YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

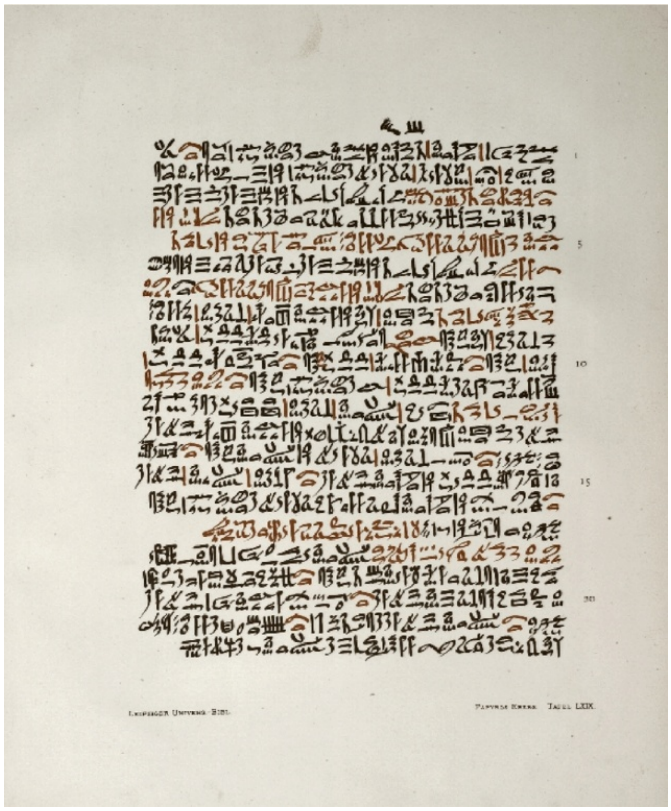
Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2022; August Vol. 79 (No. 8): pp. 747–844.

Vojnosanitetski Pregled 2022 August Vol. 79 (No. 8): pp. 747–844.



Vol. 79, No. 8, 2022

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of Vojno-sanitetski glasnik which was published between 1930 and 1941

PUBLISHER
Ministry of Defence of the Republic of Serbia, University of Defence, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720

Editorial Office: University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia. E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Serbian Citation Index (SCIndex), DOAJ. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

Vol. 79, br. 8, 2022

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine
Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Ministarstvo odbrane Republike Srbije, Univerzitet odbrane, Beograd, Srbija

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

Adresa redakcije: Univerzitet odbrane, Medicinski fakultet Vojnomedicinske akademije, Centar za medicinske naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija. Informacije o pretplati (tel.): +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu" indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Srpski citatni indeks (SCIndeks), DOAJ. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ

The Ebers papyrus, one of the oldest known medical manuscripts, originated in ancient Egypt around 1550 BC.
Much of the knowledge on which modern medicine is based has its roots in the pre-Hippocratic period. Ancient civilizations had knowledge about the functioning of the human organism and the methods of surgical and medical treatment that they applied taking into account ethical principles in treating the sick. Read more about this topic in the paper by *Glišić M.* et al. (pp. 837 – 842).

Ebersov papirus, jedan od najstarijih poznatih medicinskih rukopisa, nastao je u starom Egiptu oko 1550. godina pre nove ere.
Mnoga saznanja na kojima se zasniva savremena medicina imaju svoje korene u periodu pre Hipokrata. Drevne civilizacije imale su znanje o funkcionisanju ljudskog organizma i načinima hirurškog i medikamentoznog lečenja koje su primenjivali uzimajući u obzir i etičke principe u lečenju obolelih. Više o ovoj temi pročitajte u radu *Glišić M.* i sar. (str. 837 – 842).

ORIGINAL ARTICLES

UDC: 615.38
DOI: https://doi.org/10.2298/VSP201114021B

Adverse events associated with donor plateletpheresis: A 10-year experience from Vojvodina, Serbia

Neželjeni događaji povezani sa trombocitaferezom kod davaoca: 10-godišnje iskustvo iz Vojvodine, Srbija

Zorana Budakov Obradović, Nevenka Bujandrić, Jasmina Grujić

Blood Transfusion Institute Vojvodina, Novi Sad, Serbia; University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia

