis in the gastrointestinal tract, liver, heart, and brain [10–14]. Accelerated apoptosis in the hematopoietic and lymphoid tissues due to the reduction in the number of mature T- and/or B-lymphocytes leads to the development of immunosuppression in ongoing, and after the sepsis [15–20]. Delaying apoptosis is associated with prolonged functional survival of neutrophils, which is reflected on their respiratory burst activity [21–23]. Apoptosis of endothelial cells in the course of sepsis has increased significantly and is an important mechanism for permeability disorders of microcirculation and the development of organ dysfunctions [24–28].

The findings that plasma of septic patients can significantly inhibit apoptosis of neutrophils in the blood of volunteers, indicates that there is a soluble circulating factors which can modify apoptotic processes [29]. Fas (CD95/APO-1) receptor is the main molecule involved in apoptosis during the sepsis. It is expressed on the surface of many cell types after their activation. Hotchkiss et al. [26] described increased apoptosis in different cells and organs in patients with fatal sepsis and MODS, while Fleck et al. [30] reported significantly higher levels of circulating (soluble) Fas molecule in patients with sepsis and septic shock [26, 30].

Several studies on a small number of patients demonstrated that sFas plasma levels might correlate with MODS and survival [31–33]. However, it remains still difficult to conclude whether circulating concentrations of this molecule are related to the severity of sepsis and outcome of septic patients and whether they would have prognostic value. This was the reason why we investigated and compared the relationship of initial sFas plasma levels in septic patients with severity and outcome of sepsis.

Methods

A total of 58 patients with sepsis were enrolled in this prospective study. The study was approved by the local Ethics Committee. For each patient, we recorded clinical data in a pre-established protocol that included demographic data, sepsis score, underlying diseases, microbiology results, final diagnosis and outcome. The diagnosis and assessment of disease severity was performed on the same day, based on clinical and laboratory parameters.

The blood samples were used for monitoring laboratory standard parameters necessary for the diagnosis of sepsis, organ dysfunction and assessment of disease severity, as well as for determination of levels of sFas. The microbiological results included the results of blood cultures and cultures of any other relevant sample (urine, cerebrospinal fluid, peritoneal fluid, and others).

The diagnosis of sepsis and classification of patients

The diagnosis of sepsis and its complications was made according to the consensus guidelines of the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) [34] and all patients were enrolled into the study within 24 hours since meeting those criteria. The diagnosis of organ dysfunction and MODS was made according to sepsis-related organ failure assessment (SOFA) score [35]. The severity of illness was scored by Acute Physiology, Age and Chronic Health Evaluation II (APACHE II) score [36]. Patients who died in the hospital were included in the group named as non-survivors. All the patients were followed until hospital discharge and classified into different groups on the basis of clinical and laboratory findings: patients with sepsis, severe sepsis or septic shock, patients with or without MODS, and survivors or non-survivors.

Quantification of sFas

Blood samples were obtained from each patient within 24 hours of meeting sepsis criteria, for determination of initial plasma sFas levels. After puncture of one forearm vein, blood was collected into pyrogen free tubes, centrifuged for 10 minutes at 1000 × g aside plasma and immediately frozen and stored at -20ºC. Plasma concentrations of sFas were determined by commercially available ELISA kits (BIOTRACK, Amerslam Pharmacia Biotech, Uppsala, Sweden). In healthy persons the normal range for human plasma sFas was up to 6 U/ml. The levels of sFas were compared between corresponding groups of patients and with APACHE II score.

Statistical analysis

The results are presented as the median values ± SD. To compare two independent samples we used Mann-Whitney *U*-test. Among the sFas and APACHE II score, Pearson's correlation coefficients were calculated to estimate the linear correlation between continuous variables. All *p*-values were two-sided, and a probability of less than 0.05 was considered statistically significant.

Results

The main demografic and clinical caracteristics of a total of 58 septic patients included in the study are given in Table 1.

Table 1

Main demographic and clinical characteristics of 58 septic patients enrolled in the study

Characteristics	Values
Sex (men / women), n (%)	33 (56.9) / 25 (43.1)
Age (years), $\bar{x}$ ± SD (range)	61.3 ± 16.3 (21–81)
APACHE II score, 24 h $\bar{x}$ ± SD	19.5 ± 6.5 (8–36)
Underlying diseases, n (%)	21 (36.2)
Bacteremia, n (%)	24 (41.4)
Sepsis, n (%)	16 (27.6)
Severe sepsis, n (%)	30 (51.7)
Septic shock, n (%)	12 (20.7)
MODS, n (%)	26 (44.8)
Death, n (%)	13 (22.4)
Length of hospital stay (day), $\bar{x}$ ± SD (range)	24.7 ± 8.3 (2–46)

APACHE II – Acute Physiology, Age and Chronic Health Evaluation II;
MODS – Multiple Organ Dysfunction Syndrome; n – number of patients.

A total of 40 (69,0%) patients were treated with antibiotics prior to admission. The etiology of sepsis was demonstrated in 50 (86.2%) of the patients, of whom 24 (41.4%) had positive blood cultures. Gram negative bacteria was the cause of sepsis in a total of 22 (37.8%) of the patients, gram-positive bacteria in 14 (24.1%), a mixed and anaerobic bacterial flora in 14 (24.1%).

Levels of sFas was elevated in 31 (54.4%) of 57 patients (9.7 ± 10.1; 0–44.2 U/mL) whereas the level of sFas was below the limit of detection in 9 (15.8%) of the patients.

All the patients with septic shock had sFas concentrations > 6.0 U/mL, and the highest levels (44.2 and 43.7 U/mL) were observed in two patients with septic shock. In patients with severe sepsis 15 (50.0%) had the level of sFas < 6.0 U/mL, and in 3 (10.0%) of them the concentrations were undetectable. At the same time, the concentrations of sFas < 6.0 U/mL were observed in 10 (62.5%) of the patients with sepsis, and in 5 (31.3%) of them they were undetectable.

Unmeasurable concentrations of sFas were found in one (4.0%) of the patients with MODS, and in 8 (25.0%) of the patients without MODS. sFas concentrations < 6 U/mL were measured in 6 (24.0%) of the patients with MODS and in 20 (62.5%) without MODS.

Unmeasurable sFas levels were more frequently registered in the survivors (9 patients) compared to non-survivors (20.0% *vs* 0%). The values of sFas plasma levels and APACHE II score in different groups of septic patients are given in Table 2.

It was shown that initial plasma concentrations of sFas correlated positively with the APACHE II score ($r = 0.6046$, $p < 0.001$) (Figure 1).

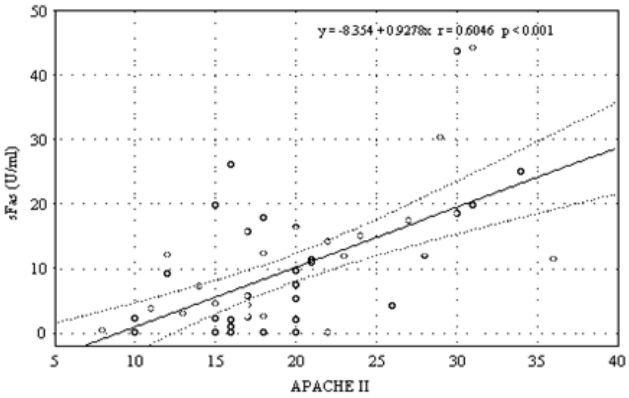

Fig. 1 – Correlation between sFas plasma levels and Acute Physiology, Age and Chronic Health Evaluation II (APACHE II) score in the patients with sepsis.

Discussion

In this study, we investigated the clinical significance of sFas in patients with sepsis and showed that, at the beginning of sepsis, the plasma levels of this biomolecule were increased in the majority of septic patients. These results are

Table 2

The relationship between sFas plasma levels and Acute Physiology, Age and Chronic Health Evaluation (APACHE II) score value in different groups of septic patients

Characteristics of patients	sFas (U/mL), $\bar{x}$ ± SD	APACHE II score, $\bar{x}$ ± SD
Sepsis	4.8 ± 6.8*** [b]	13.8 ± 2.9*** [b]
Severe sepsis	7.5 ± 6.4	18.7 ± 3.3*** [c]
Septic shock	21.1 ± 12.5*** [a]	29.3 ± 4.4*** [a]
With MODS	14.8 ± 11.5***[d]	24.5 ± 6.0*** [d]
Without MODS	5.6 ± 6.4	15.4 ± 3.3
Survivors	8.0 ± 9.2	17.9 ± 5.6
Nonsurvivors	15.8 ± 11.2**[e]	24.9 ± 6.7** [e]

a – compared to the group with severe sepsis; b – compared to the group with septic shock;
c – compared to the group with sepsis; d – compared to the group without Multiple Organ Dysfunction Syndrome (MODS);
e – compared to survivors; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

generally in accordance with few published studies related to this topic [29–33, 37, 38]. Some observed differences may be due to different etiology of sepsis, different timing of sFas monitoring or different numbers of patients included in the studies.

It is known that the Fas molecule could occur as a cell surface receptor as well as a soluble protein. sFas is derived by proteolytic cleavage of membranous Fas or by alternative splicing of membrane-bound Fas [29, 39]. sFas functions as an inhibitor of apoptosis due to the competitive binding to Fas-L, but it also down-regulates the expression of membranous Fas receptor. However, the levels of this biomolecule follow the extent of Fas expression and thus can serve as a marker of apoptosis intensity [40]. Apoptosis has been documented as an important mechanism involved in pathophysiology of septic shock and MODS and therefore determination of sFas levels might be an indirect parameter of cell death in sepsis [29–33, 37, 38].

Many factors, including pro-inflammatory mediators in sepsis increase the expression of Fas as well as Fas-L on different cells such as lymphocytes, cells of innate immunity, vascular endothelial cells and various parenchymatous cells [11–13]. It is interesting that both monocytes and monocyte-derived macrophages release TNF-α and IL-8 following Fas ligation, two important cytokines associated with many events in sepsis, suggesting that the Fas signaling pathway can also lead to proinflammatoty cytokine induction [41]. Apoptosis of endothelial cells, could be an important cause of the development of septic shock and our results showing significantly higher levels of sFas in septic shock patients compared to normotensive septic patients are in agreement with this hypothesis. Huttunen et al. [42] also demonstrated that high sFas concentrations and increased sFas/Fas-L were associated with hypotension and high SOFA score in patients with bacteremia. However, some authors found that the levels of sFas did not correlate with other apoptotic markers in sepsis implicating that these and other endothelial-damaging parameters are better indicators for the development and the severity of septic shock than sFas [31, 33, 38].

It has been documented that sFas may also influence the adaptive T-cell mediated immunity [43, 44] and thus contributing to T-cell anergy in sepsis. However, prolonged survival of neutrophils due to reduced neutrophil apoptosis was accompanied with hyperactivity of these cells and subsequent release of neutrophil elastase upon degranulation, the mechanisms which significantly contribute to the development of organ dysfunction. It has been shown that the values of neutrophil elastase and leukocyte counts correlated with serum sFas levels in patients with sepsis. Moreover, Fas-mediated neutrophil apoptosis was efficiently inhibited by serum sFas from septic patients as efficiently as recombinant sFas [23]. These results demonstrate the involvement of sFas in neutrophil-mediated pathology in sepsis and suggest that sFas may represent a target for new therapeutic approaches to prevent neutrophil hyperactivity and sepsis.

The levels of sFas in septic patients with MODS are of particular importance since MODS is a frequent complication of severe sepsis and septic shock [45]. Although massive inflammatory reaction is considered to be one of the main triggers for the development of MODS, outcome is not necessarily improved by blocking the action of these mediators [46]. Therefore, the downstream effects of these inflammatory mediators, such as the induction of apoptosis, might be pivotal in the pathogenesis of MODS [47–49].

Papathanassoglou et al. [31] examined 35 critically ill patients with MODS and analyzed association between sFas concentrations and severity of organ dysfunction, survival and levels of certain mediators. In these patients, they registered significantly elevated sFas levels during the first two weeks of hospitalization, compared with controls. Moreover, the expression of Fas and FasL on the peripheral blood mononuclear cells in the most severe patient with MODS correlated with the severity of the disease defined according to APACHE II score, and increased along with the increase in the severity of the clinical picture [33]. However, since they did not find a correlation between the concentrations of sFas and expression of Fas on the surface of mononuclear cells, they considered that in critically ill patients some other factors emerge as mediators of apoptosis in MODS [31, 33]. Similar observations about the relationship between sFas and MODS were noticed by other authors, who additionally demonstrated that recovery of patients with organ dysfunction was followed by rapid decrease of sFas concentration [29, 32]. Paunel-Görgülü et al. [23] demonstrated increased sFas levels in patients with sepsis after major trauma at day 5 and day 9 compared with patients with uneventful recovery. At the same time apoptosis of neutrophils was significantly decreased. They also showed a high correlation between sFas and SOFA or MOD scores and thus provided evidence for the clinical significance of this biomolecule as a predictor for the development of sepsis and MODS in traumatized patients. In our study we observed initially elevated concentrations of sFas in almost all the patients with MODS and its levels were significantly higher than in the patients without MODS. Similarly as reported in previous studies, a significant correlation between the concentration of sFas and APACHE II score was obtained.

The prognostic significance of sFas levels for patient survival comes from the study which demonstrated significantly higher concentrations of sFas in patients with sepsis who died and MODS, compared with survivors and from the finding that an increase in these concentrations over the time was inversely associated with the probability of survival [23]. Our results are in agreement with those, since we showed that patients with a fatal outcome had significantly higher levels of sFas in relation to the surviving patients. In addition, we observed unmeasurable concentrations of sFas only in surviving patients, suggesting that sFas concentrations may be a good prognostic parameter for outcome of sepsis. However, there were opposite conclusions resulted from a prospective cohort study in patients with bacteremia, that there were no association between maximum sFas, sFas/Fas-L ratio or minimum Fas-L levels during days 1–4 after positive blood culture had been available with increased death [42].

Conclusion

Initial sFas plasma concentrations in patients with sepsis were elevated in the majority of patients, especially in patients with complications of sepsis and positively correlated with APACHE II score values. These concentrations were significantly higher in patients with septic shock, multiple organ dysfunction syndrome, and those who died. Therefore, determination of sFas in sepsis as a parameter of apoptosis induction together with other immune-inflammatory markers might be of clinical significance.

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Different techniques of vessel reconstruction during kidney transplantation

Različite tehnike rekonstrukcije krvnih sudova prilikom presađivanja bubrega

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Abstract

Background/Aim. Multiple renal arteries (MRAs) represent a surgical challenge by the difficulty in performing anastomoses, bleeding and stenosis. MRAs should be preserved and special attention should be paid to accessory polar arteries. All renal arteries (RAs) must be reconstructed and prepared for safe anastomosis. The paper decribed the different techniques of vessel reconstruction during kidney transplantation including important steps within recovery of organs, preparation and implantation. **Methods.** In a 16-year period (1996–2012) of kidney transplantation in the Military Medical Academy, Belgrade, a total of 310 living donors and 44 human cadaver kidney transplantations were performed, of which 28 (8%) kidneys had two or more RAs. **Results.** All the transplanted kidneys had immediate function. We repaired 20 cases of donor kidneys with 2 arteries, 4 cases with three RAs, one case with 4 RAs, one case with 4 RAs and renal vein reconstruction, one case with 3 arteries and additional polytetrafluoroethylene (PTFE) graft reconstruction, one case with transected renal artery and reconstruction with 5 cm long deceased donor external iliac artery. There were no major complications and graft failure. At a minimum of 1-year follow-up, all the patients showed normal renal function. **Conclusion.** Donor kidney transplantation on a contralateral side and "end-to-end" anastomosis of the renal artery to the internal iliac artery (IIA) is our standard procedure with satisfactory results. Renal artery reconstruction and anastomosis with IIA is a safe and highly efficient procedure and kidneys with MRAs are not contraindicated for transplantation. A surgical team should be fully competent to remove cadaveric abdominal organs to avoid accidental injuries of organs vessels.

Key words:
kidney transplantation; surgical procedures, operative; postoperative period; renal artery; renal blood flow, effective; anastomosis, surgical.

Apstrakt

Uvod/Cilj. Multiple bubrežne arterije (MBA) predstavljaju hirurški izazov zbog anastomoza, krvarenja i stenoza. One se moraju sačuvati, rekonstruisati i pripremiti za sigurnu anastomozu. Posebna pažnja usmerena je na polarnu arteriju. U ovom radu prikazali smo različite tehnike koje koristimo za rekonstrukciju krvnih sudova tokom transplantacije bubrega, uključujući važne korake tokom eksplantacije, preparacije i implantacije. **Metode.** Tokom 16 godina (1996–2012.) transplantacije u Vojnomedicinskoj akademiji u Beogradu, uradili smo 310 živih i 44 kadaverične transplantacije bubrega, a kod 28 (8%) bubrega bile su prisutne dve ili više bubrežnih arterija (BA). **Rezultati.** Svi transplantirani bubrezi profunkcionisali su neposredno posle operacije. Nije bilo većih komplikacija ni gubitka grafta. Rekonstruisali smo 20 bubrega sa dve BA, 4 bubrega sa tri BA, 1 bubreg sa četiri BA, jedan sa 4 arterije i rekonstrukcijom vene, 1 bubreg sa 3 arterije i interpozicijom politetrafluoroetilenskog (PTFE) grafta i jedan bubreg sa odsečenom BA i rekonstrukcijom donorskom spoljašnjom ilijačnom arterijom. U minimalnom praćenju od 1 godine, svi transplantirani bolesnici su imali normalnu funkciju bubrega. **Zaključak.** Transplantacija donorskog bubrega na kontralateralnu stranu recipijenta i terminoterminalna anastomoza bubrežne arterije sa unutrašnjom ilijačnom arterijom je naša standardna procedura kojom ostvarujemo zadovoljavajuće rezultate. Rekonstrukcija bubrežnih arterija i anastomoza sa unutrašnjom ilijačnom arterijom predstavlja sigurnu i efikasnu proceduru za koju bubrezi sa više arterija nisu kontraindikovani za transplantaciju. Hirurški tim za kadaveričnu eksplantaciju organa mora biti u potpunosti spreman da bi se izbegle povrede organa i krvnih sudova.

Ključne reči:
transplantacija bubrega; hirurgija, operativne procedure; postoperativni period; a.renalis; krvni sudovi, prolaznost; anastomoza, hirurška.

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Introduction

Grafts with anatomic variants, especially multiple renal arteries (MRAs), are still challenging problems to the surgeons. The use of grafts with MRAs has been considered relatively contraindicated because of the increased incidence of vascular and urologic complications [1]. Injuries of renal arteries (RAs) during recovery of organs make additional problems in organ transplantation. In this retrospective study, we reviewed variation of surgical technique in reconstruction of renal allograft with multiple and injured arteries.

Methods

In the Military Medical Academy (MMA) in Belgrade during a 16-year experience (1996–2012) in kidney transplantation we performed 354 transplantation, 310 living donor and 44 deceased donor kidney transplantations, out of which 28 (8%) had two or more MRAs and some kind of reconstruction. We repaired 20 donor kidneys with 2 arteries, 4 with 3 RAs, one with 4 RAs, one case with 4 RAs and renal vein reconstruction, 1 with 3 arteries and additional polytetrafluoroethylene (PTFE) graft reconstruction, 1 with transected renal artery and reconstruction with a 5 cm long deceased donor external iliac artery. All kidney transplantation were performed to the iliac fosse and almost all anastomoses performed as "end-to-end" to the IIA using two opposite knots suture technique. Donor's renal veins were anastomosed "end-to-side" to the external iliac vein in all cases.

This technique provides good feasibility in case of short transplant renal artery. "End to side" anastomosis of the renal artery and the external iliac artery was performed in 5 cases. In deceased donor recovery of organs, RAs and veins as well as existing patches dissected from perivascular tissue and branches leaving the renal vessels were ligated. MRAs or accessory polar arteries were located and explanted on a single aortal patch, and anastomosed with external iliac artery in "end-to-side" fashion**.** In case of great distance between the arteries the patch was divided in two for sequential anastomosis. In living donor nephrectomy the most common case of multiple arteries, namely polar artery, were conjoined with renal artery and anastomosed like "end-to-end" to the IIA.

In one difficult case we used the PTFE vascular graft N^{o}V (GORE-TEX,WL Gore & Associates, Newark, Delaware, USA) as a 4 cm interposed graft between the kidney allograft with 3 conjoined short RAs and recipient's short IIA during kidney transplantation. Both anastomoses on graft were in "end-to-end" fashion. PTFE grafts in kidney transplantation are short in length and have a high blood flow. In this case, 3 arteries conjoined in one problematic ostium with tendency to stenosis, and PTFE graft secured these tiny anastomoses and held them open.

Additional problems were iatrogenic injuries of kidney vessels during recovery of organs. There were 5 cases of such injuries successfully resolved. One case of complete section of renal artery during deceased donor recovery of organs resolved with interposition of 5 cm length deceased donor external iliac artery. The second case was the injury of 4 kidney arterial branches (2 mm diameter) during living donor nephrectomy repaired with Prolen 8/0 suture. Small bleeding stoped spontaneously with a little help of Surgicell (Johnson & Johnson).

Other injury cases were resolved with multiple Prolen 6/0 suture. Probably, the most difficult case of reconstruction in our series was a case of deceased donor right kidney with injury of all 4 arterial branches and renal vein. Arterial branches conjoined in one, and the right renal vein were reconstructed with interposition of a 3 cm long part of donor's inferior vena cava.

Routine color duplex sonography was performed after renal transplantation and repeatedly during the early postoperative phase for evaluation of the perfusion. In case of any problems or doubt we perform multisliced computed tomography (MSCT) angiography with immediate reoperation if it is needed.

Results

Various techniques of vessel reconstruction during kidney transplantation are presented in Figures 1–6. Operation time was significantly longer in group with MRAs than with single RA. Mean ischemic time in living donor cases with single artery and without injuries was 59 minutes and in cases with arterial reconstruction these time was prolonged to 102 min ($p < 0.05$), but without any clinical signs of decreased kidney function. Except 4 cases with thrombosis of polar branch and 3 cases with minor intraoperative bleeding, surgery of MRAs was without other major complications. There was no difference in postoperative hospital stay and for the rate of surgical complication comparing these two groups ($p < 0.05$). At a minimum of 1-year follow-up, all the patients showed normal renal function, and color Doppler sonography indicated no thrombus formation or obstruction in the main renal artery. There were no differences in a 5-year graft function comparing these 2 groups ($p < 0.05$). Further follow-up was not done because patients scattered in all directions.

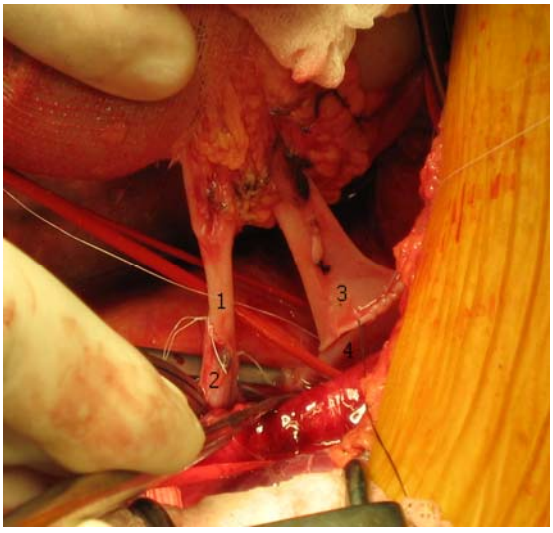

Fig. 1 – "End-to-end" anastomosis of the renal artery and the iliac internal artery. "End-to-side" anastomosis of the renal vein and the iliac external vein (1 – the renal artery, 2 – the iliac internal artery, 3 – the renal vein, 4 – the iliac external vein).

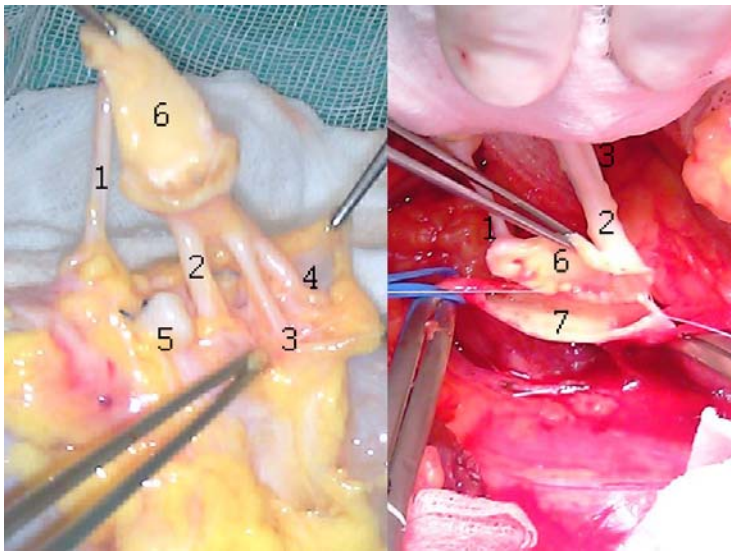

Fig. 2 – Conjoined renal artery and the accessory renal artery, and anastomosis with the IIA (1 – the renal artery, 2 – the accessory renal artery, 3 – the iliac internal artery, 4 – the renal vein, 5 – the iliac external vein, 6 – the iliac external artery, 7 – the gonadal vein).

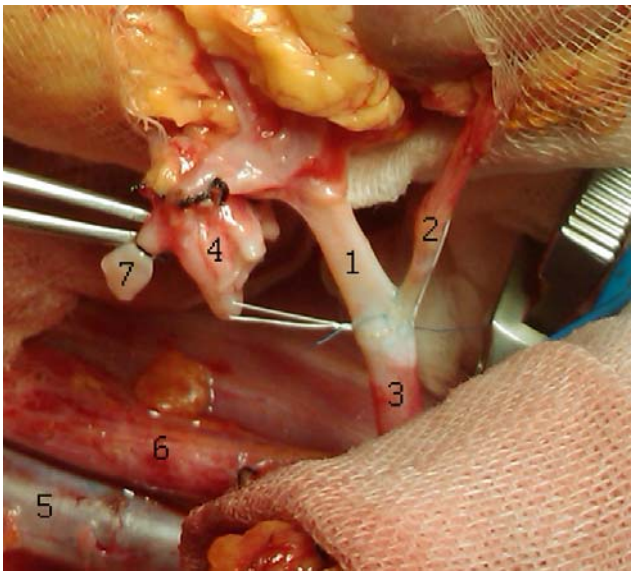

Fig. 3 – "End-to-side" anastomosis of the renal arteries on aortic patch and the external iliac artery (1 – accessory renal artery; 2, 3, 4 – renal artery branches; 5 – the renal vein; 6 – aortic patch; 7 – external iliac artery).

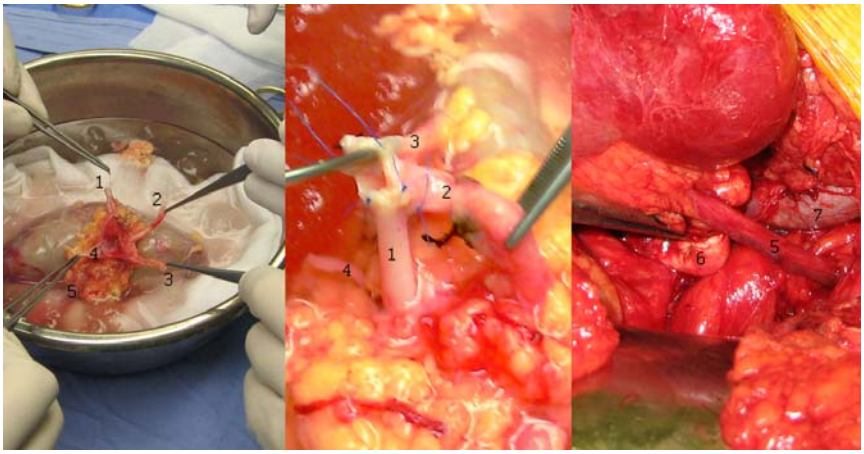

Fig. 4 – Conjoined 3 renal arteries anastomosed with polytetrafluoroethylene (PTFE) graft anastomosed with the iliac internal artery (1 – superior arterial branch, 2 –middle arterial branch, 3 – inferior arterial branch, 4 – the renal vein, 5 – the urether, 6 – PTFE graft, 7 – the iliac external vein).

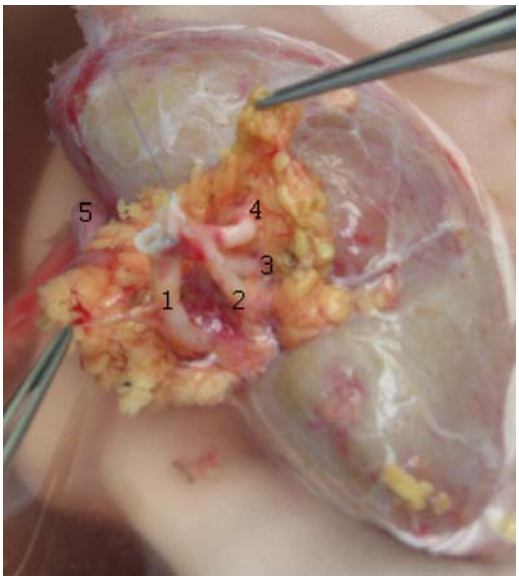

Fig. 5 – Conjoined 4 arterial branches with multiple anastomosis (1, 2, 3, 4 – arterial branches).

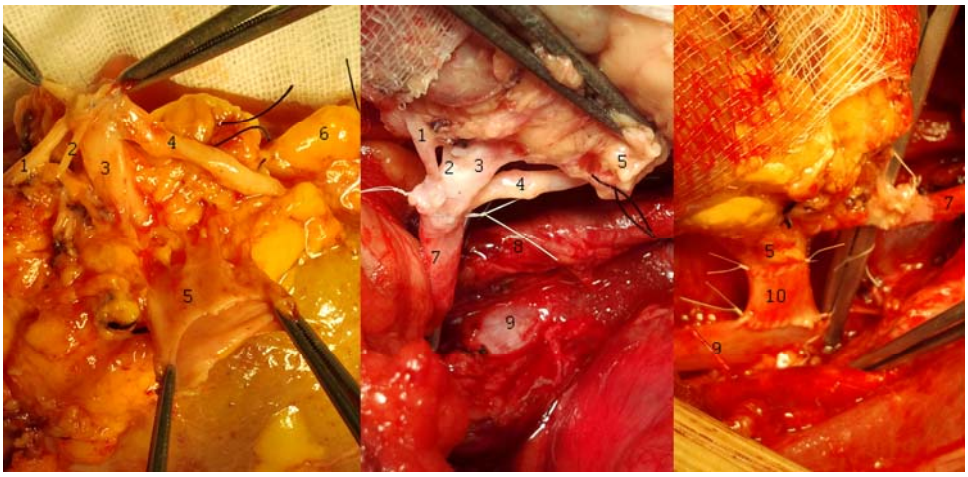

Fig. 6 – (1, 2, 3, 4- arterial branches; 5 – renal vein; 6 – the urether; 7 – the iliac internal artery; 8 – the iliac external artery; 9 – the iliac external vein; 10 – interposition of vena cava inferior conduit).

Discussion

The existence of MRAs has been considered a relative contraindication because of the incidence of vascular and urologic complications [1]. In our patient population of kidney transplantation we analyzed the outcomes of kidneys recipients with MRAs or repaired RAs in comparison to grafts with single arteries, and found no difference between these two groups [2]. Recently, studies have also shown that grafts with MRAs *vs* grafts with single artery present no differences of surgical complications and outcome [3, 4]. Respectable authors suggested some different and alternative techniques of vessels reconstruction [5]. Alternatively, a polar artery should be reconstructed to the renal artery like "end-to-side" anastomosis, but with a high risk of vascular obliteration of both vessels. If anastomosis of small polar arteries seems to be very difficult, it can be perfused and closed with a risk for distal necrosis of the ureter and small area of infarction in lower part of the kidney. In our series almost all renal anastomosis made as "end-to-end" to internal iliac artery (IIA). We found that these anastomoses offer good position for the kidney because of extended length of artery and kidney can be placed in the best position in iliac bed. Also, living donor kidneys a procured without an aortal patch for transplantation and IIA adds length to the RA. The length of the arteries is even more important than the existence of patches. Unfortunately, incongruity of the lumen often appears making anastomosis difficult and complex and the IIA bears the risk of kinking, leading to vascular occlusion [5]. In addition, the IIA does not provide possibilities for sequential anastomosis in case of MRAs as common and external iliac arteries, but conjoined MRA can be efficiently anastomosed as "end-to-end" with IIA. The external iliac artery provides good opportunity upon the existence of accessory renal or polar arteries without a common patch, and used for "end-to-side" anastomosis [5, 6]. The polar artery also can be anastomosed with the epigastric inferior artery [6]. We favor the conjoined arterial anastomosis technique in case of MRAs rather than separate or sequential anastomoses. The distal part of the IIA is ligated. In very rare cases impaired blood flow may have im-

pact on penile vascularity and erectile function in particular after second kidney transplantation to the contralateral side with both IIA ligated [5]. In our series this complication prevention was by preserving branches of IIA and ligature above bifurcation. With that, collateral circulation between branches is allowed. There was no complication as erectile dysfunction in our series (one case of bilateral kidney transplantation on IIA in a young male). Arteriosclerosic plaques were frequently found in donor organs, and mild occurrence had no effect. Along with severe arteriosclerosis the risk of intimal injury and vascular occlusion is augmented. Intimal desquamation within the patch or proximal artery requires shortening of the artery or fixation of the intimal flap to avoid dissection and vascular occlusion. Patches with severe arteriosclerosis and intimal lesions should rather be removed. Gentle dissections of the recipient's vessels with ligature of the lymphatic vessels are performed to avoid lympoceles. Injuries of donor organ vessels must be minimized, especially in deceased donor procurement. In living donor nephrectomy, in our hospital senior surgeons with wide experience in techniques rarely inflict injuries. But, in deceased donor recovery of organs, younger specialists and residents with less experience, frequently make mistakes. An abdominal team should be fully competent to remove kidneys [7]. Recently, our team of vascular surgeons and urologists has tried to avoid accidental injuries of kidneys, especially kidney's vessels. There are disagreements among transplant surgeons which side and which vessels of recipient's pelvis to use in receiving the kidney. Some respectable authors prefer placing the donor kidney in recipient's contralateral side (left kidney on the right side) to ensure the renal pelvis and ureter are anterior if future reoperation is required. In case of doubt, these authors recommended right side, because of wider choice of vessels for reconstruction [8]. Right side as better choice is recommended because right vessels are "more horizontal" and easier to use in anastomoses [9]. We recommend contralateral side in cases of RA and IIA anastomosis. As mentioned above, anterior position of the renal pelvis and the ureter ensure better approach if reoperation is required. Additional reasons are better positioning of the kidney in iliac bed, because of longer arteries "loop" and better mobility of the kidney. In these cases there is no pressure on renal vein by renal artery with probably vein congestion as in cases of "end-to-side" renal artery anastomoses with external iliac artery in contralateral position of kidney. If we plan anastomoses on external iliac artery, it is better to choose ipsilateral kidney (right kidney to right side). In this surgically easier case, there is no compression of the renal artery on the renal vein and the renal pelvis and the ureter are in anterior position, but with a reduced mobility of the kidney. If we could choose the side and the kidney, the best option would be transplantation of the left kidney to the right side, with "end-to-end" anastomosis of RA and IIA. The left renal vein is longer than the right one which makes vein anastomosis much easier. Right iliac vessels are slightly more accessible than left sided. "End-to-end" anastomosis of the kidney and IIA ensure good mobility and the best kidney positioning, with no pressure on the renal vein and excellent vein outflow. Rare incongruity of RA and IIA could be resolved by spatulation of smaller artery and meticulous surgical technique.

Conclusion

Donor organs with multiple renal arteries, missing aortic patches or severe arteriosclerosis challenge the technical skills of each transplant surgeon. Transplantation of donor kidney on contralateral side and "end to end" anastomosis of the renal artery to the internal iliac artery is our standard procedure with satisfactory results. Conjoined multiple renal arteries and anastomosis with interval iliac artery is safe and highly efficient, instead of increasing technical difficulties. A surgical team should be fully competent to remove abdominal organs to avoid accidental injuries of organs vessels.

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GENERAL REVIEW

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Multidisciplinary approach to nitric oxide signaling: Focus on the gastrointestinal and the central nervous system

Multidisciplinarni pristup signalizaciji posredovanoj azot-monoksidom: gastrointestinalni i centralni nervni sistem u fokusu

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Ključne reči:
azot monoksid; transmisija, sinaptička; duodenum; epilepsija; homocistein.

Introduction

Nitric oxide (NO), with close historical ties to cardiovascular physiology, is an important endogenous mediator, the most potent natural vasorelaxant known, involved in many biological functions [1]. NO acts as an intra/intercellular signalling molecule and is a mediator in almost all organ systems [2]. NO is known to occur in many cells types, including vascular endothelial cells, neurons, and epithelial cells [3]. The transmitter of nonadrenergic, noncholinergic (NANC) inhibitory neurons has been the subject of hundreds of investigations over the past 3 decades. Recent evidence suggests that NO may serve as a NANC inhibitory signaling molecule in the gastrointestinal (GI) tract. NO serves as the primary enteric inhibitory neurotransmitter in GI muscles, and nitrergic neurons regulate gut tone, phasic contractile amplitude and frequency, and inhibitory reflexes [4].

In the central nervous system (CNS) NO is involved in some major processes such as memory through long-term potentiation (LTP) and learning [5]. This gasotransmitter also contributes to a pathogenesis of epilepsy. The role of NO in the generation of epilepsy is contradictory since there is evidence of its proconvulsive and anticonvulsive effects [6]. In this review, we will discuss about the possible role of NO as neurotransmitter in the GI and CNS, with focus on the contribution of NO-mediated signaling pathways in the GI motility and CNS excitability.

Physiological functions of nitric oxide

In 1978, Furchgott [7], discovered a substance in endothelial cells that relaxes blood vessels, calling it endothelium-derived relaxing factor (EDRF), then he had worked out EDRF's nature and mechanism of action. It has been identified to be NO, an important compound in many aspects in both physiological and pathological conditions. NO is biosynthesied from L-arginine by the NO synthase. NO synthesis and release is mediated through the activation of NO synthase (3 isoforms of NO synthase, NOS enzymes) by an elevation of cytoplasmic Ca^{2+}, conversion of L-arginine to NO and L-citrulline, NO diffuses passively into the extracellular fluid. Although NO is made by a cytosolic enzyme, it is a highly permeable molecule that can easily diffuse out of the cell that makes it [8]. NO is a tasteless, colourless gas. It is rapidly absorbed *via* the pulmonary vasculature directly into the bloodstream. The mechanism of NO action is not fully understood, but many of its actions are mediated by the activation of guanylate cyclase, which results in an increase in the concentration of cyclic guanosine 3',5'-monophosphatate (GMP) in smooth muscle. The enhanced production of cyclic GMP that results from activation of guanylate cyclase may result in: activation of GMP-dependent protein kinase or direct actions of cyclic GMP on ion channels or other second messenger systems may also be activated by NO (non-cGMP dependent NO effects). NO displaces nitrogen and increase the volume of

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gas in body cavities such as the middle ear, sinuses, pleural space, and GI tract [9]. NO and NO donors (e.g., sodium nitroprusside) cause relaxation of vascular smooth muscle through the accumulation of cyclic GMP or through the direct activation of K^+ channels. Several studies have suggested that NO might decrease the intracellular Ca^{2+} level or reduce the Ca^{2+} sensitivity of the contractile elements, which results in smooth muscle relaxation. Furthermore, the relaxing action of NO has been indicated indirectly by inhibiting the release of the neurotransmitters acetylcholine and substance P [10].