Abstract

Background/Aim. Plateletpheresis (PLTP) is a medical procedure used for collecting donor platelets with multiple benefits for patients who will receive apheresis platelets. The procedure takes one hour and is well tolerated by donors. Nevertheless, adverse events (AEs) may occur during and after the PLTP procedure. The aim of the study was to determine the incidence and type of AEs associated with PLTP in donors. **Methods.** A retrospective analysis of AEs associated with donor PLTP was conducted at the Blood Transfusion Institute of Vojvodina from January 1, 2010, to December 31, 2019. **Results.** Out of 2,073 platelet donors, 94.84% were multiple blood donors, predominantly male (98.55%). AEs were identified during 180 (8.68%) platelet donations with no statistical significance in occurrence in the first time donors (10.28%) and repeat donors (8.59%). Mild local reactions related to venous access (42.22%) were the most common AEs. Generalized symptoms were exhibited in 16.67% of donors, 26.11% exhibited symptoms related to apheresis (citrate reactions), and 15% exhibited those related to other complications. It was found that 95.55% of AEs occurred during PLTP and only 4.45% after it. **Conclusion.** Donor PLTP is a generally safe procedure, well tolerated by donors. Understanding risk factors for a possible occurrence of AEs provides support for adopting measures to prevent them.

Key words:
blood donors; drug-related side effects and adverse reactions; plateletpheresis; risk factors.

Apstrakt

Uvod/Cilj. Trombocitafereza je medicinski postupak prikupljanja trombocita davaoca od koje pacijenti primaoci imaju mnogo prednosti. Postupak traje oko jedan sat i davaoci ga dobro podnose. Ipak, neželjeni događaji (ND) se mogu javiti, kako tokom, tako i nakon postupka trombocitafereze. Cilj rada bio je da se utvrdi učestalost i vrsta ND povezanih sa trombocitaferezom kod davaoca. **Metode.** Retrospektivna analiza ND povezanih sa trombocitaferezom sprovedena je u Zavodu za transfuziju krvi Vojvodine u periodu od 1. januara 2010. do 31. decembra 2019. godine. **Rezultati.** Od 2 073 davaoca trombocita, 94,84% su bili višestruki davaoci krvi, uglavnom muškarci (98,55%). ND identifikovani su tokom 180 (8,68%) trombocitafereza, bez statistički značajne razlike u pojavi između novih davalaca (10,28%) i višestrukih davalaca (8,59%). Blage lokalne reakcije povezane sa venskim pristupom (42,22%) bile su najčešći ND. Generalizovane simptome pokazalo je 16,67% davalaca, simptome koji se odnose na aferezu (citratne reakcije) 26,11% davalaca, dok je 15% davalaca imalo druge komplikacije. ND su se desili uglavnom tokom izvođenja trombocitafereze (95,55%), a svega 4,45% nakon nje. **Zaključak.** Trombocitafereza je, generalno, siguran postupak koji davaoci dobro podnose. Razumevanje faktora rizika od moguće pojave ND omogućava donošenje mera za njihovo sprečavanje.

Ključne reči:
krv, davaoci; lekovi, neželjene reakcije; trombocitafereza; faktori rizika.

Introduction

Plateletpheresis (PLTP) is a procedure used for collecting donor platelets. The procedure involves removing whole blood from a donor, centrifugation to separate the blood into individual components, removing platelets in the separated – standardized platelet bags, and reinfusing the remaining blood components into the donor's bloodstream. PLTP is

Correspondence to: Nevenka Bujandrić, Blood Transfusion Institute Vojvodina, Hajduk Veljkova 9A, 21 000 Novi Sad, Serbia.
E-mail: nevenka.bujandric@mf.uns.ac.rs

performed by the apheresis machine and takes between one and two hours [1]. The platelets can also be separated from the whole blood unit collected from the donor by way of the traditional mode. Apheresis platelets are collected from a single donor and are equivalent to 4–8 pooled units obtained from whole blood. An apheresis platelet concentrate contains 200–400 mL of plasma and a minimum of 3.0×10^{11} platelets [2–4]. The benefits of PLTP are decreased risk of transfusion-transmitted infections, allergic transfusion reactions, bacterial contamination, as well as prevention of alloimmunization with platelets and leukocyte antigens due to the reduction of the number of donors a recipient is exposed to. Another significant benefit is providing leukocyte-reduced platelets by a modern generation of apheresis machines [5].

Donors can donate apheresis platelets more frequently than whole blood – a maximum of twice in 7 days or 24 times a year. A specially designed machine ensures the safety of the platelet donor during the procedure so that the procedure is well tolerated by the donor. Nevertheless, adverse events (AEs) of variable severity may occur not only during but also after the PLTP procedure [6, 7]. An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medical treatment or procedure that may or may not be considered related to the medical treatment or procedure [8, 9]. Local and systemic AEs during PLTP may be the result of a number of causes, but they almost always occur as mild reactions, very well tolerated [10, 11].

This study was performed at the Blood Transfusion Institute (BTI) of Vojvodina, Novi Sad, Serbia, one of the largest Serbian blood transfusion services and blood donation centers, which collects a total of 48,000 blood units and 200 platelet collections by PLTP annually. The BTI of Vojvodina meets the blood supply demand of secondary hospitals located in Vojvodina (north part of Serbia) and tertiary hospitals located in the city of Novi Sad.