We shall list a number of physiological functions of NO and we can distinguish the following: it is involved in the regulation of blood flow, maintenance of vascular tone, control of platelet aggregation, modulation of the activity of the mastocytes, as a neurotransmitter in the CNS and peripheral nervous system (NANC, neurons), in the nervous control of the cerebral blood flow and in the neuroendocrine regulation or synaptic plasticity [11–15].

On the other hand, NO plays a role in memory formation. NO is a retrograde messenger at N-methyl-D-aspartate (NMDA) receptors mediated synapses. Inhibitors of NO synthesis in any case, block initiation of LTP which involves an NMDA receptor mediated intracellular cascade finishing in lasting modulation of synaptic morphology [16, 17]. Inversely proportional relationship between NO and glutamate, is also described. *In vivo* and *in vitro* studies with NO donors, NO synthase inhibitors and glutamate receptor antagonists have shown that NO increases the release of glutamate in several regions of the brain (hippocampus, striatum, hypothalamus and locus ceruleus) and spinal cord [18, 19].

To maintain the postsynaptic activation, a retrograde communication with the presynaptic component must exist. It has been suggested that the retrograde NO molecule triggers the release of glutamate *via* cyclic GMP-dependent way. The effects of NO on glutamate release depends on the level of NO. Thus, when the concentration is low, NO reduces the release of glutamate in spite of elevated levels of cyclic GMP. But when NO increases the levels of cyclic GMP, an inhibitory effect on glutamate release reverses, indicating that cyclic GMP showed biphasic effects [20]. Morphine-induced impairment of memory formation can be prevented by NO donor [21].

Nitric oxide in gastrointestinal smooth muscle

Opinions concerned with GI system is polarised as to whether or not NO causes nausea and vomiting. It can certainly increase intestinal and middle ear volumes, which may in turn lead to nausea [9]. On the other hand, ghrelin, a gastric peptide, which possesses orexigenic effects, is the endogenous ligand for the growth hormone with stimulating effects on growth hormone and GI motility. Gaskin et al. [22], demonstrated that a sub-threshold dose of N(omega)-nitro-L-arginine methyl ester (L-NAME) significantly blocked the ghrelin-induced increase in food intake. Ghrelin increased NO synthase levels in the hypothalamus-supporting the hypothesis that ghrelin's effects are NO dependent.

In GI smooth muscle, NO or NO donors evoke different responses, including relaxing, contractile effects, relaxations followed by contractions or contractions followed by relaxations, which depend on the compound, tissue and species [23–25]. The nerves whose transmitter function depends on the NO release are called "nitrergic" and such nerves are recognized to play major roles in the control of smooth muscle tone and motility [26]. NO is likely an inhibitory neurotransmitter in the human jejunal longitudinal smooth muscle, acting *via* mechanism mediated by guanylyl cyclase [27]. It was suggested that basal release of NO caused an oscillatory patterns of electrical and mechanical activities. NO is a vasodilator and mediates gastric blood flow and it is responsible for helping to maintain the integrity of the gastric epithelium and the mucus barrier [28].

There are numerous data supporting the hypothesis that NO plays a pivotal role in NANC relaxation or inhibitory junction potential associated with electrical field stimulation or nicotinic agonists in the duodenum, jejunum, and ileum from a variety of mammals, including the human and rat [27, 29]. It was reported that the ability of the nitrergic neurotransmitter to induce relaxation of the rat gastric fundus was influenced by the mechanism used to induce tone, and sarcoplasmic/endoplasmic reticulum Ca^{+2} ATPase appeared to play a role in nitrergic relaxation [30].

Giant migrating contractions of the rat colon, possibly mediated by neuronal release of acetylcholine, appear to be partially suppressed by constitutive release of NO [31]. Nitrergic innervation also contributes to the regulation of the smooth muscle tone in the rat rectum [32].

At the GI level, 2 constitutively expressed isoforms, namely endothelial NO synthase (eNOS) and neuronal NO synthase (nNOS), are expressed basally at the vascular endothelium and the enteric nervous system of the GI tract [33].

However, NO shows a dual behavior: at physiological concentrations, released through the constitutive synthase (cNOS), it regulates house-keeping functions and is responsible for production of NO in physiological context. In physiological conditions, NO acts as an endogenous mediator modulating both, the repairing and integrity of the tissues, and exhibits gastroprotective properties against different types of aggressive agents. In contrast, its overproduction by the inducible isoenzyme (iNOS) exhibits cytotoxic activity because interacting with reactive species producing peroxinitrites and other compounds, which are highly damaging for the tissues. iNOS produces NO in pathophysiological circumstances. High concentrations of NO are related to numerous pathological processes of GI tract including peptic ulcer, chronic gastritis, GI cancer, bacterial gastroenteritis, and celiac or chronic inflammatory bowel diseases. Indeed the adverse action of cigarette smoking on ulcer healing is largely dependent on the deficiency of cNOS and a subsequent depression of gastric blood flow and angiogenesis. To this end, NO may act as a crucial signal to promote endothelial cell differentiation into vascular tubes [34].

Endogenous NO also contributes to the inhibition of acid secretion in the stomach. NO is implicated in mechanisms

maintaining the integrity of the gastric epithelium. In this connection, it regulates gastric blood flow and directly stimulates gastric mucus secretion by activating soluble guanylate cyclase [35]. A blockade of NO production resulted in an impairment of the vascular response and the subsequent alkaline flux in the lumen. This would impair the restitution process. Intragastric administration of hydrochloric acid stimulated a subpopulation of nitrergic, but not cholinergic, myenteric plexus neurons, which might play a role in secretion, vasodilatation, and muscle relaxation [36]. Expression of nNOS in parietal cells suggests a participation of endogenous NO in the regulation of gastric acid secretion [37]. Helicobacter pylori increased pepsinogen secretion from dispersed human peptic cells through a Ca^{2+}- and NO-mediated intracellular pathway [38].

In the GI tract NO participates in the modulation of the smooth musculature tone, such as the regulation of intestinal peristaltism, gastric emptying and antral motor activity. It also regulates acid and gastric mucus secretion, alkaline production, and is involved in the maintenance of mucosal blood flow. NO biology can influence nutrition and be nutritionally modulated to affect mammalian (patho)physiology. NO as modulator of feeding behavior and mediator of GI homeostasis could be used for supplementation as a therapeutic modality for preserving GI health [39]. Presumed mechanisms of relaxation of NO in GIT tract are:

– Cyclic GMP seems to be a key substance for nitrergic inhibitory responses in the most mammalian GI tracts, including that of humans. Cyclic GMP-dependent reduction of cellular free Ca^{2+} without changing the membrane potential;

– Reduce the Ca^{2+} sensitivity of the contractile element [40];

– Cyclic GMP-dependent opening of apamin-sensitive K^+ channels or other types of ion channels to produce hyperpolarization and relaxation;

– Indirectly by inhibiting the release of the neurotransmitters acetylcholine and substance P [41];

– Cyclic GMP-independent mechanisms, such as actions of NO on ion channels involved in muscle contractility, either directly or *via* membrane hyperpolarization.

Stimulation of these nerves, elicits hyperpolarization of postjunctional smooth muscle membranes referred to as inhibitory junction potentials and relaxation [42–44]. These neurons mediate the majority of inhibitory responses in the GI tract and regulate many important physiological reflexes, such as relaxation of the lower esophageal sphincter after swallowing, receptive relaxation of the proximal stomach during eating, and descending inhibition in response to distension [45].

Clear species variations in the functioning of nitrergic nerves were also seen in the distal colon [46].

L-NAME has been used by many investigators to determine the role of endogenous NO in various physiological and patophysiological conditions. In our experiments L-NAME shows increasing of the resting tone, amplitude and frequency of the contractions of the isolated duodenal segments. It has been shown that L-arginine reversed the action of L-NAME. These data confirm the evidence for the participation of the L-arginine-NO pathway in the relaxation of isolated rat duodenum [47].

Nitric oxide signaling modulation by homocysteine in the gastrointestinal system

We wanted to examine the effects of D,L-homocysteine thiolactone (HCT) on duodenal motility, proved to have a prokinetic effect. Homocysteine is a sulfhydryl amino acid derived from catabolism of methionine. As GI smooth musculature is similar to blood vessel's muscles, we investigated how elevated homocysteine levels affect NO mediated neurotransmission in the gut. HCT leads to immediate increase in tone, amplitude and frequency of spontaneous movements of isolated rat duodenum [47]. In the presence of L-NAME, treatment with HCT caused significant increase of resting tone, amplitude and the frequency of the contractions. These results suggest that mechanism of acting of HCT on the duodenal segments contractions are based on the modulation of nitrergic neurotransmission in the gut [47]. We found that NANC relaxations induced by low frequencies of electrical field stimulation were significantly changed in duodenal preparations obtained from duodenal segments treated with HCT. These findings suggest that homocysteine causes an impotrant impairment on NANC innervation of the rat duodenum [47]. Our results show that HCT increases the motility of isolated rat duodenum. They are consistent with the results of Park et al. [48] which suggest that sulfur-containing amino acids like D,L-homocysteine potentiates depolarisation of murine proximal colon cells. These effects include increasing the amplitude and frequency of spontaneous contractions of murine colonic stripes.

Choe et al. [49] in their study investigated the effects of methionine on the contractile activity of human colon smooth muscle *in vitro*. Methionine is a sulfur containing amino acid that is transformed into homocysteine during biometabolism. Their results indicate that methionine increases the amplitude contractions of colonic muscle strips, which supports our results [49].

Nitric oxide-mediated neurotransmission in the central nervous system: focus on hyperexcitability and epileptogenesis

Disorders of the CNS are one of the primary categories in health care system funds expenditures [50, 51]. With the prevalence of 1–2% in the general world population, epilepsy is among the leading neurological disorders [52]. Epilepsy is characterized by paroxysmal occurrence of motor seizures in behavior (different and specific semiology: from tonic-clonic *via* myoclonic to atonic seizurs) and ictal activity in electroencephalography (EEG) (different forms of spiking activity from isolated spikes to generalized bursts/trains of spikes or spike-and-wave discharges depending on the type of epileptic acitivity). Imbalance between excitatory and inhibitory phenomena within the CNS is thought to be the primary mechanisms involved in the process of epileptogenesis [53]. However, many other mechanisms are recognized as potential

parts of epileptogenesis mosaic. Recently, inflammation, as well as gasotransmitters-mediated signaling processes have been identified as one of the important pathways in modulation of epileptic activity [54–57]. Our understanding of the process of epileptogenesis relies on adequate experimental models of epileptic disorder. Therefore, experimental models are unequivocal tools for investigations in epileptogenesis. It should be pointed out that no single model system could be useful for all types of epilepsy [58]. Having that in mind, a number of very useful experimental models of epilepsy were developed up to now. Recently, Stanojlović et al. [59] showed that acute administration of HCT to adult rats significantly alters neuronal circuits, leading to epileptogenic activity in the EEG with characteristic spikes–and-wave discharges (SWD), and convulsive episodes (manifested through 5-grade descriptive rating scale addapted by Stanojlović et al. [59]) in animal behavior. HCT-induced seizures are accepted as a suitable model of generalized epileptic seizures in which coexistence of convulsive and absence-like seizures were proven [60, 61]. This is one of the advantages of this particular epilepsy model which allowed reliable investigations of variety seizure activity modifications, like paradoxical sleep deprivation [62].

NO displays pleiotropic effects in the CNS. All the three NO synthase isoforms have been expressed in the brain. Neurons produce NO mostly by activation of nNOS [63]. Constitutive isoforms of NO synthase are responsible for the synthesis of physiologically vital amounts of NO [64], while inducible NO synthase (iNOS) produces high amounts of NO lasting hours or days [65]. nNOS is found to be mostly expressed in the hippocampus, cerebral cortex, corpus striatum and cerebellum, as well as in some cells of the autonomic nervous system [66]. iNOS has been found to be a major contributor to initiation/exacerbation of the CNS inflammatory/degenerative conditions through the production of excessive NO [67]. iNOS is reported to be highly expressed in brains of humans with epilepsy. In some spontaneously epileptic mouse overexpression of iNOS is also found [68, 69].

The role of NO-mediated neurotransmission in the process of epileptogenesis is highly unpredictable and contradictor in the existing scientific literature, since numerous evidences exists for both its proconvulsive and anticonvulsive activity [6]. The results on the role of NO in epileptogenesis have been recognized to depend, among other factors, on the source of NO production [70–72]. Moreover, different NOS modulation upon generalized seizures along the anterioposterior axis of the brain have been recently proved, showing dependence on vicinity of original epileptic focus [73]. Recently, we showed that the systemic administration of increasing doses of L-arginine in a dose–dependent manner significantly decreased seizure incidence and the number of seizure episodes and the prolonged latency time to the first seizure elicited by the convulsive dose of HCT [74]. On the other hand, pretreatment with L-NAME, in a dose–dependent manner, increased seizure incidence and severity and shortened latency time to the first seizure following the injection with the subconvulsive dose of HCT. In the same study, L-arginine decreased, while L-NAME increased the median number of SWD *per* rat, while duration of individual SWD was not modified. These results showed the functional involvement of NO in the HCT-induced epileptic activity [74].

Further studies have been undertaken in order to investigate the involvement of nNOS in HCT – induced seizures. With this aim, pharmacological inhibition of nNOS by 7-nitroindazole has been applied [75]. Systemic administration of 7-nitroindazole showed tendency to increase seizure incidence, decrease latency time to first seizure, increase number of seizure episodes *per* rat and increase severity of seizures induced by HCT in rats in a dose dependet manner. Therefore, these results were congruent with those obtained using non-selective NOS inhibition. Contribution of iNOS-derived NO in the process of epileptogenesis elicited by HCT was recently demonstrated in this model of seizures using aminoguanidine, as selective iNOS inhibitor [57]. The results of that study showed that pretreatment with aminoguanidine (applied in the three doses) increased convulsive properties, i.e. seizure incidence, the number of seizure episodes *per* rat and severity of HCT-induced seizures, as well as the number and duration of SWDs in EEG. Also, aminoguanidine decreased the latency time to the first seizure episode induced by HCT in the same dose-dependent manner. Quantitative analysis of ictal activity in EEG showed congruent results with those from behavioral assessment in that study. Namely, aminoguanidine pretreatment significantly increased the number and duration of SWD induced by HCT in that study [57].

The interaction between NO and HCT is supposed to involve NMDA receptor complex, as well as interaction of both NO and HCT with glutamate and gamma-aminobutyric acid. These relationships, discussed in details in Hrnčić et al. [74], could contribute to obtained results on NO effects in HCT-induced seizures.

Conclusion

Nitric oxide in low concentration derived from constitutive nitric oxide synthase is cytoprotective by directly acting as an inducer of defense responses in the gastrointestinal tract. However, higher concentrations of nitric oxide from inducible nitric oxide synthase exhibit toxic effects through nitrosative and oxidative stress. These findings suggest that the cholinergic and nonadrenergic noncholinergic inhibitory nerves play important roles in regulating contraction and relaxation of the gut, and nitric oxide plays an important role in nonadrenergic noncholinergic inhibitory nerves of the digestive tract. In addition, a decrease of the action of cholinergic nerves and an increase of the action of nonadrenergic noncholinergic inhibitory nerves by nitric oxide may be largely related to the low pressure in some part of the gut. However, the findings related to the nitrergic innervation may provide us a new way of understanding gastrointestinal tract physiology and pathophysiology and might result in the development of new therapies of gastrointestinal diseases.

We have shown that nitric oxide causes anticonvulsive effects in the experimental model of epilepsy induced by D, L-homocysteine thiolactone.

Further studies are needed to elucidate all nitric oxide effects on the central nervous system, since new clues for dissolving the role of nitric oxide in the central nervous system and especially its disorders, like epilepsy, is of significant importance from physiological, pathophysiological and pharmacological viewpoint.

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Current concepts of radioimmonotherapy for lymphoma

Aktuelni koncepti radioimunoterapije za limfom

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Abstract

Key words:
lymphoma, non-hodgkin; radioimmunotherapy; clinical protocols; remission induction; treatment outcome.

Apstrakt

Ključne reči:
limfom, nehodžkinov; radioimunoterapija; protokoli, klinički; remisija, indukcija; lečenje, ishod.

Introduction

Non-Hodgkin's lymphoma (NHL) represents a heterogeneous group of lymphocyte malignancies. In general, despite being sensitive to radiation therapy and chemotherapy, low grade NHL remains an incurable disease. In most cases, patients respond to the initial treatment, but half of those relapse within 10 years. Relapsed patients are retreated but there is often a shorter duration of each remission. In addition, some patients become refractory to the treatment regimen [1–3]. A great deal of effort has been made to improve the treatment of NHL to achieve a longer duration of response.

However, despite new chemotherapeutic agents that have been introduced and established into the clinical treatment during the last several decades, overall survival for NHL patients has not essentially changed over the past 40 years [4]. In the 1970's, Köhler and Milstein [5] developed the technique for large-scale production of monoclonal antibodies making possible the anti-tumor therapy using monoclonal antibodies. The first B-cell specific antibody B1 was identified in 1981 by Nadler et al. [6]. Subsequent investigations demonstrated the therapeutic potential of anti-CD 20 immunoglobulins [7, 8]. In the late 1990's, another anti-CD 20 monoclonal antibody, rituximab, was developed and subsequently approved by the United States Food and Drug Administration (FDA) for treatment of patients with low grade B-cell lymphoma who have relapsed. Rituximab is a chimeric monoclonal antibody which is derived from the murine antibody, ibritumomab, and is marketed as Rituxan®/Mab Thera®. Initially, rituximab was used in combination with standard chemotherapeutic options and as a single agent for treatment of elderly NHL patients. The results showed improvement in outcome, overall response rate and duration of remissions [9–12].

Recent advances in molecular medicine have provided a novel approach to the treatment of NHL. During the early decades of the 21st century, therapy with radiolabeled monoclonal antibodies became a treatment option. Radioimmunotherapy (RIT) is based on the concept of conjugation of a radionuclide to a monoclonal antibody that would deliver localized radiation to an antigen that is expressed on tumor cells. RIT targets the cytotoxic radiation to the tumor cells with minimal irradiation of normal cells. RIT the most appropriate for treatment of multiple tumor sites that cannot be readily excised surgically or irradiated using external beam radiation or brachytherapy.

The US FDA has approved 2 RIT protocols: the first in 2002, Zevalin® – rituximab and ^{90}Y-ibritumomab, and the second in 2003, or Bexxar® – tositumomab and ^{131}I-tositumomab. The primary indication for these agents was treatment of relapsed, refractory, or transformed CD20+B-cell NHL. Treatment with either agent is based on monoclonal antibodies specific for the CD20 surface antigen found on normal B-cells and more than 90% of B-cell NHL. In fact, CD20 is an epitope (antigen) expressed on pre-B and mature B-cells, but not on early precursors, stem cells or

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plasma cells [13–17]. This represents an excellent target as B-cell precursors and plasma cells are not targeted by anti-CD20 antibodies [18]. Moreover, the bound antibody is not shed and is minimally internalized resulting in antibody-dependent and complement-mediated cytotoxicity as well as apoptosis [19–21].

Both RIT agents target the CD surface antigen. Zevalin® utilizes ^{90}Y, and is a pure β emitter with a 2.7 days of half-life, energy of 2.3 MeV, and maximal tissue penetration of 5 mm. Since there are no gamma emissions in the decay spectrum of this isotope, it is poorly visualized on gamma cameras. Therefore, ^{111}In was used until recently in the US for pretreatment imaging and evaluation of biodistribution. In contrast, Bexxar®, is a directly radiohalogenated β and γ emitter; with a γ emission spike of 0.36 MeV, β energy emission of 0.6 MeV. Thus, it can be readily visualized on a gamma camera. Furthermore, Bexxar® involves a covalent bond between I-131 and monoclonal antibody, while Zevalin® uses chelation complex thus providing non-covalent linkage for the radiometal (Table 1) [22].

Zevalin® increases the efficacy of anti-CD20 antibody therapy due to the conjugation of monoclonal antibody with a beta-emitting radionuclide (^{90}Y) [15, 23, 24]. This specific treatment is based on direct toxicity delivered to the cell bound by the antibody and to the neighboring tumor cells *via* cross-fire effect. Beta particles thus kill cells in the nearby environment which are either not accessible to the monoclonal antibody, or may not express CD20, and/or that may be resistant to the immune-mediated or direct apoptotic effects of the unlabeled antibody [25].

Two products, Bexxar® and Zevalin® had been approved in the US and Canada for the treatment of refractory low grade and transformed intermediate grade NHL (follicular lymphoma). Zevalin® regimen is available only in Europe. Both products involve infusions of both unlabeled (cold) antibody and a radiolabeled (hot) antibody: Zevalin® consists of rituximab and ^{90}Y-ibritumomab tiuxetan. Rituximab is non-labeled component of Zevalin®. Ibritumomab is a murine monoclonal antibody component of Zevalin® – labeled with ^{90}Y. Bexxar® consists of tositumomab and ^{131}I-tositumomab. Tositumomab is a murine monoclonal antibody component of Bexxar® – labeled with ^{131}I. In fact, both regimens include the combination of cold, unlabeled antibody infusions followed by infusion of radiolabeled antibody (Zevalin® includes ^{90}Y-labeled anti-CD20 antibody ibritumomab; while Bexxar® includes the ^{131}I-labeled anti-CD20 antibody tositumomab) [18].

Nevertheless, despite the excellent clinical responses, the utilization of these products has been disappointing. Consequently in early 2014, Bexxar® was withdrawn from the market by the manufacturer and is no longer available.

Table 1

Chemical and physical characteristics of Zevalin® and Bexxar® [22]

Characteristics	^{90}Y Ibritumomab Tiuxetan (Zevalin®)	^{131}I Tositumomab (Bexxar®)
Epitope	CD20	CD20
Antibody used for labeling	Ibritumomab-murine Ab	Tositumomab-murine Ab
Linking molecule	Tiuxetan-chelation complex (noncovalent bond)	None (direct halogenization) (covalent bond)
Pretreatment imaging	Optional one	Requested, three
Cold antibody	Chimeric rituximab	Murine tositumomab
Imaging agent	^{111}In ibritumomab tiuxetan	^{131}I tositumomab
Aim of pretreatment imaging	Biodistribution	Dose estimation

Eligibility criteria

RIT is approved for patients with low-grade follicular lymphoma that relapsed after the treatment with rituximab or are refractory or failed to respond to rituximab. As stated, recently the indications for use have been extended to include consolidation therapy in patients with a complete response or at least partial response. This treatment is also used in patients with large B-cell lymphoma that express the CD-20 epitope. Before administration of RIT, patients have to meet the following criteria: initial biopsy confirmation of NHL with the expression of CD20 epitope; recent (within 4-6 weeks) bone marrow biopsy to confirm less than 25% involvement, because treatment of patients with 25% or more bone marrow involvement is associated with severe bone marrow toxicity; history of allergies or medications; and recent (within 1-2 weeks) complete blood count (a platelet count greater than 150,000 justifies full dose, while platelet counts between 100,000 and 150,000 require a modified amount of radiolabeled antibody). This treatment should not be performed in patients younger than 18 years or pregnant and lactating women [18].

Pretreatment imaging and predosing

Until recently, pretreatment imaging in the Zevalin® regimen was required in the US to confirm normal biodistribution despite the fact that altered biodistribution had been reported in less than 1% of patients. The imaging protocol involved administration of rituximab and ^{111}In ibritumomab tiuxetan [2]. Since 2013, this imaging requirement has been abandoned in the US. In Europe, the imaging component of the Zevalin® regimen was never required but predosing with rituximab one week prior to the combination of rituximab and ^{90}Y ibritumomab remains a component of the Zevalin® protocol.

For both regimens, Zevalin® and Bexxar®, an initial infusion of cold, unlabeled anti-CD20 antibody is necessary to

saturate binding sites on normal lymphocytes and improve the more specific targeting to the malignant cells [22]. Without prior infusion of cold antibody, administration of radiolabeled antibody would result in rapid binding of activity by circulating lymphocytes and clearance by those stored in the spleen. When the radiolabeled antibody is injected after the cold antibody, the sites in the spleen have been already saturated and a greater portion of injected labeled antibody remains in the circulation and increases the percentage of the administered dose in tumor [18].

Treatment regimens

The protocol of the Zevalin® regimen is described in detail in our previous reviews [26, 27]. The dosing regimen for treatment with Zevalin® starts with a pre-dose of rituximab 250 mg/m^2 on the first day. The same dose of rituximab (250 mg/m^2) is repeated one week later, followed by the ^{90}Y-ibritumomab tiuxetan infusion in a dose dependent on the platelet counts (30 MBq/kg if platelets exceed 150,000/mL; 22.5 MBq/kg if platelets are > 100,000/mL, < 150,000/mL). The maximum dose should not exceed 1.18 GBq (Figure 1).

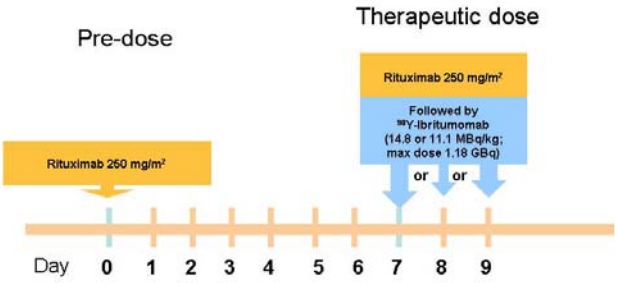

Fig. 1 – The Zevalin® treatment regimen for non-Hodgkin lymphoma.

Patients receiving Bexxar® should be premedicated with a saturated solution of potassium iodide, to block or to reduce thyroidal iodine uptake (3 drops saturated solution of potassium iodide diluted in water 2 times per day, beginning the day before the protocol initiation and continuing for 3 weeks). The Bexxar® regimen consists of 2 steps, the first being, dosimetric and the second, therapeutic [27]. The dosimetric phase involves 3 whole body scans (24–48 h apart) during the week after a dosimetric dose of 185 MBq of ^{131}I-tositumomab preceded by an infusion of 450 mg of unlabeled tositumomab. The Bexxar® regimen is completed seven to nine days after the initial infusion, with the infusion of 450 mg of unlabeled tositumomab followed by the ^{131}I-tositumomab therapeutic dose (Figure 2). For dosimetry, whole body counts are calculated from the total counts on the anterior and posterior whole body scans performed one h after the infusion, 2 and 4–5 days after the initial infusion. The residency time is determined by setting the initial whole body counts as 100% and plotting the other data on a semilog plot. Residence time is at the 37% intercept. This value is used in the calculation of the dose of radioactivity to be administered. Patients with a platelet count exceeding 150,000/mL would receive the maximum tolerated dose of 75 cGy whole-body radiation absorbed dose, while 65 cGy whole-body radiation absorbed dose is optimal for patients with platelet counts between 100,000/mL and 150,000/mL [18].

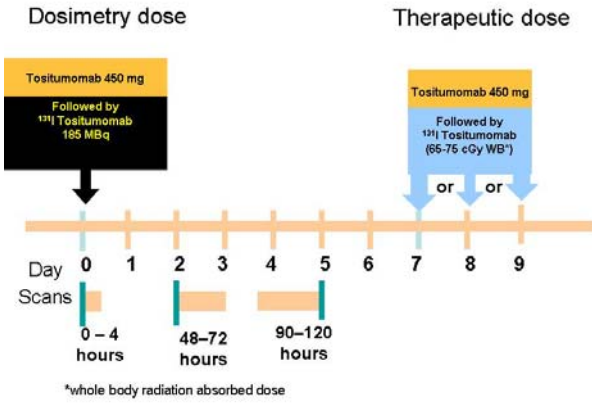

Fig. 2 – The Bexxar® treatment regimen for non-Hodgkin lymphoma.

Clinical efficacy

For both Zevalin® and Bexxar® there are similar clinical outcome. The overall response rates vary between 60% and 80%, with a complete response (CR) of 20–50%, and with one year duration of response for indolent B-cell NHL [16]. Iagaru et al. [28] compared treatment with Bexxar® and Zevalin® in 67 patients with low-grade refractory/relapsed NHL. Objective responses were similar: 70.9% for Bexxar® *vs* 77.8% for Zevalin®. In this report, however, Zevalin® induces more CR than Bexxar® (41.7% *vs* 35.5%).

Clinical efficacy results in several studies using the Zevalin® regimen in NHL patients are shown in Table 2. A single-arm phase II clinical trial was performed on 57 follicular B-cell NHL patients who relapsed or were refractory to the prior rituximab treatment. Patients achieved 74% overall response rate (ORR) and 15% CR. Estimated median duration of response (MDR) was 6.4 months and time to progression (TTP) was 6.8 months for all patients and 8.7 months for responders [24]. A randomized, phase III multicenter study involving 27 institutions and 143 patients with relapsed or refractory low-grade, follicular, or transformed NHL was performed. All patients had advanced disease with a median of two prior chemotherapy regimens. Seventy three patients received Zevalin®, and 70 patients received 4 doses of rituximab. RIT group was pretreated with two rituximab doses to improve biodistribution. An 80% of ORR for Zevalin® group *vs* 56% for the rituximab group ($p = 0.002$), and CR of 30% for Zevalin® group *vs* 16% for rituximab group ($p = 0.04$) were observed. The median duration of response was 14.2 *vs* 12.1 months while TTP was 11.2 months or 10.1 months for Zevalin® *vs* rituximab, respectively [15]. Updated results of the trial reported in 2004 indicated 80% ORR and 56%, and CR rates of 34% *vs* 20%, for Zevalin® compared to rituximab, respectively. Results of this trial suggested a longer estimated MDR (16.7 *vs* 11.2 months) and median TTP (15 *vs* 10.2 months) in the Zevalin® group

Table 2

Clinical efficacy of the Zevalin® regimen in treatment of patients with non-Hodgkin's lymphoma (NHL)

Study	Patients (n)	ORR (%)	CR (%)	MDR (months)	TTP (months)
Witzig et al, 2002. [24]	57	74	15	6.4	6.8
Rituximab-refractory follicular NHL					8.7*
Witzig et al, 2002[15]					
Rituximab-refractory follicular or transformed NHL	143				
Zevalin group	73	80	30	14.2	11.2
Rituximab group	70	56	16	12.1	10.1
Gordon et al, 2004. [29]					
Rituximab-refractory follicular or transformed NHL	143				
Zevalin group	73	80	34	16.7	15
Rituximab group	70	56	20	11.2	10.2
Gordon et al, 2004. [30]					
Follicular and diffuse large B-cell lymphoma, low-grade or mantle-cell lymphoma	51	73	51	11.7*	12.6*
Wieseman et al, 2005. [31]					
Relapsed, refractory or transformed indolent CD20+ B-cell NHL	211	73–83	15–51	6.4–13.9	NA
phase I/II	51	73	51	11.7	
phase II	30	83	47	11.5	
phase II	54	74	15	6.4	
phase III	73	80	34	13.9	
Emmanoulides et al, 2007. [32]					
Relapsed or refractory CD20+ B-cell NHL	211				
patients <60 years	113	78	35	9.9	9.3
patients 60–69 years	58	71	33	11	8.4
patients ≥70 years	40	80	38	9.4	8.8
Vaes et al, 2012. [33]					
Previously treated CD20+follicular B-cell NHL	26	88	65	8.7*	29.6

***Responder patient population; ORR – overall response rate; CR – complete response; MDR – median duration of response; TTP – time to progression.**

vs rituximab group, respectively. CR were highly durable in the Zevalin® group with a median TTP of 24.7 months compared to TTP of 13.2 months in rituximab group with ongoing responses of more than 5 years [29]. The same authors [30] performed a clinical phase I/II trial on 51 patients with follicular and diffuse large B-cell lymphoma, low-grade or mantle-cell lymphoma with a long-term follow-up of more than 5 years. They showed 73% ORR and 51% CR, with the mean TTP and the duration of response in responders of 12.6 and 11.7 months, respectively [30]. Wiseman et al. [31] reported durable long-term responses in 37% of 211 patients with relapsed, refractory or transformed indolent B-cell NHL who were treated with Zevalin® in 4 clinical trials. They obtained ORR of 73–83%, CR of 15–51%, with MDR of 6.4–13.9 months. Patients with TTP of 1 year or longer were characterized as long-term responders; 65% of those patients achieved CR with median TTP of 31 months. Emmanoulides et al. [32] analyzed data from clinical trials of Zevalin® performed in a total of 211 relapsed or refractory NHL treated in 4 different centers. Patients were divided into there different age groups: < 60; 60–69; and ≥ 70 years. The authors obtained different results in different age groups: ORR, between groups ranged from 71–78%; CR, 33–38%; MDR, 9.4–11 months; and TTP, 8.4–9.3 months. In a study performed on 26 patients (CD 20+ B-cell lymphoma), ORR was 88%, CR was 65%, while estimated median progression-free survival (PFS) was 9.1 months after a median follow-up of 29.6 months. Responders had estimated MDR of 8.7 months [33].

Morschhauser et al. [34] studied 414 patients with advanced stage follicular lymphoma who had been enrolled in 77 centers. A large group of 208 patients who received consolidation therapy [chemotherapeutic induction of CR and partial response (PR)] in contrast to a similar group of patents after first line induction treatment without additional treatment were compared. They detected that consolidation induced significantly longer median PFS in the control group (36.5 *vs* 13.3 months). Moreover, 77% of patients with PR after induction treatment converted to CR after consolidation treatment. The final CR rate was 87.4% after consolidation with Zevalin® compared to 53.3% to the control group.

Table 3 shows the clinical efficacy in several single arm trials on the Bexxar® regimen in previously treated NHL patients. Kaminski et al. [35] performed a pivotal study on 60 patients with chemotherapy-refractory low-grade or transformed low grade B-cell NHL. They compared the efficacy of Bexxar® regimen to the last qualifying chemotherapy obtaining ORR of 65% *vs* 28%, respectively. Twenty percentage of patients achieved CR on ^{131}I tositumomab with MDR of 6.5 months and TTP of 8.4 months. Fisher et al. [36] enrolled 250 previously treated relapsed or refractory low-grade, follicular, or transformed low-grade NHL patients in five clinical trials. Bexxar® regimen was administered in NHL patients who were previously treated with chemother-

Table 3

Clinical efficacy of Bexxar® in previously treated patients with non-Hodgkin lymphoma (NHL)

Single arm trials with Bexxar® in previously treated NHL, without rituximab	Patients (n)	ORR (%)	CR (%)	MDR (months)	TTP (months)	Follow-up (months)
Kaminski et al, 2001[35] *Low-grade/ transformed low-grade CD20+ B-cell, at least 2 prior chemotherapy*	60	65	20	6.5	8.4	NR
Fisher et al., 2005 [36] *Relapsed or refractory low-grade, follicular, or transformed low-grade NHL*	250	56 (47–68)	30 (20–38)	12.9	15	41.5
	81*	100*	77*	45.8*	48.5*	61.2*
Kaminski et al. 2000 [37] *Relapsed/ refractory to chemotherapy,CD20+ B-cell NHLs*	59	71	34	NR	12	37.2
Vose et al. [38] *Low-grade/ transformed low-grade CD20+ chemotherapy relapsed/refractory*	47	57	32	9.9	11.6	NR
Davies et al. 2004. [13] *B-cell NHLs in first or second recurrence*	41	76	49	15	9.6	36

***Durable Response Population; NR – not reached; ORR – overall response rate; CR – complete response; MDR – median duration of response; TTP – time to progression.**

apy or rituximab (50% of them did not respond to the last treatment). Data of the integrated patient population indicated ORR and CR rates of 56% and 30%, respectively, with MDR of 12.9 months and median TTP of 15 months (in patients with a complete response, MDR was 58.4 months and TTP 48.5 months). ORRs and CR differ among each of five clinical trials, ranging from 47–68% and 20–38%, respectively; with the median follow-up of 41.5 months. For the durable response population (32% of the entire patient population), CR was 77%; MDR 45.8 months with a median follow-up of 61.2 months [36]. Updating the long-term data on chemotherapy-relapsed/refractory patients treated with Bexxar® in the phase 1–2 single-center study, Kaminski et al. [37] detected 71% ORR and 34% CR with TTP of 12 months during the follow-up of 37.2 months. They showed better ORR in transformed low-grade lymphoma compared to newly diagnosed intermediate-grade tumors (79% *vs* 41%), with 50% CR rate in a group of transformed NHL patients. A multicentric phase II study with a single dosimetric and therapeutic dose of Bexxar® was conducted in 47 patients with relapsed or refractory low-grade (79%) or transformed low-grade (21%) B-cell NHL. Patients were heavily pretreated with median of four prior chemotherapy cycles and showed extensive disease. The study obtained 57% ORR of all the treated patients and 32% CR. The median TTP was 11.6 months and MDR was 9.9 months [38]. Davies et al. [13] performed a phase II study to assess the efficacy of Bexxar® at first or second recurrence on 41 patients with indolent or transformed indolent B-cell NHL. During the follow-up of 3 years, they obtained ORR of 76%, CR of 49%, with 9.6 months of TTP and 15 months of MDR.

Table 4

Clinical efficacy of Bexxar® in previously untreated patients with non-Hodgkin lymphoma (NHL)

Single arm trials with Bexxar in previously untreated NHL	Patients (n)	ORR (%)	CR (%)	MDR (months)	TTP (months)	Follow-up (months)
Kaminski et al., 2001[39] *Follicular advanced stage*	76	95	75	NR	73.2	61.2
Press et al., 2003 [40] *Follicular, II–IV stage*	90	90	67	NR	NR	27.6
Leonard et al., 2004 (abs) [41] *Advanced low-grade*	35	100	83	NR	NR	52.8
Leonard et al., 2005 [42] *Stage III/IV follicular grade*	35	100	86	NR	NA	58
Wahl et al, 2004[43] *Relapsed NHL responders to Bexxar®*	32	56	25	35	NA	NA

NR – not reached; ORR – overall response rate; CR – complete response; MDR – median duration of response; TTP – time to progression; NA – not applicable.

The Bexxar® regimen was also performed in previously untreated NHL patients (Table 4). In a study on 76 previously untreated stage III or IV follicular NHL patients receiving Bexxar® as a sole treatment, after a median follow-up of 5.1 years, Kaminski et al. [39] observed a 95% ORR, 75% CR with a TTP of 73.2 months and a median PFS of 6.1 years. Among patients who achieved complete remission,

70% remained in remission for 4.3–7.7 years. Press et al. [40] conducted a phase 2 trial that included six cycles of CHOP chemotherapy followed by Bexxar® in 90 patients with advanced stage follicular NHL. The results of this trial shows 90% ORR and 67% CR, while 57% of patients who achieved less than CR with CHOP improved their response after Bexxar® therapy (49% patients converted from PR to CR/unconfirmed CR (Cru), while 4.9% patients changed from CRu into CR). With a median follow-up of 27.6 months, the 2-year PFS was estimated to be 81%, with 97% of the 2-year overall survival. Another Bexxar® trial in patients with previously untreated non-Hodgkin lymphoma reported 100% ORR and 83% CR with follow-up of 52.8 months [41]. In different study, Bexxar® regimen was administered after a short chemotherapeutic course of Fludarabine for 3 cycles in 35 previously untreated, stage III or IV follicular grade NHL CD20 lymphoma. The results of this study showed 100% of ORR to the complete regimen, and 86% of CR during the median follow-up of 58 months. The median duration of response was not reached. PFS was also not reached but was estimated to exceed 48 months [42]. A single-arm open-labeled multicenter phase II trial was performed on 32 patients who had initially responded to Bexxar® and were retreated with Bexxar® after relapse of the disease. Authors [43] reported 56% of ORR and 25% of CR, with 35 months of MDR in patients with CR. Retreatment with Bexxar® was also studied in 16 relapsed NHL patients who initially responded to the first regimen and achieved ORR and CR in 56% and 31% of patients, respectively [38].