The aim of the study was to present single-center experiences in order to determine the incidence and type of AEs associated with donor PLTP.

Methods

A retrospective analysis of AEs associated with donor PLTP in the BTI of Vojvodina, Serbia, was conducted from January 1, 2010, to December 31, 2019. Data from the register of AEs related to donation were used. During the observation period, 2,073 donors underwent PLTP. PLTP was done using two mobile cell separators: the Haemonetics MCS®+ cell separator (Braintree, MA, USA) and the Trima Accel® Automated Blood Collection System (Terumo BCT).

PLTP was performed on healthy non-remunerated first-time volunteers or repeat blood donors (BD), some of whom donated platelets for the first time. After the donors completed a questionnaire with standard questions relating to their general health, lifestyle, travel history, past medical history, and medication, they were physically examined, and the donated samples underwent serological and molecular tests for markers of four transfusion-transmitted pathogens (human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and *Treponema pallidum*).

Criteria for blood donor selection

In addition to the general criteria for the BD selection, platelet donors should be selected in accordance with the following criteria: the donor must have more than 60 kg of body weight; must be between 18 and 60 years old; the hemoglobin level of red cells must be greater than 125 g/L for a female and greater than 135 g/L for a male; the minimum pre-donation platelet count must be 150×10^9/L; an interval of 2 months between donations of whole blood must exist; an interval of 15 days between apheresis platelet donations must exist [12].

Classification of donor AEs

According to the time of occurrence, AEs are divided into AEs occurring during procedures and AEs occurring after the procedures.

All AEs were recorded and classified according to the International Haemovigilance Network categories of donor AEs in the following complications: complications mainly with local symptoms; complications mainly with generalized symptoms; complications related to apheresis such as citrate reaction, hemolysis, generalized allergic reaction, and air embolism; other complications related to blood donation [9].

Local reactions related to venous access are the following: hematomas (caused by incorrect placement of the needle during the venipuncture), pain, hyperemia, swelling; pain due to the subcutaneous nerve irritation/injury; local phlebitis and thrombophlebitis; delayed bleeding; local allergy.

Systemic reactions include vasovagal reactions (immediate/delayed), pallor, sweating, dizziness, gastrointestinal disorders, nausea, hypotension, and bradycardia.

Differentiation based on the severity of the AEs

Based on the severity of the AEs, the following division was made: Grade 1 – mild (high blood pressure, vein collapse, poor vein flow, lip tingling, tongue tingling, facial tingling, weakness and fainting, urticaria at the injection site); Grade 2 – moderate (sweating, nausea); Grade 3 – severe (collapse).

Personal information concerning the donors' age, gender, address of residence, types of donations (first time/repeat), dates of all previous donations, previous and current deferrals, screening test results, and platelets donation history (yes/no) were obtained from the information system.

Statistical analysis

The Fisher's exact test and χ^2 test were used for assessing the occurrence of AEs in two donor groups (who donated blood/platelets for the first time or multiple times, men and women of different ages, relating to using different machine models). A *p*-value of 0.05 and less was considered statistically significant. Prism (GraphPad) statistics program was used for statistical analysis.

Results

During the study period, 2,073 persons donated platelets. Just 107/2,073 BDs donated platelets for the first time (5.16%). The majority of platelet BDs (1,966/2,073) were multiple BDs (94.84%).

The AEs were identified in 180/2,073 (8.68%) platelet BDs. Out of 107 first-time BDs, 11 (10.28%) suffered AEs. Out of 1,966 repeated BDs, 169 (8.59%) suffered AEs. The Fisher's exact test (p = 0.4845) showed no statistically significant difference in the incidence of AEs in BDs who donated blood for the first or multiple times.

The majority of donors who suffered AEs (93.89%) belonged to the repeat BDs (169/180). Out of 169 repeated BDs with AEs, 45 (26.63%) underwent PLTP for the first time, and 124 (73.37%) had already undergone PLTP. Ten BDs had already experienced AEs during the previous PLTP.

Out of the 2,073 donor PLTP, 1,151 (55.54%) were performed using Trima Accel® and 922 (44.46%) using Haemonetics MCS®+ cell separator. The analysis showed AEs in 112/1,151 (9.74%) platelet donors on the Trima Accel® and in 68/922 (7.42%) platelet donors on the Haemonetics MCS®+ cell separator. The Fisher's exact test (p = 0.06) showed no statistically significant differences between the groups and outcomes.

The most common cause of AEs associated with donor PLTP was venipuncture in 76 (42.22%) donors. Types of AEs during donor PLTP are shown in Table 1.