Safety

RIT using either molecular regimens, Zevalin® and Bexxar® seems to be a safe. The most frequent toxicity reported was bone marrow suppression, transient and reversible. Hematologic toxicity includes neutropenia and thrombocytopenia, was delayed in onset with nadir between 7–9 weeks after the regimen and recovery after 2–3 weeks. More than a half of treated patients show platelet nadir below 50,000 per mm^3, and approximately 20% will have a nadir below 25,000 per mm^3 and may require platelet transfusion. Absolute neutrophil counts below 500 per mm^3 may be detected in about 25–30% of treated patients with a month nadir duration. Hospitalization for febrile neutropenia or similar hematopoetic suppression develops in less than 10% [22]. Some authors [44] reported that Bexxar® causes significantly less severe declines in platelet counts than Zevalin® and thus may be better treatment option for patients with limited bone marrow reserve. Press et al. [40] conducted Bexxar® regimen and obtained grade 3–4 neutropenia, thrombocytopenia and anemia in 15.8%, 13.4% and 2.4%, respectively. In contrast, the most recent study with the Zevalin® regimen, reported 34% incidence of grade 3–4 neutropenia, 38% of thrombocytopenia and 8% of anemia, however, patients spontaneously recovered [33].

Compared to cytotoxicity caused by chemotherapy, nonhematologic adverse effects from either Zevalin® or Bexxar® are very mild. These toxicities are related mostly to minor allergic reactions to the protein components of the cold antibody, generally greater for patients treated with rituximab than those treated with tositumomab. In these situations, infusion should be adjusted to a slower rate. Asthenia or nausea was reported in about 20–40% after receiving either of the anti-CD20 compounds. Side effects such as hair loss, severe mucositis and persistent nausea or vomiting were not detected [44].

Potential long-term adverse effects might be hypothyroidism, development of human antimouse antibodies and secondary malignancies. Human antimouse antibody was reported in 10% of patients following Bexxar®; human antichimeric antibody was detected in about 1–2% following Zevalin® [22]. However, this adverse effect is without serious clinical consequences [38]. Hypothyroidism develops in about 10–20% of patients treated with Bexxar® despite the pretreatment of thyroid-blocking medications [22].

The most important late effect of RIT is secondary malignancy which includes myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Witzig et al. [45] reported 1–2% incidence of MDS/AML for lymphoma patients treated with Zevalin®. Emmanoulides et al. [46] detected annual incidence of 0.34% for MDS and 0.7% for AML since initial treatment with Zevalin® for the period 1993–2002. However, the review of the literature showed 4–8% rate of secondary malignancy in NHL patients treated with chemotherapy alone or combined with radiation therapy [47–49]. In a large study on 1,071 NHL patients treated with Bexxar®, MDS and AML was reported with an annualized incidence of 1.4% per year (95% CI; 1–2% per year) [50]. In another study, there were no cases of MDS or AML [13]. These late toxic effects of bone marrow occur late in patients with B-cell NHL no matter how they are treated. Patients treated with either regimen, Bexxar® or Zevalin®, did not show increased incidence of MDS/AML [22]. In the extensive literature review Cheung et al. [51] reported that secondary MDS and AML had been reported of 0–8% in treated patients, and 0–3% in untreated patients.

Radiation exposure

RIT with either agents, Zevalin® and Bexxar®, is generally considered an outpatient therapy in the US. This treatment, in general, should not be performed in children and adolescents under 18 years of age, in pregnant and in lactating women.

The Zevalin® regimen includes radionuclide ^{90}Y, which is pure beta emitter without gamma radiation. However, the bremsstrahlung emission radiation (which is emitted out of beta particle loosing energy process) is below the limit of exposure and is not hazardous for health personnel and family members. However, patients are provided by written instructions about contact with household members. They are suggested to avoid transmission of excretions such as saliva, blood, urine, seminal fluid and stool [52, 53]. According to the data published from another study, the Zevalin® regimen includes minimal exposure to treated patients (0.00295 mSv/h at 1 m immediately after dosing). Exposure to patient's fam-

ily members (first week 0.035 mSv) is in the range of European background radiation (0.04–0.15 mSv/week) [54].

According to the Nuclear Regulatory Commision and specific dose-calculations, virtually all patients can be released at the end of the therapeutic infusion. A study in Nebraska included family members of patients who had received 1–5 GBq of Bexxar® to deliver 30–75 cGy. Measurement of monitoring devices of 26 family members from 22 patients showed radiation absorbed dose differ from 17 to 409 mrem (below 500 mrem limit applicable to general public members), based on patients receiving Bexxar® (limit exposure to the total effective dose does not exceed 500 mR) [55].

Bexxar® therapeutic regimen can be administered safely with minimal additional exposure to healthcare professionals. Harwood et al. [56] studied exposure on professional workers who were involved in 300 administrations of Bexxar® treatment: radiopharmacists, nuclear medicine technologists, nurses and physicians at four different institutions during 2–4.5 years. They reported that additional average radiation monthly exposure per healthcare worker involved in Bexxar® regimen was 5.8 mrem. Before release, patients are given detailed instructions on the duration and proximity to others to minimize exposure such as: avoiding sleeping with other individuals for a week or more, not traveling by air for several days, and avoiding children and pregnant women for a week or longer. These instructions are based upon patient specific variables including administered dose, measured emission from the patient at the body surface and at 1 m, and biologic turnover rate calculated from dosimetry measurements [51].

Other radioimmunotherapy compounds

In Australia, due to the lack of availability of the RIT regimen, Zevalin® and Bexxar®, Leahy et al. [57] developed a new hybrid regimen consisting of rituximab as the cold antibody and rituximab labeled with ^{131}I in patients with indolent non-Hodgin's lymphoma. In a recent study, they achieved ORR of 76% and CR of 53% with a median survival over 4 years. At 6–7 weeks, they reported side effects such as grade 4 thrombocytopenia and neutropenia in 4%, and 16%, respectively.

Linden et al. [58] developed an anti-CD22 monoclonal antibody, radiolabeled with ^{90}Y and evaluated as ^{90}Y-Epratuzumab in combination with cold Epratuzumab for treatment of indolent NHLs. An ORR in 62% and CR in 25% of patients was achieved; ORR of 75% in indolent NHLs and 50% in aggressive NHL. Subsequently, Leonard et al. [59] reported a 24% response in patients with follicular NHL with median duration of the objective response of 79.3 weeks and median time to progression for responders of 86.6 weeks. The treatment was well tolerated with manageable hematologic toxicity.

Recently, a multicenter, fractionated dose phase I/II study with ^{90}Y-epratuzumab was performed on 64 patients with relapsed or refractory NHL. The results indicated that for 61 patients, a median PFS was 9.5 months, while ORR and CR were 62% and 84%, respectively. In addition, 17 patients previously treated with autologous stenc cell transplantation, ORR of 71% and 55% CR were achieved. On the other hand, in patients with indolent follicular lymphoma, the ORR was 100% with CR of 92% and a PFS of 18.3 months [60]. Sharkey et al. [61] suggested that combining anti-CD 20 and anti-CD 22 antibodies might be more efficient for NHL patients in future clinical trials. They also suggested the possible role of ^{177}Lu or an alpha particle emitter in the setting of minimal or occult disease.

Conclusion

Two radiolabeled antibodies (with different radiolabels) were approved as therapeutic agents in low grade non-Hodgkin's lymphoma. There was no direct comparison between the two agents and currently Bexxar® is unavailable. In general, clinical responses (complete response, partial response) with radioimmunotherapy after relapse are better, and of greater duration, than alternative or repeat chemotherapies. Radioimmunotherapy regimen is safe and effective even after multiple relapses following chemotherapy and/or rituximab (Rituxin®) therapy. The complete response and overall response rate is even better when used in conjunction with first-line chemotherapy ("consolidation" treatment). The principle toxicity is hematologic, secondary to bone marrow irradiation from labeled antibody in blood and specific deposition on tumor cells in the bone marrow. Radioimmunotherapy should not be performed in patients younger than 18 years, pregnant or lactating women. Radiation exposure of family members and health care personnel is low. In the event of relapse, patients tolerate subsequent therapy as well or better than equivalent populations who have not received radioimmunotherapy.

During the last few years, new agents for radioimmunotherapy have been developed such as ^{131}I-rituximab therapy and ^{90}Y-epratuzumab, showing impressive results. Hopefully, future trials should investigate the combination of immunoglobulins and introduce new radionuclides including alpha emitters for radioimmunotherapy of patients with non-Hodgkin lymphoma.

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CASE REPORTS

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Decompression as an effective primary approach to large radicular cyst in the maxillary sinus – A case report

Dekompresija kao delotvorni primarni pristup radikularnoj cisti u maksilarnom sinusu

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Abstract

Introduction. Therapeutic approach to jaw cysts may depend on their dimensions and localization. Enucleation of cystic lesion is not always preferable in the first act, especially if large cysts are in close proximity to important anatomical structures. The aim of this paper was to present the outcome of the treatment protocol comprising preoperative decompression and subsequent enucleation of a large maxillary cyst. **Case report.** A 21-year-old male patient with large asymptomatic radicular cyst in the right maxillary sinus was presented to our clinic. Cone-beam computed tomography (CBCT) showed a large cyst, which perforated the right anterior maxillary wall by 1.5 cm, and was in the intimate contact with the orbital floor. Surgical treatment of the cystic lesion comprised: preoperative decompression with biopsy in the first act and enucleation, performed under general anesthesia, 6 months after the observation period. **Conclusion.** Decompression with subsequent enucleation proved to be effective treatment of large radicular cyst in maxillary sinus with low-morbidity.

Key words:
maxillary sinus; radicular cyst; cone-beam computed tomography; oral surgical procedures; treatment outcome.

Apstrakt

Uvod. Terapijski pristup cistama vilice može zavisiti od njihovih dimenzija i lokalizacije. Enukleacija cistične lezije često nije pogodna u prvom aktu ako se velika cista nalazi u blizini važnih anatomskih struktura. Cilj ovog rada bio je da se prikaže ishod lečenja velike ciste u maksilarnom sinusu koji je podrazumevao preoperativnu dekompresiju i odloženu enukleaciju. **Prikaz bolesnika.** Prikazan je bolesnik, star 21 godinu, sa velikom, asimptomatskom, radikularnom cistom u maksilarnom sinusu. Radiografska dijagnostika pomoću *cone-beam* kompjuterizovane tomografije (CBCT), pokazala je prisustvo velike cistične lezije koja je probila prednji maksilarni zid (1,5 cm) i bila u bliskom kontaktu sa podom orbite. Hirurški tretman cistične lezije uključio je preoperativnu dekompresiju i biopsiju u prvom aktu i enukleaciju u opštoj anesteziji nakon 6 meseci. **Zaključak.** Dekompresija i odložena enukleacija pokazale su se efikasnim terapijskim pristupom kod lečenje velike radikularne ciste maksilarnog sinusa uz mali morbiditet.

Ključne reči:
maksilarni sinus; cista, radikularna; kompjuterizovana tomografija konusnog zraka; hirurgija, oralna, procedure; lečenje, ishod.

Introduction

Radicular cysts are the most common odontogenic inflammatory jaw cysts. The remnants of Malassez epithelium in combination with intracanal infection as an initial stimulus are necessary for radicular cysts development [1]. The main feature of cystic lesion is its constant growth, with bone resorption and displacement of surrounding structures [2]. The growth of a cyst is slow, and may develop without any symptoms, so it may reach impressive dimensions.

Treatment of radicular cysts is mainly surgical. However, in some strictly selected cases, there are still open questions

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about radicalism in surgical approach in order to preempt probable postoperative complications and morbidity. Therefore, the therapeutic approach to cyst treatment sometimes depends on their dimensions and localization. Enucleation of a cystic is not always possible in the first act, especially when large cysts are in close proximity to important anatomical structures [3]. Therefore, in order to avoid anatomical structures damage, the conservative method involving decompression with subsequent enucleation might be preferabble [4,5]. Decompression of a cyst comprises making a small opening in the cyst keeping it open with a drain [3,6]. Decompression has to stop cystic growth by eliminating inflammation and intracystic pressure, to allow thickening of the cystic wall and to reduce cyst size for easier removal in the second stage.

We presented a patient with a large maxillary cyst treated by preoperative decompression and subsequent enucleation.

Case report

A 21-year-old healthy male patient came to the Clinic of Oral Surgery, Faculty of Dental Medicine in Belgrade, complaining of swelling in the right maxillary vestibule. Clinical examination confirmed the complaint and the painless swelling was noticed in the region of the right maxillary vestibule from the right upper canine to the third molar (region 13 to 18). No local signs of infection were seen. Dupuytren's phenomenon and fluctuation were present on palpation. Ortopantomogram (OPG) showed large cystic lesion in the right maxillary sinus, 8–10 cm in diameter, with clearly defined borders, comprising the region 13–18. On the Water's projection, the whole lumen of the right maxillary sinus was embedded with the cystic lesion. Additional radiographic procedure, which included cone-beam computed tomography (CBCT) showed a large cyst perforating the right anterior maxillary wall by 1.5 cm: the cyst was in intimate contact with the orbital floor (Figure 1). Furthermore, the cyst was also in close contact with the content of the pterygopalatine fossa in posterior aspect and with the content of the infraorbital canal in the frontal aspect (Figure 1).

On the basis of clinical characteristics and radiological findings, the treatment plan included the following:

Stage one: *Decompression and biopsy*

Cystic lesion decompression and biopsy were performed in local anesthesia (4% articaine with 1:100 000 epinephrine; Septanest®, Septodont, France). The mucoperiosteal flap was raised and one part of the cyst excised and sent for histopathological analysis. In addition, tooth 14 was extracted and the slight re-

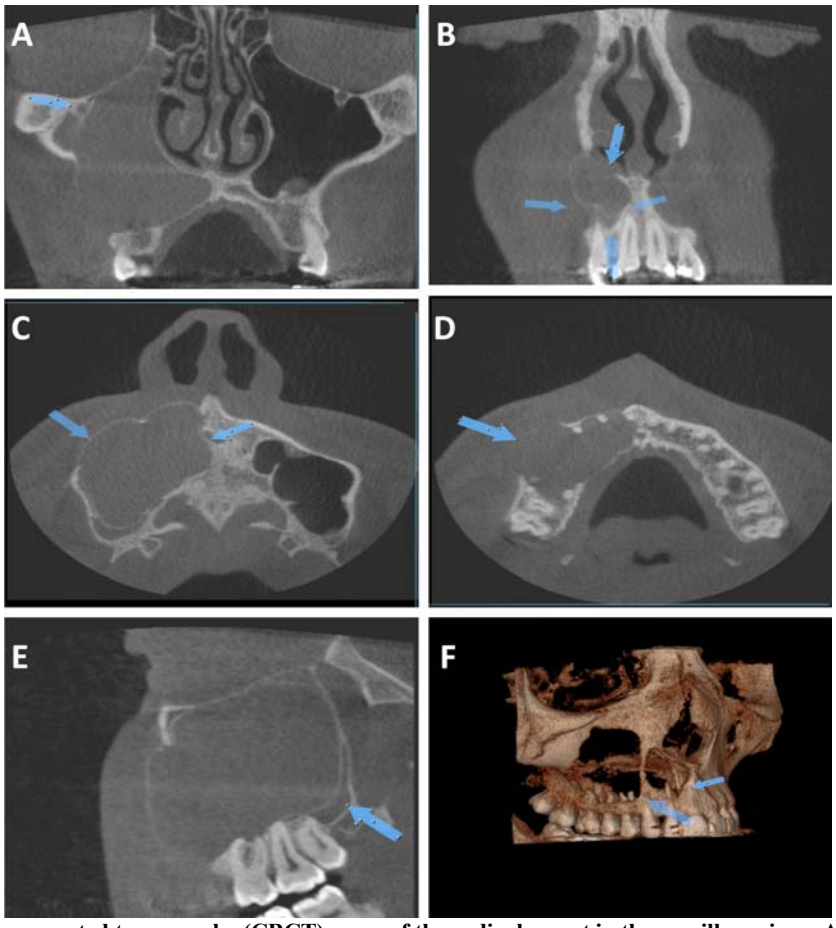

Fig. 1 – Cone-beam computed tomography (CBCT) scans of the radicular cyst in the maxillary sinus. A) The coronal view clearly shows the right maxillary sinus filled with cyst in the proximity to the infraorbital canal and the floor of the orbit; B) The coronal view shows growth of the cyst to the region of tooth 12; C) The axial view clearly shows the cystic lesion in the maxillary sinus, with thinning and perforation of the cortical plate on the medial aspect of the maxilla; D) The axial view clearly shows alveolar process of the maxilla deformity; E) The sagittal view of the maxilla shows the expansive lesion with posterior growth in the maxillary tuber; F) The 3D image of the affected site shows ovoid destruction of the bone.

sorption of its roots was observed. A drainage tube was placed in the socket of the tooth 14 and cyst rinsed with saline three times per week in the following 6 months. There was no infection during that period.

Histopathological finding

The multilayered squamous epithelium with the signs of parakeratosis was observed by histopathological analysis of the sample. There was granulation tissue proliferation with inflammatory infiltration of polymorphonuclear leukocytes beneath the epithelium. Histopathological analysis confirmed the diagnosis of radicular maxillary cyst.

Stage two: *Enucleation*

CBCT control 6 months thereafter showed the cystic lesion significantly reduced and the cystic wall thickened (Figure 2). The teeth 15, 16 and 17 were devitalized prior the surgery. The operative procedure to remove the cystic lesion was performed under general anesthesia. All the necessary laboratory analyses prior to general anesthesia were done at the Pharmacological Laboratory, Faculty of Dental Medicine. To enucleate the cyst, we made incision after the Wassmund-Rerhman's procedure. The incision encompassed region from the tooth 13–18. After raising mucoperiosteal flap, we noticed that the cyst 1.5 cm in diameter resorbed the maxilla in the premolar region, on the vestibular side. This opening was extended by a drill and served to approach the cystic lesion. Another trephination in the region 13 was made to make enucleation easier. After curettage, the lumen of the defect was rinsed with saline to enable the sight of the cystic wall remnants. Peripheral ostectomy was performed and the overlying attached to mucosa was excised. The cystic wall and specimens of the adjacent bone were sent for histopathological analysis. The sinus cavity and cystic defect which remained after enucleation were merged into one cavity. The defect was buffered with iodoform gauze, which was pulled through the drainage canal in the vestibule of the opposite side. The mucoperiosteal flap was relaxed after periosteum incision and than sutured with horizontal mattress sutures to the palatal mucosa.

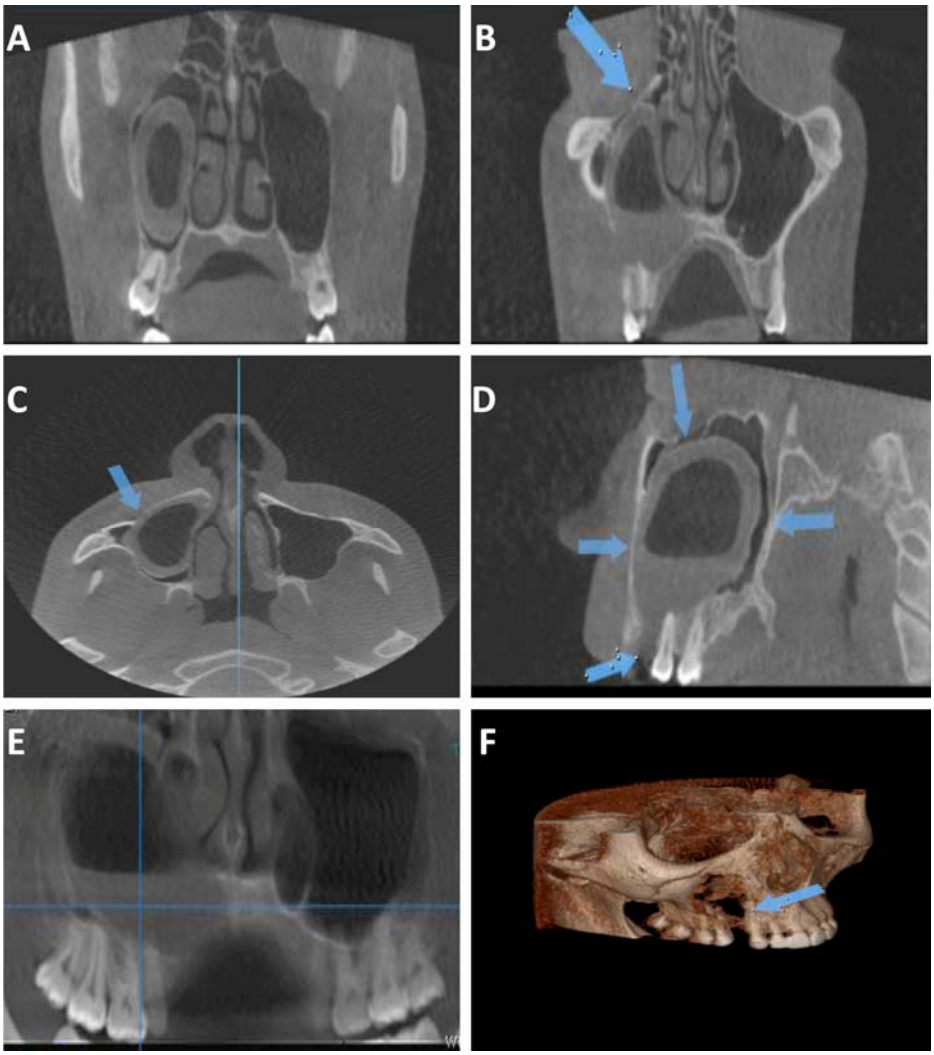

Fig. 2 – Cone-beam computed tomography (CBCT) scans 6 months following decompression. A) The coronal view clearly shows the reduced size and thickening of the cystic lesion; B) The coronal view shows decompression of the infraorbital canal and the orbit; C) The axial view shows the thickened cystic sheath and its separation from the maxillary sinus walls; D) The sagittal view demonstrates cystic reduction and canal decompression; E) Panoramic thin-slice image shows the maxillary sinus; F) The 3D image of the affected site shows the reduced bony defect.

Postoperatively, the patient was prescribed antibiotic (amoxicilline) and analgesic therapy (ibuprofen). Iodoform gauze was partially removed on the third day. Sutures and the rest of the iodoform gauze were removed on the 7th postoperative day. The wound healed uneventfully.

Discussion

Odontogenic cysts originate from the remains of the epithelium associated with odontogenesis. Radicular cysts are the most common odontogenic cysts. Enucleation is described as a basic way of treatment [2]. On the other hand, treatment of large radicular cysts still remains a challenge for clinicians. Dandotikar et al. [7] show that endodontic therapy is successful in complete resolution of large radicular cysts. Seno et al. [8] performed the endoscopic sinus surgery in the treatment of radicular cysts in the maxillary sinus. However, the same authors completely removed radicular cysts only in five out of ten cases. Kubota et al. [9] demonstrate that decompression is effective in reducing the size of cysts prior to enucleation as a definitive surgery of large cysts. Moreover, surgical decompression prior to enucleation considerably reduces intraoperative and postoperative complications [10]. However, treatment of large cysts still remains without a strict protocol.

It is unusual for radicular cysts to develop in that size. Although enucleation of odontogenic cysts is the treatment of choice, it seemed to us that insisting on enucleation of such a large cyst in the first act in the presented patient would possibly provoke several complications: first, injury to the orbital floor and the content of the infraorbital canal, with concomitant bleeding and future neural deficit, then injury to the nasal cavity and maxillary bone walls. Moreover, cystic distal outgrowth toward the maxillary tuber brings it in close proximity to the pterygopalatine fossa and maxillary artery. Injury to the artery could lead to bleeding with fatal consequences. There was also a possibility of failing to remove all parts of the cyst, causing recurrence of the lesion. For that reason we chose to perform decompression, the method suggested as first-phase therapy of large odontogenic cysts [9, 11], being described as an effective procedure in reduction of the size of radicular cysts and keratocysts [11–13]. The results of the study of Kubota et al. [9] show that speed of cystic shrinkage after decompression directly correlates to the size of radicular cysts, and that the mean decompression time is 6 months. On the other hand, Anavi et al. [12] find that the period of decompression is 9.2 months. Brøndum and Jensen [6] report the mean decompression time of 10 months. We decided to rinse cystic cavity only for 6 months, having in mind that the patient was young, with high regenerative potential. After that period, a significant reduction of the cyst was observed (Figure 2). We also noticed thickening of all cystic walls, which was important for easy surgical removal at the second stage.

The diagnosis of radicular cysts is completed by clinical examination and orthopantomogrom OPG in most cases. In case of large odontogenic cysts, additional radiographic method, such as CBCT, is needed [14]. CBCT allows precise determination of the cystic borders and its proximity to adjacent anatomical structures, as well as evidence of cortical perforation [15]. Likewise, CBCT allows the insight into the three-dimensional position of the cyst [15]. That was the reason to choose CBCT as radiographic method for our patient.

One of the disadvantages of the decompression method is a long decompression time [5]. Therefore, the patients should be disciplined and motivated for regular rinsing of the cystic cavity for a long period. The presented patient was such a person.

Surgical treatment was completed without complications. The cystic wall was thick and separated from the maxillary sinus walls, so that enucleation was easy to perform. This is in accordance with the study of Gibson et al. [16] who enucleated a large radicular cyst from the maxillary sinus after decompression, without postoperative complications. Our approach to this large radicular cyst shows that the decompression period of 6 months is quite enough for safe and easy enucleation of the cyst.

Conclusion

Having in mind that decompression with subsequent enucleation is effective treatment of large radicular cysts, it seems that decompression, as primary approach, should be considered as the treatment of choice for large odontogenic cysts in close proximity to important anatomical structures.

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CASE REPORT

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A misdiagnosed myasthenia gravis with anti-muscle-specific tyrosine kinase antibodies with possible childhood onset

Pogrešno dijagnostikovana miastenija gravis sa antitelima prema tirozin kinazi specifičnoj za mišić sa mogućim početkom u detinjstvu

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Abstract

Introduction. Childhood onset myasthenia gravis associated with anti-muscle-specific tyrosine kinase antibodies is very rare and atypical in presentation. **Case report.** As a baby, the presented patient was choking and sleeping with open eyes. She had weak cry and breathing difficulties. In childhood, there were frequent falls and fluctuating swallowing difficulties. At the age of 19 she was misdiagnosed with Miller Fisher syndrome due to the presence of diplopia, ataxia and hyporeflexia with spontaneous recovery. Repetitive nerve stimulation test was normal. Four years later, after several relapses, there was significant decrement on facial muscles. Neostigmine test was negative, provoking muscle fasciculations. Serum anti-muscle-specific tyrosine kinase antibodies were positive. With cyclosporine therapy she achieved the minimal manifestations status. **Conclusion.** The presented case confirms that childhood onset myasthenia gravis associated with anti-muscle-specific tyrosine kinase antibodies is often with atypical presentation and spontaneous remissions, so it could be easily misdiagnosed.

Key words:
myasthenia gravis; child; diagnosis, differential; antibodies; protein-tyrosine kinases.

Apstrakt

Uvod. Miastenija gravis udružena sa antitelima prema tirozin kinazi specifičnoj za mišić u dečijem uzrastu veoma je retka i sa atipičnom prezentacijom. **Prikaz bolesnika.** Kao beba, prikazana bolesnica gušila se i spavala sa otvorenim očima. Slabo je plakala i imala je smetnje sa disanjem. Tokom detinjstva, imala je česte padove i fluktuirajuće smetnje sa gutanjem. U uzrastu od 19 godina pogrešno joj je postavljena dijagnoza Miller Fisher sindroma zbog pojave diplopija, ataksije i hiporefleksije sa spontanim oporavkom. Test repetitivne stimulacije bio je negativan. Četiri godine kasnije, posle nekoliko relapsa, registrovan je značajan dekrement na mišićima lica. Neostigmin test bio je negativan, provocirajući mišićne fascikulacije. Serumska antitela prema tirozin kinazi specifičnoj za mišić bila su pozitivna. Uz terapiju ciklosporinom postignut je status sa minimalnim manifestacijama. **Zaključak.** Ovaj prikaz ukazuje na činjenicu da je miastenija gravis sa antitelima prema tirozin kinazi specifičnoj za mišić u dečijem uzrastu često atipične kliničke prezentacije i sa spontanim remisijama, tako da se može lako prevideti prilikom postavljanja dijagnoze.

Ključne reči:
miastenija gravis; deca; dijagnoza, diferencijalna; antitela; protein kinaze.

Introduction

Anti-muscle-specific tyrosine kinase antibodies (anti-MuSK Ab) have been found in arround 50% of acetylcholine receptor (AChR) negative myasthenia gravis (MG) patients [1]. MuSK positive MG is a clinically separate entity [1] and it usually affects young adults. It is extremely rare in pediatric population and it has been described in only several case reports and one small case series [2] so far.

We presented misdiagnosed patient with MuSK positive MG, who might have the childhood disease onset.

Case report

The presented female patient was born prematurelly, in the 7th month. She was choking during breast feeding and was sleeping with her eyes open. She did not cry and there were difficulties in breathing. She had slower motor development

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and started walking at the age of 2 years. During the whole childhood, there were fluctuating swallowing difficulties and frequent falls without loss of consciousness. At the age of 11, she had Henoch Schonlein purpura.

She was examined in our Clinic for the first time at the age of 19 years. At that time, after the flu, she presented with double vision, right eyelid drop, slight dysphagia and nasal speech with more severe presentation in the evenings.

Neurological examination revealed right eyelid ptosis, diplopia, nasal speech, mild hypotonia, diminished muscle stretch reflexes and mild ataxia of the right leg. Repetitive nerve stimulation test of the deltoid and nasal muscle was normal. At that point, the patient was diagnosed with Miller Fisher Syndrome, but aditional investigations showed normal nerve conduction studies and cerebrospinal fluid findings. The spontaneous recovery appeared with the persistence of only mild right eyelid ptosis.

During the next several years the patient had several relapses of the same symptoms, always with spontaneous recovery. One of the most severe exacerbations started after respiratory infection four years after the first admission.

At that time, neurological examination revealed bilateral ptosis, facial muscle weakness, nasal speach, dysarthria, dysphagia and severe tongue weakness. Neostigmine test was negative, provoking severe muscle fasciculations. Repetitive nerve stimulation test showed decremental response on nasal muscle (47%), while it was normal on deltoid muscle. Electromyography revealed myopathic pattern in the facial muscles. Single fibre electromyography showed mildly incresed jitter only in *orbcularis oculi* muscle and normal finding in *extensor digitorum communis* muscle. Brain magnetic resonance imaging showed extensive cortical atrophy of the right cerebellar hemisphere. At this point, the diagnosis of myasthenia gravis was made. Serum anti-AChR antibodies were negative (< 0.2 nmol/L), while anti-MuSK antibodies were positive (0.43 nM; normal < 0.05 nM). Chest computed tomography scan was normal. Treatment with small dose of pyridostigmine and prednisone was initiated and 6 months later she has significantlly improved with the persistence of only mild facial muscle weakness. The favourable effect of this therapy was temporary, so in the later course of the disease, due to frequent relapses, cyclosporine was introduced in the dosage of 300 mg *per* day. Since then, our patient has significantly improved, achieving the minimal manifestations status. For the last five years the presented patient has been without any symptoms, and in the neurological examination only with signs of persistant mild facial muscle weakness and mild right leg ataxia due to the right cerebellar hemisphere atrophy. We also performed serum anti-low density lipoprotein receptor related protein 4 (Lrp4) antibody analysis and it was negative.

Discussion

MuSK MG is a rare form of this disease and it usually affects adults. There have been only a few cases of childhood onset MuSK MG reported so far. We presented a case of initially misdiagnosed MuSK MG with the possible childhood onset. We are aware of the fact that most of the symptoms present at birth were most likely due to the prematurity, but fluctuating swallowing difficulties throughout the years spoke in favour of MG. When our patient was admitted to our Clinic for the first time, the clinical expression was atypical, partially due to the unknown presence of cerebellar atrophy, which led to the eroneus diagnosis of Miller Fisher syndrome. In the later course of the disease, the patient had variable symptoms, which were consistant with MuSK MG: ptosis, double vision, facial muscles weakness, dysphagia, dysphonia and tongue weakness [1]. Before establishing the correct diagnosis, the patient had several episodes of exacerbations of these symptoms, but always with spontaneous recovery. There was incomplete response with hypersensitivity to anticholinesterases and variable response to corticosteroids, which is also consistent with MuSK MG [1]. A significant improvement was achieved after introduction of cyclosporine. This reactivity to immunotherapy and the presence of anti-MuSK antibodies ruled out the congenital myasthenic syndrome. Also, our patients mother did not have MG, she was negative for both anti-AChR and anti-MuSK antibodies, so our patient did not have transient neonatal MG.

During the first admission, repetitive nerve stimulation test was negative and it became positive several years later, which is consistent with the fact that almost half of MuSK MG patients have no decrement on repetitive nerve stimulation test [1]. Another finding in our patient, consistent with MuSK MG was the presence of decremental response to repetitive nerve stimulation test and increased jitter on single fiber electromyography only in the facial muscles and normal findings in the extremity muscles. Facial muscles are known to be among most severly affected in MuSK MG patients and electrophysiological tests are often positive only in these muscles [1], which implicates the necessity of their electrophysiological examination. The presented patient also showed signs of myopathic lesion in the facial muscles on electromyography, which was the reason for their persistant weakness. Such myopathic pattern is also typical for MuSK MG [1].

We assume that the onset of the disease in the presented patient was in early childhood. Most of the symptoms present at birth could be explained by the prematurity, but fluctuating swallowing difficulties throughout the whole childhood and adolescence and the absence of any symptoms presently speak in favour of MG. After extensive investigations and clinical follow up we concluded that our patient had childhood onset MuSK positive MG with incomplete response to anticholinesterases and variable response to corticosteroids. A significant and long-term improvement was achieved after introduction of cyclosporine.

The presented case, together with other cases of childhood onset MuSK MG published so far, signifies that MuSK MG is not an adult onset disease and that it can affect also very young children. At that age, it is often with atypical presentation, so it could be easily misdiagnosed, as was in the presented patient. Also, another charactestic of childhood onset MuSK MG is the presence of spontaneous and some time long remissions, present also in our patient and other published cases [3–5]. On the other hand, such spontaneous remissions are extremly rare in adult onset MG associated with anti-MuSK antibodies.

Conclusion

MuSK MG is very rare in pediatric population and it often has certain peculiarities different from adult onset disease. Due to often atypical clinical presentation and spontaneous remissions it can easily be misdiagnosed. So, cases like the presented one are very valuable for better understanding of the very broad spectrum of MuSK MG.

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Synchronous gastric and colonic cancer – A case report

Istovremeni karcinom želuca i debelog creva

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Abstract

Introduction. Synchronous gastric and colorectal cancer is present in 4% of cases, commonly as additional finding. This is the case of invasive, synchronous gastric and sigmoid colon cancer. **Case report.** A 63-years-old male patient admitted to our institution complaining on pains in epigastrium, vomiting, rapid weight loss and occasional constipation. Using the method of esophagogastroduodenoscopy (EGD) the presence of ulcero-infiltrative tumor of gastric fundus was verified, and colonoscopy revealed stenosing tumor of sigmoid colon. Undergoing a multislice computed tomography (MSCT) of the thorax and the abdomen the changes on the patient's right lung appeared, while video-assisted thoracoscopy (VATS) and biopsy of the right lung excluded malignant dissemination. A total gastrectomy with omentectomy, splenectomy, D2 lymphadenectomy and typical left hemicolectomy were also performed. Histopathological examination verified invasive, diffuse gastric adenocarcinoma and invasive, tubular colon adenocarcinoma. The patient underwent systemic postoperative chemotherapy. Two years after the surgical procedure, the patient is alive, with no signs of recidivism. **Conclusion.** In patients with symptomatology which does not correspond to primary malignancy, just like in the presented case, additional diagnostics is required. Combined resection is adequate surgical procedure for synchronous gastric and colonic cancer.

Key words:
neoplasms, multiple primary; stomach neoplasms; colonic neoplasms; diagnostic techniques and procedures; histology; digestive system surgical procedures.

Apstrakt

Uvod. Istovremeni karcinom želuca i debelog creva javlja se kod 4% bolesnika, obično kao uzgredan nalaz. Prikazali smo primer invazivnog, sinhronog karcinoma želuca i sigmoidnog kolona. **Prikaz bolesnika.** Bolesnik, star 63 godine, primljen je u našu ustanovu zbog bola u epigastrijumu, povraćanja, naglog gubitka telesne mase i povremene opstipacije. Ezofagogastroduodenoskopijom (EGDS) verifikovan je ulceroinfiltrativni tumor fundusa želuca, a kolonoskopski stenozirajući tumor sigmoidnog kolona. Multislajsnom kompjuterizovanom tomografijom (MSCT) grudnog koša i abdomena utvrđeno je postojanje promene u desnom plućnom krilu. Videoskopskom torakoskopijom (VATS) i biopsijom promene desnog pluća isključena je diseminacija. Učinjena je totalna gastrektomija sa omentektomijom, splenektomijom, D2 limfadenektomijom i tipična leva hemikolektomija. Patohistološkim pregledom verifkovan je invazivni, difuzni adenokarcinom želuca i invazivni, tubularni adenokarcinom kolona. Primenjena je sistemska postoperativna hemoterapija. Bolesnik je živ, bez znakova recidiva, 2 godine posle operacije. **Zaključak.** Postojanje simptomatologije koja ne odgovara primarnom malignitetu zahteva dodatnu dijagnostiku. Kombinovana resekcija je adekvatna hirurška procedura.

Ključne reči:
neoplazme, multiple, primarne; želudac, neoplazme; kolon, neoplazme; dijagnostičke tehnike i procedure; histologija; hirurgija digestivnog sistema, procedure.

Introduction

The presence of two or more simultaneous primary malignances of different digestive organs is relatively rare in everyday surgical practice. So far, there have been descriptions of synchronous tumors of the larynx and the stomach [1, 2], the stomach and the duodenum [3], the stomach and the colon [4], the stomach, the gall bladder and colon [5, 6]. The most common form of synchronous digestive tract tumor is colorectal (CRC) and gastric cancer (GC). Among the most of described cases, synchronous gastric and colorectal cancers were the early carcinomas and discovered in preoperative endoscopic examination. We pre-

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sented the case of invasive GC and CRC, treated by radical surgical method and followed during a 2-year period.

Case report

A 63-years-old male patient, admitted at the Clinic for Gastroenterology suspecting on the gastric tumor after emergency esophagogastroduodenoscopy (EGD). The patient noted the problems as sharp pain in epigastrium followed by vomiting, immediately after taking food, rapid weight loss (10 kg for one month), as well as occasional constipations without blood in stool. A longtime smoker with the history of alcohol abuse, the patient had no significant chronic diseases. Early, he had the operation of benign uvula polyps. Family anamnesis pointed on the hereditary inclination to malignant diseases, i.e. the mother died from leukemia, the father from small intestine carcinoma and the aunt from CRC. There was no conducted diagnostic examination for familial adenomatous polyposis of close relatives.