The largest number of AEs occurred due to local symptomatology in 76/2,073 (3.66%) donors.

Mild AEs occurred in 166 (92.22%), moderate in six (3.33%), and severe in eight (4.45%) donors. The most common AE was a collapsed vein that occurred in 41 (22.78%) BDs.

The demographic characteristics of platelet donors (gender, age) are shown in Table 2.

No statistically significant differences were observed in the occurrence of AEs between men and women (Fisher's exact test, p = 1).

AEs were statistically significantly more frequent in platelet donors aged 36-45 (the χ^2 statistic was 51.767, p < 0.00001).

The study showed that 172 (95.55%) AEs occurred during PLTP, while eight (4.45%) AEs occurred after PLTP.

Table 1

Types of adverse events (AEs) during donor plateletpheresis (PLTP)

Adverse events	Platelet donors with AEs		Total
	first time BDs	repeated BDs	n (%)
Local symptoms			
high blood pressure during returning blood		5	5 (2.78)
collapsed veins		41	41 (22.78)
poor blood flow	3	27	30 (16.66)
Total			76 (42.22)
Generalized symptoms			
weakness, fainting		16	16 (8.89)
nausea and sweating		6	6 (3.33)
vasovagal syncope	2	6	8 (4.45)
Total			30 (16.67)
AE related to apheresis – citrate reaction			
lip tingling	6	32	38 (21.11)
tongue tingling		3	3 (1.67)
facial tingling		6	6 (3.33)
Total			47 (26.11)
Other complications			
lipemic plasma		6	6 (3.33)
icteric plasma		3	3 (1.67)
injection site urticaria		3	3 (1.67)
instrument failure		15	15 (8.33)
Total			27 (15.00)

BDs – blood donors.

Table 2

The demographic characteristics of platelet donors with adverse effects (AEs)

Parameter	Platelet donors	Platelet donors with AEs
	n (%)	n (%)
Gender		
male	2,043 (98.55)	178 (8.71)
female	30 (1.45)	2 (6.67)
Age (years)		
18–24	427 (20.60)	14 (3.28)
26–35	777 (37.48)	63 (8.10)
36–45	629 (30.34)	93 (14.78)
46–60	240 (11.58)	10 (4.17)

The study did not analyze the most common causes of blood donor deferral, although 1,891 BDs were rejected. Out of them, 252/359 (70.19%) were first time BDs and 1,639/3,605 (45.47%) were repeated BDs; 189/219 (86.30%) were female BDs and 1,702/3,745 (45.45%) were male BDs. The total number of platelet donations compared to the number of donors who tried to donate platelets was 2,073/3,964 (52.29%).

Discussion

The study found that the AEs occurred in 8.68% of platelet donors as well as that most of them occurred during PLTP. Mild local reactions related to venous access were the most common AEs observed with apheresis procedures. The highest occurrence of AEs was recorded in platelet donors aged 36–45. During the observation period, the study found that repeat male BDs were mostly selected for platelet donors. Although no statistically significant difference was found in the incidence of AEs in platelet donors who donated blood/platelets for the first or multiple times, the majority of donors who suffered AEs (93.89%) were repeated donors (169/180). No statistical difference in the occurrence of AEs between men and women was found. However, AEs happened significantly more frequently in donors who experienced AEs during the previous PLTP. No significant difference was noted in AEs related to the two cell separators used for PLTP.

Platelet transfusions are used for prophylaxis and treatment of platelet-related bleeding. The indication for platelet transfusion depends on the platelet count and function, bleeding pathology, risk factors for bleeding, as well as the underlying disease [13]. Nowadays, the use of platelet concentrates as well as the use of apheresis platelet concentrate is increasing because collecting the platelets from a single donor improves the chance of a successful transfusion [2, 14]. Platelet supply management is not easy due to variable daily demand and short shelf life. Furthermore, a significant impact on the future platelet supply could have an increase in the demand for platelets as well as a reduction of the active donor base. British Society for Hematology Guideline pointed out that the majority of platelets in the UK are collected from approximately 14,000 registered platelet donors (apheresis platelets) while the whole BD base is steadily dropping by a 35% reduction in 15 years [15]. In Vojvodina, the entire blood collection dropped by 15% in two years, which reflects on the management of patients who require platelet transfusion. Minimizing platelet waste as well as minimizing AEs in order to achieve donor retention has become an essential requirement in guaranteeing optimal patient care.