On physical examination no abnormality was discovered. At admission, the patient was in good general condition. Laboratory findings were in the normal average, including C-reactive protein (CRP), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), prostate-specific antigen (PSA) and CA 72-4. On EGD an infiltrative change on the gastric fundus, without clear borders, dimension of about 5 cm and of ulcero-infiltrative type, spreading toward the small curve was found. Biopsy indicated gastric adenocarcinoma, histological gradus II, tubular type, intestinal type according to Laurén [7].

Due to suffering from the mentioned pains and positive family anamnesis, colonoscopy examination was made and infiltrative change found, 3 cm long, approximately 30 cm from the anocutaneous line, of a slightly narrowing lumen. Biopsy indicated tubular, invasive adenocarcinoma of a low grade and intestinal type. Multislice computed tomography (MSCT) of the stomach and lesser pelvis confirmed the endoscopic findings, without the signs of lymphadenopathy and long distance metastasis. Thoracic sections demonstrated the existence of several micronodular solid changes in the lung parenchyma of the right lower lobe, suspected on secondary deposits, without lymphadenopathy in thorax. Video assisted thoracoscopy (VATS) biopsy of changes on the right lung was made. Following patohistologic examination of biopsies, malignant etiology was excluded.

Posterior to the preoperative preparation, the patient underwent the open surgical procedure through medial laparotomy. Intraoperatively, ulcero-infiltrative tumor was detected of the gastric fundus posterior wall (Bormann III), of about 5 cm, penetrating all the wall layers, without infiltrating surrounding structures. In the distal third of the sigmoid colon, the presence of vegetant tumor was found, of robust consistency and gray color, partially and circularly limiting the colon lumen, of about 35 mm, penetrating all the layers of the wall without infiltrating around structures. In the first surgical act, total gastrectomy with omentectomy, splenectomy and D2 lymphadenectomy were performed. The resection border of the distal esophagus was sent to *ex tempore* histopathological analysis (frozen section histopathological examination) with the response – benign. In the second surgical act, typical left hemicolectomy was carried out. The digestive tract continuity was established by creating esophagojejunal end-to-side anastomosis, using isolated Roux loop and circular stapler, CEEA N°25. Postoperative care during the first 7 days included total parenteral and partially parenteral nutrition (TPN, PPN), from the second day the patient started with enteral nutrition and finally after the day 7, was on the oral food intake.

Histopathological analysis of GC (Figure 1) demonstrated the following: infiltrative (Ming), diffusive (Laurén) adenocarcinoma, stage pT3 N2 (9/25) Mx, histopathological grade G3, with the presence of lymphatic and perineural invasion. For CRC (Figure 2) we got the following answer: vegetant, tubular adenocarcinoma, stage B sec. Dukes (B2 sec. Astler Coller) pT3 N0 (0/11) Mx, histological grade G2, no vascular, perineural invasion. For technical reasons immunohistochemical analysis was not carried out. The patient underwent systemic postoperative chemotherapy. A 2-year postoperative follow-up did not shown any recidivism, but satisfying general condition and life quality.

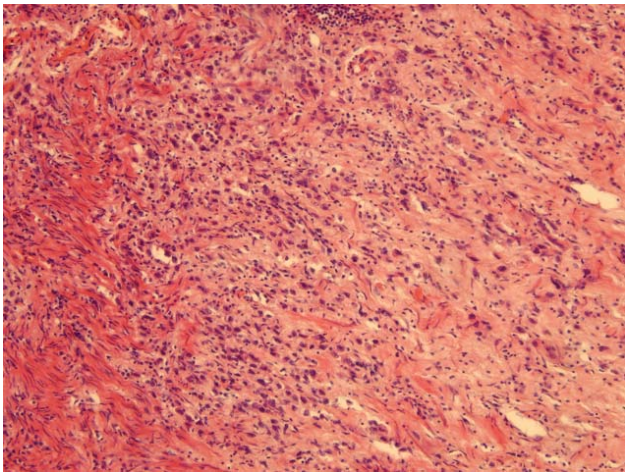

Fig. 1 – Poorly differentiated gastric adenocarcinoma – diffuse type (HE, 4×).

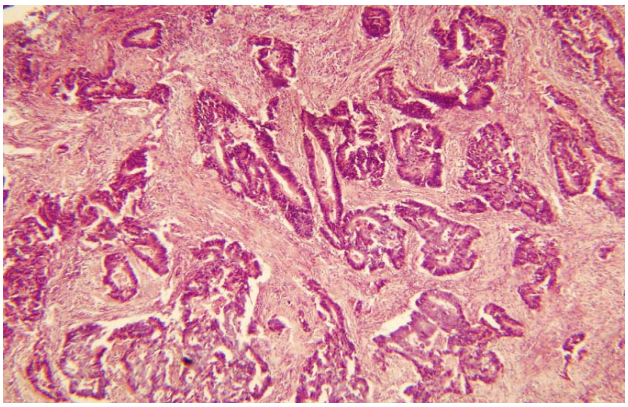

Fig. 2 – Moderately differentiated, tubular colon adenocarcinoma (HE, 4×).

Discussion

In the last decade, probably with the development of advanced diagnostic and therapeutic methods, there was a positive trend in synchronous primary carcinoma [8]. Warren and Gates [9] were the first to define synchronous carcinoma: lesion malignancy should be histopathologically proved, lesions clearly differentiated and locally isolated, while the possibility that one of the tumors is metastatic must be excluded. Conclusions made by Lee at al. [10] in retrospective analysis demonstrate that 3.4% of patients had synchronous carcinoma of the other digestive organ, with the most common CRC of 37.2% of all patients with synchronous carcinoma. After routine colonoscopy performed in patients suffering from GC Saito et al. [11] found CRC in 4% of cases. In our country, colorectal carcinoma incidence rate is 33.5/100,000 persons and 12/100,000 persons for GC [12].

According to the literature, the incidence rate for synchronous CRC and GC in our country may cover about 1/100,000 of newly discovered CRC.

Surgical resection with D2 lymphadenectomy represents the standard oncology treatment procedure for gastric and colorectal carcinoma; therefore, simultaneous resection is indicated for all cases where curative resection is to be expected [13, 14]. Although radical oncologic surgery involves the risks from early and late postoperative complications, in case of synchronous resection there is a sure increase of morbidity and mortality rate. Considering there are no randomizing studies proving that, synchronous resections are reserved exclusively in patients with good general condition, low ASA score, as in the presented patient. A study performed by Eom et al. [15] including 4,593 patients with operated GC shows a 5-year survival rate in 55,2% of cases having combined resection due to synchronous GC and CRC, and 43,8% from that number due to GC dissemination without postoperative mortality. It has been considered that survival depends directly on GC stadium while not on other synchronous carcinoma [15, 16].

In the presented patient clinical profile coincided with the epidemiological characteristics described in the literature – male, between 51 and 81 years old, heavy smoker with alcohol abuse [11, 17]. From the histopathological aspect, in the majority of cases one of the tumors is early and well differentiated carcinoma [10, 11]. In the presented case both carcinomas are invasive with the histological gastric grade G3 and colon grade G2. The dominant symptomatology in the patient addressed on gastric etiology, with the presence of colon disease incipient symptomatology. In most of cases, there was the dominance of obstructive colon symptoms, while the gastric disease symptoms were moderate or overlapping [18], leading to a mistake in preoperative diagnostics, and the synchronous gastric carcinomas were incidentally noticed intraoperatively [19]. The majority of synchronous GC are early carcinomas, localized in the antrum while CRC in the left colon and the rectum [20–22]. In the presented case, it was invasive, infiltrative carcinoma of the gastric fundus, thus explaining the fast development of gastric symptoms and a difference in symptomatology.

Preoperative EGD and colonoscopy represent standard procedures in some institutions [10]. The advancement and availability of radiological diagnostic methods evaluated during years, as well as the possibility of locating lesions, especially in early phase.

Conclusion

The presented patient suggests that in case of any doubt on symptomatology not corresponding with the diagnosis of digestive tract primary tumor, a possibility of synchronous multiple carcinomas should be considered. The detection of synchronous gastric cancer and colorectal cancer, obtained by easily available diagnostic methods, enabled us to treat both cancers simultaneously and thus beneficially influence the prognosis and the quality of life of the patient. Therefore, simultaneous resection represents the adequate approach to surgical treatment, although a longer follow-up is required to demonstrate oncological adequacy.

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CASE REPORT

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The effect of whole-body vibration and resistance training on muscle strength in a 13-year-old boy with *m. biceps femoris* lesion and posttraumatic calcification

Efekti primene vibracionog treninga i treninga sa dodatnim spoljašnjim opterećenjem na razvoj snage kod 13-godišnjeg dečaka nakon lezije *m. biceps femoris* i posttraumatske kalcifikacije

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Abstract

Introduction. Skeletal muscle atrophy is a common adaptation after major muscle lesion of *m. biceps femoris* that results in numerous health-sport related complications. Resistance strength training and whole-body vibration (WBV) have been recognized as an effective tool, which attenuates atrophy and evokes hypertrophy. **Case report.** We presented a 13-year-old boy with a lesion of *m. biceps femoris* and post-traumatic calcification sustained in soccer training session 6 month prior participation in this study. The patient underwent training 3 times a week for 7 weeks, including unilateral progressive WBV + resistance training (RT) of the right hamstrings muscle group using WBV and weights. Hamstrings muscle strength was measured using a Cybex isokinetic dynamometer. At the end of week 4, the patient peak torque value of the involved leg increased from 39% body weight (BW) to 72% BW and bilateral deficit decreased from -64% to -35%; at the end of week 7 the participant's peak torque value of the involved leg increased from 72% BW to 98% BW and bilateral deficit decreased from -35% to -3%, respectively. **Conclusion.** Unilateral WBV + RT protocol evokes strength increase in the hamstrings muscle group. This case study suggests that adding WBV, as well as the RT program have to be considered in the total management of strength disbalance. Further studies are needed to verify the efficiency of WBV + RT protocol over the classic physical therapy exercise program.

Key words:
athletic injuries; young adult; muscular atrophy; femur; physical therapy modalities.

Apstrakt

Uvod. Atrofija skeletnih mišića česta je promena nakon lezije mišića *m. biceps femoris* koja rezultira brojnim zdravstvenim problemima nastalim u sportu. Trening snage sa dodatnim spoljašnjim opterećenjem (RT) i vibracioni trening (WBV) su dokazane metode smanjenja mišićne atrofije i povećanja hipertrofije mišića. **Prikaz bolesnika.** Prikazali smo dečaka, uzrasta 13 godina, sa lezijom *m. biceps femoris* i post-traumatskom kalcifikacijom koja je nastala na fudbalskom treningu šest meseci pre početka lečenja. Primenili smo unilateralno progresivno rastuće WBV i RT opterećenje mišića zadnje lože natkolenice desne noge. Trening je sprovođen u vremenskom periodu od sedam nedelja, tri puta nedeljno. Snaga mišića zadnje lože natkolenice merena je izokonetičkim dinamometrom (Cybex). Na kraju četvrte nedelje relativna vrednost momenta sile u odnosu na telesnu težinu (BW) desne noge povećala se sa 39% BW na 72% BW, dok se bilateralni deficit smanjio sa -64% na -35% u odnosu na početno merenje. Na kraju sedme nedelje lečenja, relativna vrednost momenta sile prikazanog dečaka u odnosu na BW desne noge povećala se sa 72% BW na 98% BW, dok se bilateralni deficit smanjio sa -35% na -3%. **Zaključak.** Unilateralni WBV i RT povećali su snagu mišića zadnje lože natkolenice. Ovo istraživanje ukazuje na mogućnosti uključivanja WBV i RT u protokole prilikom lečenja mišićnog disbalansa. Postoji opravdana potreba za daljim istraživanjima koja bi potvrdila veću efikasnost WBV + RT protokola nad klasičnim pristupom fizikalne terapije.

Ključne reči:
povrede, atletske; mlade osobe; mišići, atrofija; femur; lečenje vežbanjem.

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Introduction

Hamstring strain injuries mostly occur in activities that include elements of running or sprinting. In his study about hamstrings injuries Agre [1] suggests several causes that lead to injury of the hamstring musculotendinous unit. For example, the lack of flexibility, imbalances in hamstrings muscle strength ratio, poor running biomechanics, dyssynergic muscle contraction during running, improper warm-up activities and premature return to activities after uncompleted rehabilitation program are the factors that initiate such condition. According to Kujala et al. [2] in the lengthening phase of contraction, muscle is not always capable to adequately respond to stimuli, which results mainly in partial hamstrings muscle tear, but the authors also leave the possibility of other injury mechanisms. Muscle impairment prevalence due to quick extension of the lower leg at the knee joint is common occurrence. The reason could be found in motion which provokes elongation of hamstrings muscle fibers in order to decelerate the forward movement of the shin bone in the late swing phase. A paper by Petersen and Holmich [3] suggests that the moment of instantaneous change of muscle action from eccentric to concentric caries the highest incidence for injury. However, Croisier et al. [4] arise the question whether strength imbalances are the result of previous muscle injury, or inducement element for reinjury occurrence, or both.

Structural changes of myofascial tissue after injury consequently lead to a decrease in hamstring muscle strength [5]. Positive effects of resistance training (RT) on physical condition of both children and adolescents are achievable with suitably prescribed and supervised exercise programs [6]. Fyfe et al. [7] explain that positive effects of eccentric training and underlying physiological mechanisms were clarified over the last seven decades. Eccentric training took its necessary place in strength training. The authors also state the existence of a pile of evidence in the literature, in recent years, supporting benefits of eccentric exercise in treatment of number of tendinopathies, muscle strains, and in the anterior cruciate ligament (ACL) rehabilitation protocols. However, in recent years, whole-body vibration (WBV) draw a lot of attention of scientists and practitioners, and it was used as an additional tool in exercise prescription in order to improve neuromuscular properties in sport performance and rehabilitation, as well [8]. Also WBV has been proposed as the exercise method for injury prevention and rehabilitation [9–11].

RT and WBV have been suggested as effective interventions to address the loss of hamstring muscle strength following injury but the combination of both has not been reported in the literature. We studied the effect of combining RT and WBV on changes in hamstring muscle strength for the presented patient with a lesion of *m. biceps femoris*.

Case report

In this case report we investigated the effects of combining RT and WBV in a 13-year-old boy with a lesion of *m. biceps femoris* and post-traumatic calcification sustained in soccer training session 6 month prior to participation. The patient underwent training 3 times a week for 7 weeks, including unilateral progressive WBV and RT of the right hamstrings muscle group using WBV and weights.

Whole body vibration was provided by power plate next generation vibration platform (Power Plate North America, Chicago, IL), strength training was provided by a Space gym multigym unit (Space gym, Novi Sad, Serbia).

The intervention protocol for this study was intended to be progressive in RT as well as in WBV in regard to WBV frequency, amplitude, and duration, since this has previously been shown to be effective in healthy population [12]. The protocol consisted of supersets of RT exercise without vibration combining with WBV of the same biomechanical pattern.

Eccentric hamstring curl is performed using a leg-curl machine. A subject in lying position lifts weight with two legs and lowers the weight with the involved leg. The pelvic bridge is performed using a power plate. A subject lies with the knees bent 90° with the foots on a vibration platform. His/her performs a full double leg bridge hold in the top position and extend non-involved knee to full extension, then his/her as flexed in hips doing hip thrusts.

The Nordic hamstring exercise (NHE) is a bodyweight exercise. In the presented patient we used the NHE protocol described by Mjolsnes et al. [13]. The patient's starting position was kneeling from, then the patient lowered his upper body towards the ground by using eccentric contraction of the hamstrings muscle group, while the ankles were held down by a partner. We assumed that the NHE increase eccentric hamstring torque.

Due to insufficient muscle strength single legged squat was executed with the patient standing with one arm extended out in front and with the other one holding a vibration machine. The balance of the involved leg with the opposite leg extended straight leg forward as high as possible. The subject squats down as far as possible, while keeping leg elevated off the floor, keeping back straight and supporting knee pointed the same direction as foot supporting. Raising body back up to the start position until knee and hip of the supporting leg are straight. Progression for squatting while holding vibration machine with hand was 6, 8, 10, 12, 15 repetitions. When the patient was able to perform 15 repetitions holding a vibration machine with the hand he started to perform single legged squat, standing with both arms extended out in front and without holding thevibration machine. Single legged isometric stance was performed standing on a platform with flexion in knees of the involved leg of 110 degrees with the opposite leg extended straight leg forward as high as possible according to the modified protocol of de Ruiter et al. [14] (Table 1).

The patient underwent the WBV training protocol following progression parameters shown in Table 1.

Isokinetic measurement of concentric/concentric hamstring/quadriceps torque was measured using an isokinetic (Cybex – NORM – CSMI, Stoughton, Massachusetts) dynamometer. Testing had four sets. For the first two tests angular velocity was set at 60°/s with five repetitions of trial test, before four repetition tests. For the third and fourth sets, angular velocity was set at 180°/s with 4 repetitions and 15 repetitions, respectively. Test was performed for each leg. These sets were performed with a 2 min rest between the sets. The patients was

Table 1

Whole-body vibration (WBV) training protocol and WBV progresion parameters

Parameters	Exercise	Tempo	Repetitions (n)	Sets (n)	Rest (s)		
1st superset							
A1	Eccentric hamstring curl	61×	6	3	30		
A2	WBV pelvic bridge	31×	6	3	30		
2nd superset							
A1	Nordic hamstring exercise	61×		3	30		
A2	WBV isometric pelvic bridge			3	30		
3rd superset							
A1	Single legged squat	31×	6	3	30		
A2	WBV single legged isometric stance			3	30		
WBV progresion parameters							
Frequency (HZ/a)	Week 1 30Hz/2mm	Week 2 35Hz/2mm	Week 3 30Hz/4mm	Week 4 35Hz/2mm	Week 5 35Hz/2mm	Week 6 35Hz/2mm	Week 7 35Hz/2mm

n – number; s – seconds; × – movement is performed explosively with full acceleration; a – amplitude.

seating on the Cybex with his hip joint at approximately 90° flexion, the upper body secured with dual crossover straps and the waist secured by a waist strap. The range of motion of the knee was set at 90° of full extension, with the upper leg secured using the thigh strap to limit excess movement of the knee and the limb. The main variables tested were peak torque, total work, endurance ratio and average power. Before the commencement of each testing speed, the patient was allowed to familiarize himself with 3 trials. The non-involved limb was tested first. Verbal encouragement at a conversational level was given during testing. The testing apparatus was regularly calibrated according to the manufacturer instructions.

The peak torque deficit for both legs of extensor muscles strength was in the acceptable range ≤ 10% [15], but bilateral deficit of peak torque of flexors muscles showed the deficit of -64% for the speed 60/60 and -47% for the speed 180/180, respectively.

We noticed a decrease in flexors muscles deficit for the speed 60/60, -35%; 180/180, -8%, respectively two months later.

The third measurement showed the increase in the right leg flexors peak torque although it was a noticeable decrease in the left leg peak torque (Table 2).

Discussion

This protocol was conducted to determine the effect of combined WBV+RT on changes in the knee muscles strength in those with the lesion of *m. biceps famoris*. The in-

Table 2

Isokinetic values for the first, second and third measurements

Measurement	Extensors (Con)		Deficit	Flexors (Con)		Deficit
	Value	%BW	%	Value	%BW	%
First measurement						
Peak Torque (Nm - average)						
speed 60/60 Right	89	200	-10%	18	39	-64
speed 60/60 Left	99	220		49	107	
speed 180/180 Right	66	146	-8	26	57	-47
speed 180/180 Left	72	161		49	107	
Second measurement						
Peak Torque (Nm - average)						
speed 60/60 Right	98	218	-8	33	72	-35
speed 60/60 Left	89	200		50	110	
speed 180/180 Right	68	152	-4	33	72	-8
speed 180/180 Left	71	158		35	77	
Third measurement						
Peak Torque (Nm - average)						
speed 60/60 Right	103	229	-11	45	89	-3
speed 60/60 Left	92	206		46	92	
speed 180/180 Right	71	158	-5	41	89	-3
speed 180/180 Left	75	168		45	92	

BW – body weight.

tervention produced positive changes in the peak torque and the percentage of body weight values in the involved leg during tree testing phases both flexor and extensor muscles. Positive changes were noticed in the non-involved leg also but in fewer amounts. During the third testing we noticed a decrease in healthy leg flexor muscle peak torque, which could be attributed to internal factors of a subject's motivation.