PLTP is generally considered safe, although some AEs of varying severity may occur during or after the PLTP procedures. The incidence of AEs related to PLTP is usually low, which points to the fact that the procedure is well tolerated by donors. It is an important factor for donor recruitment and retention. PLTP has a lower incidence of AEs compared to whole blood donation, which can be explained by the longer-lasting donor preparation [14]. Additionally, platelet donors are selected not only based on general criteria for whole blood donation but also on specially defined criteria for PLTP. The results concerning the total number of platelet donors who had some type of AEs (8.68%) presented in the study are slightly more frequent than literature data (6.06%) which confirm that PLTP, although invasive, is relatively well tolerated [16]. The fact identified in the study indirectly indicates the possibility of donors' safety level improvement. At the same time, the study showcases that mild AEs were most common.

Understanding PLTP-related risk factors for AEs assists in the prevention of the occurrence of AEs. In the study, the majority of repeated BDs with AEs who had already undergone PLTP had their previous experiences on Haemonetics MCS®+ cell separator, as the first apheresis machine was used in the BTI of Vojvodina, Serbia. It took them a while to get used to a different style of machine, even if the machine had continuous blood flow with less procedure time, less volume processed, and less volume of used anticoagulant citrate dextrose (ACD). Ultimately, both donor recruitment and donor retention showed that the donors were comfortable using both separators.

The study has several strengths related to the design: long-term study, detailed information about the study participants, and the link between routine practice and later outcomes. Our study also has one limitation. The limitation is the non-notation of the type of AE associated with each individual cell separator. As both cell separators are used worldwide, we have overlooked that those procedural problems related to the new separator can affect the appearance of AEs. However, the findings of this study offer new, potentially useful information for our future work. Finally, we could not control which separator would be associated with icteric or lipemic plasma type complications as well as local symptoms related to venous access.

The study found that the largest number of AEs was due to local symptomatology (42.22%). Vein collapse and poor vein flow during the apheresis procedure were the most common AEs associated with venipuncture. In a four-year study examining the occurrence of AEs, Diekamp et al. [17] reported that discontinued collections due to venous access problems, repeated venipuncture, and small hematomas were the most common AEs. In order to prevent such occurrences, the vein must be of a certain caliber, and the placement of the needle during the venipuncture must be correct as the same vein in the arm is used for the inflow and return of blood. Although the platelet donors with AEs who participated in this study did not attach special importance to these events, in order to prevent the risk of these AEs and to increase the return rate of platelet donors, the appropriate selection of donors according to the given criteria is necessary, as well as the evaluation of the quality of the cubital vein of both arms. Therefore, more rigorous selection criteria than those for whole BD are required [8, 18].

The study identified mild forms of AEs attributed to citrate in the form of tingling of lips, tongue, and face, which comprise one-quarter of all AEs identified in the study participants. The overall incidence of citrate reaction

during PLTP (47/2,073) remains low (2.27%), and these findings seem comparable to those found in the literature incidence rate reports, which range between 2.7% and 3.03% [18]. Citrate intoxication during PLTP was caused by the administration of the citrate anticoagulant ACD-A, which chelates calcium ions, leading to a decrease in their plasma concentration. Despite compensatory mechanisms that reduce the concentration of citrate in the extracellular fluid (intensive metabolism of citrate in the kidneys, liver, and muscles, as well as the return of blood to the circulation during apheresis), symptoms caused by a decrease in the concentration of calcium ions were realistically possible [19]. Routine determination of calcium ion concentration during the preparation of platelet donors was not performed. However, to prevent AEs of citrate etiology, donors were supplemented with calcium salts (calcium lactate gluconate and calcium carbonate) before and during the procedure. In a similar study performed in Southern India, similar citrate-related toxicity reactions (2.43%) were seen [20]. Citrate toxicity due to hypocalcemia may cause perioral paresthesia of the extremities, tremors, dizziness, chills, tetany, and seizure.

Most studies have shown that vasovagal reactions are usually of mild intensity, in the form of weakness and fainting, and, in most cases, allow the performance of platelet procedures in their entirety [21]. The study found that mild forms of syncopal reactions were the most common but that moderate and severe forms of syncopal reactions were also reported. It must be taken into account that these types of reactions arise as an effect of psychological factors prompted by the dynamics and the length of the procedure. For this reason, appropriate donor selection and proper psychophysical preparation for PLTP could be essential factors for preventing syncopal AEs. Although we are certain that vasovagal reactions occur more frequently among female donors because of the smaller circulatory volume [20], we were unable to show it in our study due to a relatively small sample size of female donors.

Technical aberrations due to machine malfunction in 15/2,073 (0.72%) donors were the least frequent causal factor of the AEs. In a study from Iraq by Bassi et al. [22], 0.94% technique-related AEs were found, while 1.40% AEs associated with defective kit/equipment were recorded. These types of errors should be minimized, but in reality, their occurrence cannot be ruled out.

Donor care is ensured by recognizing and diagnosing AEs, which occur during and after donor PLTP, as well as appropriately investigating and treating them. Systematic records, collation, and analysis of AEs, as well as continued monitoring and reporting, will establish a platform for evaluating the occurrence of AEs and ensure timely response as necessary. We have noted the need for professional and post-qualification staff training, as well as educating and helping donors prepare for platelet donation.

Conclusion

The low incidence of usually mild AEs related to PLTP indicates that the procedure is generally safe and well tolerated by donors. Understanding the PLTP-related risk factors for AEs provides support for the adoption of measures to prevent their occurrence.

Conflict of interest

None to declare.

R E F E R E N C E S

1. *Dogu MH, Hacioglu S*. Analysis of plateletpheresis donor deferral rate, characteristics, and its preventability. J Appl Hematol 2017; 8: 12–5.
2. *Kaufman RM, Djulbegovic B, Gernsheimer T, Kleinman S, Tinmouth AT, Capocelli KE,* et al. Platelet transfusion: a clinical practice guideline from the AABB. Ann Intern Med 2015; 162(3): 205–13.
3. *Estcourt LJ, Malouf R, Hopewell S, Doree C, Van Veen J*. Use of platelet transfusions prior to lumbar punctures or epidural anaesthesia for the prevention of complications in people with thrombocytopenia. Cochrane Database Syst Rev 2018; 4: CD011980.
4. *Squires JE*. Indications for platelet transfusion in patients with thrombocytopenia. Blood Transfus 2015; 13(2): 221–6.
5. *Ferber T, Hannan M*. Donor Apheresis. In: *Linz W, Chhibber V, Hoffman J, Torloni A, Vrielink H, Mason H,* et al, editors. Principles of Apheresis Technology: Technical Principles of Apheresis Medicine. 6th ed. BC. Vancouver, BC: ASFA; 2017. p. 143–62.
6. *Grujic J, Gulan Z, Budakov Z*. Importance of hemovigilance and reports on transfusion reaction in blood component therapy. Med Pregl 2012; 65(1–2): 50–3. (Serbian)
7. *Heuft HG, Fischer E, Weingand T, Burkhardt T, Leitner G, Baume H,* et al. Donor Safety in Haemapheresis: Development of an Internet-Based Registry for Comprehensive Assessment of Adverse Events from Healthy Donors. Transfus Med Hemother 2017; 44(3): 188–200.
8. Standard for Surveillance of Complications Related to Blood Donation. Working Group on Donor Vigilance of the International Society of Blood Transfusion (ISBT); Working Party on Hemovigilance in collaboration with The International Haemovigilance Network and The American Association of Blood Banks (AABB) Donor Hemovigilance Working Group, December 11, 2014. Available from: http://www.aabb.org/research/hemovigilance/Documents/Donor-Standard-Definitions.pdf.
9. *Goldman M, Land K, Robillard P, Wiersum-Osselton J*. Development of standard definitions for surveillance of complications related to blood donation. Vox Sang 2016; 110(2): 185–8.
10. *Das SS, Sen S, Chakrabarty RI*. Machine and man individualities in apheresis adverse events. Global J Trans Med 2018; 3(1): 30–3.
11. *Bialkowski W, Bruhn R, Edgren G, Papanek P*. Citrate anticoagulation: Are blood donors donating bone? J Clin Apher 2016; 31(5): 459–63.
12. Low on I transfusion medicine. ("Sl. glasnik RS", br. 40/17, 113/17).
13. *Guan L, Tian X, Gombar S, Zemek AJ, Krishnan G, Scott R,* et al. Big data modeling to predict platelet usage and minimize wastage in a tertiary care system. Proc Natl Acad Sci USA 2017; 114(43): 11368–73.