To our knowledge, this is the first Serbian study of combined WBV and RT treatment in a 13-year-old boy with *m. biceps femoris* lesion and post-traumatic calcification. There are relatively few studies that investigate effects of combined WBV and RT in children. Relatively heterogeneous findings in studies could be attributed to different training protocols, WBV machines (vertical *vs* pivotal tilting platform), subject's condition, etc, which make it hard to compare the outcomes. For instance, Stark et al. [16] conducted WBV, physiotherapy, resistance training and treadmill training in bilateral spastic cerebral palsy children. The results of that study showed that after 6 months of training the combined method resulted in the increased maximal force in extension totally 7.9%. Mahieu et al. [17] observed isokinetic knee muscle strength of healthy young skiers aged 9–15 after a 6-week training period. The authors noticed changes in hamstring peak torque (pre 66.36 Nm, post 74.25 Nm, 11.88% increase at angular velocity of 60°/s; pre 56.46 Nm post 64.17 Nm 13.65% at angular velocity of 180°/s). Comparing our findings and the results from a study of Mahieu et al. [17] greater strength increase could be noticed in our study in hamstring peak torque after the treatment. The reasons could be found in the fact that Mahieu et al. did not superset WBV exercise with RT. Also the subjects from the study of Mahieu et al. were young athletes already in training so the amount of strength increase was expectedly lower, than with a subject with *m. biceps femoris* lesion. Supersets combined of WBV and RT make a distinction between this study and other investigations.

In a study by Clark et al. [18] the minor changes in hamstring peak torque (dominant leg pre 98.61 Nm, post 99.00 Nm; nondominant leg pre 97.30 Nm, post 103.64 Nm) was noticed after 4 weeks of Nordic eccentric training in healthy amateur football players, but the authors noticed the improvement in vertical jump which they attributed to the changes in the position of peak hamstring torque. Rauch [19] describes advantageous effects of WBV on the gain of muscle functions over regular activities. In a recent study of Moawd et al. [20] healthy adults performed RT after WBV sets of similar biomechanical pattern (half squat) followed by polymetric jump type exercises, placebo group performed WBV sets on a platform without vibrations. The authors noticed a superior, 8% increase in isometric strength knee flexion in the WBV group compared to the placebo. In a study by Karatrantou et al. [21] the authors show 11.77% increase in isokinetic hamstring strength after < 2 months WBV training in healthy adult females. Comparing our results with the results of Karatrantou et al. [21] a greater strength increase in this study is evident, which can be attributed to the different methodological approach. In orthopedic rehabilitation the improvement in knee stability and proprioception *via* increased effectiveness in muscle reflex excitability has been found [22]. In addition to rehabilitation purpose Semler et al. [23] found improved mobility in motor impaired children after WBV treatment.

One of possible mechanisms of strength increase due to combining WBV+RT could be found in a study of Davis et al. [24] where the authors explain that greater forces in muscle are generated by placement of a participant on a vibrating platform. In their study, Bressel et al. [25] notice that children have greater vibration transmissibility than adults in the ankle and hip area. Mechanical vibrations evoke reflex muscle contractions which are according to Cardinale and Bosco [26] "mediated not only by monosynaptic but also by polysynaptic pathways". In a recent study by Pollock et al. [27] recorded recruitment thresholds from 38 motor units (MU) before and after WBV. The authors noticed that lowest MU increased their threshold while in higher MU firing threshold was decreased. This information indicates that WBV has preferential effect on higher MU, which is responsible for strength and power output. The authors also indicate that such response on higher threshold MU exists due to the use of polysynaptic pathways, which are not related with low-threshold MU.

While it is not appropriate to generalize to all persons with a lesion of *m. biceps femoris* muscle and post traumatic calcification, based on the results of this case, the results support earlier literature with regard to improvement in strength ratio by resistance training alone [28] and WBV alone [29]. The results from the first measurement supported the literature findings regarding decreased hamstring muscle strength following injury [30, 31]. It seems that combining RT and WBV copies the effects of both training methods. The results of this case report might suggest the direction for future studies. The single greatest limitation of this investigation is that it is a case study and should not be generalized to other individuals with *m. biceps femoris* lesion and post-traumatic calcification. Progressive resistance training applied combined with WBV is similar or in a way modified to the protocols used by others [13, 31].

Lastly, the schedule of intervention was established to meet the demands of the patient (primary school pupil), which had a flue 10 days from the week 4 until the week 5 across the study. These factors, related to the individual participant, might have affected the outcomes of this case report.

Conclusion

The unilateral whole-body vibration + resistence training protocol provided strength increase in the hamstrings muscle group. The findings of this case report suggest that adding whole-body vibration, as well as the resistance training program, must be considered in the total management of muscle strength imbalance. More studies are needed to verify the efficiency of whole-body vibration + resistence training program over the classic physical therapy exercise program. Furthermore, on the basis of the evidence in this study it is possible to conduct one randomized controlled trial

which will determine differences among the groups exercising only resistence training, only whole body vibration and resistence training + whole-body vibration. Further studies should investigate what is optimal dose response of intervention, i.e. intensity, duration, and frequency of whole body vibration. The dosage of resistence training regarding sets, repetitions, and resistance is widely understood but in a combination with whole-body vibration is still unclear.

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CASE REPORT

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Cervical poorly differentiated adenocarcinoma with dominant choriocarcinomatous pattern – A case report

Slabo diferentovani cervikalni adenokarcinom sa preovlađujućom formom horiokarcinoma

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Abstract

Introduction. Gestational trophoblastic neoplasm (GTN), choriocarcinoma in coexistence with primary cervical adenocarcinoma, is a rare event not easy to diagnose. Choriocarcinoma is a malignant form of GTN but curable if metastases do not appear early and spread fast. **Case report.** We presented choriocarcinoma in coexistence with primary cervical adenocarcinoma in a 48-year-old patient who had radical hysterectomy because of confirmed cervical carcinoma (Dg: *Carcinoma porto vaginalis uteri* FIGO st I B1). Histological findings confirmed cervical choriocarcinoma with extensive vascular invasion and apoptosis but GTN choriocarcinoma was finally confirmed after immunohystochemical examinations. Preoperative serum human gonadotropine (beta hCG) level stayed unknown. This patient did not have any pregnancy-like symptoms before the operation. The first beta hCG monitoring was done two months after the operation and found negative. According to the final diagnosis the decision of Consilium for Malignant Diseases was that this patient needed serum hCG monitoring as well as treatment with chemotherapy for high-risk GTN and consequent irradiation for adenocarcinoma. **Conclusion.** The early and proper diagnosis of nonmetastatic choriocarcinoma of nongestational origine in coexistence with cervical carcinoma is curable and can have good prognosis.

Key words:
uterine cervical neoplasms; adenocarcinoma; choriocarcinoma; comorbidity; histology; gynecologic surgical procedures.

Apstrakt

Uvod. Gestacijska trofoblastna neoplazma (GTN), horiokarcinom zajedno sa primarnim cervikalnim adenokarcinomom retko se javlja i nije ga lako dijagnostikovati. Horiokarcinom je maligni oblik GTN i izlečiv je ako se ne pojave rane metastaze koje se brzo šire. **Prikaz bolesnika.** Kod bolesnice, stare 48 godina, sa primarnim karcinomom grlića materice i istovemeno horiokarcinomom urađena je radikalna histerektomija zbog potvrđenog karcinoma cerviksa (Dg: *Carcinoma porto vaginalis uteri* FIGO st I B1). Histološkim pregledom potvrđen je horiokarcinom cerviksa sa rasprostranjenom vaskularnom invazijom i apoptozom, ali je definitivna dijagnoza GTN – *choriocarcinoma* potvrđena nakon imunohistohemijskog pregleda. Preoperatvne vrednosti humanog horiogonadotropina (beta hCG) u serumu nisu bile poznate. Pre operacije bolesnica nije imala simptome slične onim u trudnoći. Prvi beta hCG kontrolisan je dva meseca nakon operacije i bio je negativan. U skladu sa konačnom dijagnozom, preporuka konzilijuma za maligne bolesti bila je hemioterapija prema protokolu za GTN visokog rizika uz zračnu terapiju za adenokarcinom, kao i redovne kontrole serumskog hCG. **Zaključak.** Pravovremena i tačna dijagnoza nemetastatskog horiokarcinoma negastacijskog porekla zajedno sa karcinomom cerviksa je izlečiva i može da ima dobar ishod.

Ključne reči:
grlić materice, neoplazme; adenokarcinom; horiokarcinom; komorbiditet; histologija; hirurgija, ginekološka, procedure.

Introduction

Gestational trophoblastic neoplasms (GTNs) represent the disturbance of fertilization and usually appear in women younger than 20 and older than 40. In the majority of instancies GTNs are developed as benign forms (complete or partial hydatidiform mole). Malignant forms of GTNs can seriously damage reproductive health and even have a letal outcome. Choriocarcinoma is a highly potent malignant form most often localized in uterine tissue. Trophoblast invasion

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and fast metastases spreading is following high risk choriocarcinoma with poor prognosis. Choriocarcinoma is usually of gestational origine and diagnosed after normal or molar pregnancy as well as after delivery. It can also be a nongestational event. Choriocarcinoma in coexistence with primary cervical adenocarcinoma is a rare event [1–5]. Cases of GTN in coexistance with primary cervical adenocarcinoma are very complex and it is not easy to make a fast and proper diagnosis. Serum human chorionic gonadotropine (hCG) is of a great value for diagnosing GTNs. Serum hCG level depends of syncyciotrophoblast activity and hormone secretion. It is necessary to check it during the follow-up programme after molar evacuation, surgical treatment and chemotrerapy [4–6].

In cases with coexsisting malignancies hystological examination could not give a final relevant answer and immunohistochemical techniques should be of a great value [5, 6].

Case report

A 48-year-old patient, 4 gravida, 2 para, was hospitalized for operative treatment because of cervical carcinoma (Dg: *Ca portio vaginalis uteri* FIGO st I B1). Radical hysterectomy was done.

Department of Histopathology Birmingham. The planomorphic tumor that invaded endocervical tissue with biphasic intimate mixture of cytotrophoblast and syncytial trophoblast was found. Some mononuclear cells were positive for human placental lactogen (HPL) which was likely to be an intermediate trophoblast component.

The differential diagnosis was between choriocarcinoma of the cervix, an epithelioid trophoblastic tumor of the cervix, and an adenocarcinoma of the cervix with the dominant choriocarcinoma pattern. The final diagnosis was: poorly differentiated adenocarcinoma with a dominant choriocarcinomatous pattern.

The patient did not have preoperative hCG monitoring, no any symptoms suggestive of pregnancy.

The first hCG monitoring was done two months after the operation and found negative result, < 1 IU/L, and a week later 2.7 IU/L. Chest and head radiography excluded metastases.

According to the final diagnosis the patient needed careful monitoring of the hCG levels as well as treatment with chemotherapy for high–risk GTN.

The patient rejected chemotherapy treatment and left after one month in good condition when the third hCG level was 58.3 IU/L.

Methotrexate + folinic acid (MTX+FA) was used to treat this nonmetastatic trophoblastic neoplasm (5-day treatment

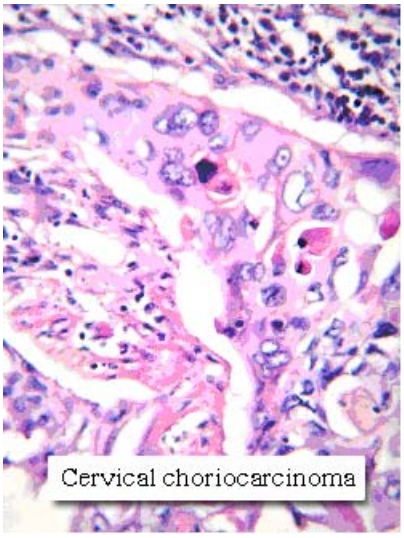

Fig. 1 – Histological finding of cervical choriocarcinoma (HE, ×20).

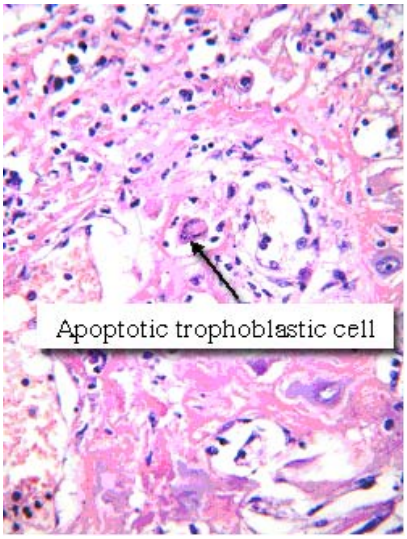

Fig. 2 – Apoptotic trophoblastic cells (HE, ×20).

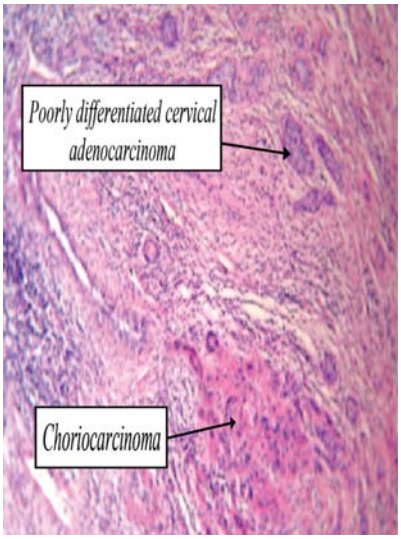

Fig. 3 – Poorly differentiated cervical adenocarcinoma (above) with the dominant choriocarcinomatous pattern (below) (HE, ×20).

Hystologycal findings confirmed pleomorphic tumor which invades through the endocervical tissue with extensive hemorrhage and necrosis. Cervical choriocarcinoma with extensive vascular invasion and apoptosis was also described (Figures 1 and 2). Immunohystochemical examination was performed and cervical adenocarcinoma and malignant gestational trophoblastic neoplasm was finally confirmed (Figure 3). Because of the unclear GTN diagnosis choriocarcinoma *vs* placental site trophoblastic tumor, tissue blocks of cervical tumour were sent to Consultant Histo/Cytopathologist-

cycle with the window of 10–14 days between the last day of one course and the first day of the next one).

After the first MTX+FA treatment the hCG level was negative, as well as after the second chemotherapy treatment.

The consiliar decision for this patient was to receive two MTX+FA regimens before irradiation therapy for adenocarcinoma. The third MTX+FA regimen was suggested two weeks after the irradiation treatment. The patient rejected the third MTX+FA treatment. Seven months after the operation, the patient was alive with the negative hCG level.

Discussion

It is believed that nongestational choriocarcinoma can develop from gonadal pluripotent germ cells and can be found in the liver, lungs, colon, bladder, breast, and other sites [1–5]. According to the literature cervical localization is very rare [6]. Serum hCG is the most relevant parameter in the diagnosis of GTN but it depends of secretion of syncyciotrophoblast which is a hormone active component of choriocarcinoma [1, 4–6]. Nongestational choriocarcinoma usually follows significantly lower hCG level than choriocarcinoma of postgestational origine [5]. Cytopathology and immunohistology is of a great help in the differential diagnosis. In excluding metastases, chest and brain radiography has to be done before any treatment.

In cases with complex malignancy as in the presented case treatment and follow-up must be performed not only for choriocarcinoma but also for adenocarcinoma [6].

Conclusion

The early and proper diagnosis of nonmetastatic choriocarcinoma of nongestational origine in coexistence with cervical carcinoma is curable and can have good prognosis.

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B O O K R E V I E W

Behavioural effects of the inverse agonists of benzodiazepine receptors

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Author/autor: Janko Samardžić
Publisher/Izdavač: Andrejević Endowment, Belgrade/ Zadužbina Andrejević, Beograd
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"Behavioural effects of the inverse agonists of benzodiazepine receptors" is an up-to-date monograph of highest interest for the experts in neuropsychopharmacology. The monograph is the result of a significant scientific research that the author, Dr. Janko Samardzic, did in the field of behavioural pharmacology, and is based on his doctoral thesis, defended on November 1, 2013 at the Medical Faculty, University of Belgrade. The book is a 110-page bilingual monograph, written in Serbian and English, with the following chapters: Introduction, Methodology in behavioural pharmacology, Behavioural effects of benzodiazepine receptor ligands, Development and prospect of benzodiazepine ligands research and Conclusions and clinical implications. It includes 12 figures and more than 160 references.

Introduction presents a review of recent findings involving γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the central nervous system (CNS), with the special review of the types of GABA receptors, the structure and function of $GABA_A$ receptor complex and benzodiazepine binding site (BDZ receptor). The new term is being introduced and explained in detail, for the first time: $GABA_A$ -BDZ-chloride channel receptor complex. The author then emphasizes the importance of studying behavioural effects of agonists, antagonists and inverse agonists of the BDZ receptor, and particularly their influence on the learning process, memory and motivation, anxiety and depression, and pharmacological characterization of these effects, as well.

Further, a review of animal models for the characterization of behavioural effects of BDZ receptor ligands, and, in detail, four tests complementary and widely used in the field of behavioural pharmacology, are presented: elevated plus maze (EPM), forced swimming test (FS), active avoidance of aversive stimuli (AA) and Morris water maze (MWM). Using this methodology, the results of various behavioural studies are presented, including the original results of the author on the subject of all-comprising influences of the inverse agonists of BDZ receptor on the learning process in rats, in the reaction of active avoidance of aversive stimuli, degree of motivation, anxiety and antidepressant activity. Besides, within the mechanism of action analysis of the used inverse agonist: methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM), a specific binding site is identified within $GABA_A$-BDZ-chloride channel of the receptor complex using the antagonist of benzodiazepine binding site – flumazenil. The concluding results of studying the influence of the inverse agonists of BDZ receptor on various behavioural effects of benzodiazepine in rats, and also the importance of the obtained results for clarifying the importance of BDZ receptor within $GABA_A$-BDZ-chloride channel receptor complex are presented and discussed.

This valuable book is very useful for the experts in the field of behavioural pharmacology, because it provides them with a guidance and proposals for current and future benzodiazepine ligands research and development. The reviewers pointed out that "…The monograph provides a significant contribution to the development of Medical Pharmacology. It represents one of the first reports on the influence of inverse agonists of BDZ receptor on the degree of motivation and depression. In clinical sense, negative modulation of GABA-ergic neurotransmission opens up a new field of research in the area of neurobiology of depression and learning which could represent a different approach in treatment of depression and cognitive disorders associated with them..."

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Academy, University of Defence, Belgrade, Serbia.

CORRIGENDUM

The article „End-of-life costs of medical care for advanced stage cancer patients”. Vojnosanit Pregl 2015; 72(4): 334–341 (DOI:10.2298/VSP1504334K).

The authors were listed as follows: Aleksandra Kovačević, Viktorija Dragojević-Simić, Nemanja Rančić, Milena Jurišević, Florian Gutzwiller, Klazien Matter-Walstra, Mihajlo Jakovljević

The fifth author Florian Gutzwiller should be listed as: Florian S. Gutzwiller

The correction has been made to the online version of that issue of the Journal which is available at: http://www.vma.mod.gov.rs/eng/vojnosanitetski-pregled/archive/2015#.VYPCZlKdfKE

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

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

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

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

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

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

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