14. *Yu C, Lau JT, Zhong W, Huang X, Pan C, Chen Y*, et al. Why some donors are more willing to donate platelets? – a qualitative study on 25 regular platelet donors in Guangzhou, China. BMC Public Health 2019; 19(1): 1671.
15. *Estcourt JL., Birchall J, Allard S, Bassey JS, Hersey P, Kerr JP,* et al. British Committee for Standards in Haematology. Guidelines for the use of platelet transfusions. Br J Haematol 2017; 176(3): 365–94.
16. *Khajuria K, Sawhney V, Sharma R, Gupta S*. Adverse donor reaction during and after plateletpheresis in a tertiary care centre. Int J Res Med Sci 2017; 5(4): 1221–3.
17. *Diekamp U, Gneißl J, Rabe A, Kießig ST*. Donor Hemovigilance with Blood Donation. Transfus Med Hemother 2015; 42(3): 181–92.
18. *Navkudkar A, Desai P, Rajadhyaksha S*. Effect of citrate on ionized calcium levels during plateletpheresis procedures. Vox Sang 2021; 16(1): 26–30.
19. *Dogra K, Fulzele P, Rout D, Chaurasia R, Coshic P, Chatterjee K*. Adverse Events During Apheresis Procedures: Audit at a Tertiary Hospital. Indian J Hematol Blood Transfus 2017; 33(1): 106–8.
20. *Shanthi B, Sudhir Kumar V, Mahesh K, Krishna BM*. Adverse Events Associated with Plateletpheresis: A Tertiary Care Hospital Experience in Southern India. J Clin Diagn Res 2019; 13(5): EC10–EC12.
21. *Philip J, Sarkar RS, Pathak A*. Adverse events associated with apheresis procedures: Incidence and relative frequency. Asian J Transfus Sci 2013; 7(1): 37–41.
22. *Bassi R, Thakur KK, Bhardwaj K*. Plateletpheresis adverse events in relation to donor and plateletpheresis session profile. Iraqi J Hematol 2017; 6: 38–42.

Received on November 14, 2020
Revised on February 22, 2021
Accepted on February 23, 2021
Online First March 2021

ORIGINAL ARTICLE

UDC: 616-001-083.98::617.576
DOI: https://doi.org/10.2298/VSP200308035N

Evaluation of hand injury management at the emergency department – are we getting better?

Zbrinjavanje povreda šake u urgentnom centru – da li napredujemo?

Jelena Nikolić*†, Marija Marinković*†, Dragana Leković†, Isidora Djozić†

***University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; †University Clinical Center of Vojvodina, Clinic for Plastic and Reconstructive Surgery, Novi Sad, Serbia**

Abstract

Background/Aim. Hand injuries are one of the most common injuries seen in emergency departments. Inadequate treatment can lead to prolonged healing, complications, significant morbidity, and serious disability. The aim of this study was to evaluate the epidemiology, risk factors, and treatment of hand injuries in one tertiary care level clinical center. **Methods.** This study was designed as a descriptive retrospective epidemiological study that involved all patients with hand injuries treated at the University Clinical Center of Vojvodina, Novi Sad, Serbia for seven years. The authors collected sociodemographic and clinical data such as age, gender, mechanism of injury, type of injury, days of hospitalization, type of defect reconstruction, the time of injury, the timing of surgery, and reasons for operative treatment delay. For every hospitalized patient the Modified Hand Injury Severity Score (MHISS) was calculated. All data were analyzed using SPSS IBM 21.0 software. **Results.** From 2012 to 2018, 34,796 patients were treated for hand injury at the University Clinical Center of Vojvodina, with 554 (1.6%) hospitalized patients. The mean age of patients was 43.2 years; the majority of them (87.55%) were men, and most (47.2%) were injured at home. Most injuries occurred during knife handling. The average length of stay for hospitalized patients was 4 days. MHISS score for most patients was over 50 and was classified as severe. It was noticed that the waiting time for operation became shorter throughout the selected years. **Conclusion**. Hand injuries present a complex problem that can sometimes be underestimated by patients. The requirement of highly specialized hand surgeons, sometimes special equipment (e.g., microscope), multiple operations, prolonged rehabilitation, possible invalidity, and high cost of treatment calls for careful evaluation of the problem and the development of proper strategies in order to be able to lower the costs and obtain better medical care for all people with higher injury risk.

Key words:
emergency service, hospital; hand injuries; reconstructive surgical procedures; risk factors; trauma severity indices; treatment outcome.

Apstrakt

Uvod/Cilj. Povrede šake spadaju među najčešće povrede koje se sreću u urgentnim centrima širom sveta. Neadekvatno lečenje može dovesti do produženog zarastanja, komplikacija, značajnog morbiditeta i invaliditeta. Cilj rada bio je da se sagledaju epidemiologija, faktori rizika i tretman povreda šake u jednom tercijarnom kliničkom centru. **Metode.** Studija je dizajnirana kao deskriptivna retorspektivna epidemiološka studija koja je obuhvatila sve pacijente sa povredama šake lečene u Univerzitetskom kliničkom centru Vojvodine u Novom Sadu, Srbija u sedmogodišnjem periodu. Analizirani su opšti sociodemografski i klinički podaci: starost, pol, mehanizam povrede, tip povrede, dužina hospitalizacije, način rekonstrukcije defekta, vreme povrede, dužina čekanja na operativno zbrinjavanje i razlozi za odlaganje operativne intervencije. Za svaku povredu lečenu u hospitalnim uslovima izračunat je modifikovan skor za procenu težine povrede šake (MHISS). U obradi podataka korišćen je softverski paket SPSS IBM 21.0. **Rezultati.** U analiziranom periodu (2012.–2018. godine) tretirano je 34 796 pacijenata sa povredama šake, od kojih je hospitalizovano 554 (1,6%) pacijenata. Prosečna starost hospitalizovanih povređenih pacijenata bila je 43,2 godine; 87,55% pacijenta činili su muškarci, a povrede su većinom (47,2%) nastale u kućnim uslovima. Najčešći uzrok povreda bilo je rukovanje oštricom noža. Prosečna dužina hospitalizacije iznosila je 4 dana. Vrednost MHISS kod većine je iznosila preko 50, što se klasifikuje kao teška povreda. Uočen je trend smanjenja dužine čekanja na operativno lečenje tokom posmatranih godina. **Zaključak.** Povrede šake predstavljaju kompleksan problem koji pacijenti nekada potcenjuju. Potreba za hirurzima visoko specijalizovanim za šaku i, ponekad, specifičnom opremom (npr. mikroskop), višestruke operativne intervencije, dugotrajna rehabilitacija, potencijalni invaliditet i visoki troškovi lečenja ukazuju na po

Correspondence to: Jelena Nikolić, University of Novi Sad, Faculty of Medicine, University Clinical Center of Vojvodina, Clinic for Plastic and Reconstructive Surgery, Hajduk Veljkova 1–9, 21 000, Novi Sad, Serbia. E- mail: JELENA.NIKOLIC@mf.uns.ac.rs

trebu da se ovaj problem pažljivo proceni i kreira adekvatna strategija kako bi se smanjili ukupni troškovi i pružio bolji tretman osobama izloženim većem riziku od povrede.

Ključne reči:
hitna služba, bolnica; šaka, povrede; hirurgija, rekonstruktivna, procedure; faktori rizika; povrede, indeksi težine; lečenje, ishod.

Introduction

Hand injuries are one of the most common injuries seen in emergency departments (EDs). As almost every human activity involves hands, they are the most exposed part of the body and thus are often prone to different kinds of injuries such as lacerations, cuts, crush injuries, amputations, sprains, infections, fractures, burns, etc. Most hand injuries are minor and usually heal without problems. People are used to getting small burns while cooking, cuts while working, or being scratched while playing with animals, so one may often underestimate the level of injury and try to solve problems with inadequate home remedies or improvised treatment in a nonsterile environment. Postponed or inadequate treatment can lead to prolonged healing, complications, long-term morbidity, and even serious disability. As in leisure and home activities, occupational hand injuries contribute significantly to the total count of injuries [1].

It is estimated that between 16% to 30% of all emergency visits occur due to hand injuries in the USA [1]. The United States Bureau of Labor Statistics reported that hand injuries are the second most common injury resulting in days away from work (DAFW). Incidence rates for non-fatal hand injuries involving DAFW per 10,000 full-time workers for 2018 in the USA report the highest rates for the upper arm in total (28.6/10,000 workers) and 12.3/10,000 workers just for hand [2]. In the national statistical analysis for occupational injuries in the Republic of Serbia, the upper arm was the most often (46.64%) affected part of the body, with fingers being injured in 18.41% of all cases [3]. Finger injuries were also the most frequent (38.4%) injuries of upper extremities observed in EDs in the USA, as published by Ootes et al. [4] in a broad epidemiological study that involved 92,601 patients. The same study estimated that the average USA resident had a 1 in 88 chance of presenting in ED with upper arm injury during their lifetime.

Among many hazardous occupations, according to statistics from the USA, crop harvesting with machinery (106.4/10,000 workers) and working with narrow fabric mills (112.9/10,000 workers) are considered the most dangerous jobs [2]. This is taken as a very important risk factor at work, as the region of Vojvodina is a typical agricultural area with many workers employed in such a risky occupational environment. These injuries are often highly mutilating and involve multiple finger amputations and defects of vital neurovascular structures, leaving limited surgical options for reconstruction (Figures 1 and 2).

The aim of this study was to get a closer insight into the treatment of hand injuries at the University Clinical Center of Vojvodina, Novi Sad, Serbia, a tertiary care level center, and to present epidemiological data on hand injuries in previous years in order to analyze potential risk factors that could lead to injury. Another aim was to evaluate medical treatment strategies that patients received upon ED admission so that targeted strategies for prevention, risk management, and better medical treatment can be suggested. Creating public health initiatives based on the national injury registry could allow professionals to target current problems and thus better allocate limited resources.

Methods

This study was designed as a descriptive retrospective epidemiological study that included patients treated for hand injury at the ED of the University Clinical Center of Vojvodina for seven years (2012–2018). All data was obtained from medical documentation and the local electronic database. Authors collected sociodemographic and clinical data such as age, gender, education level, qualification for the job that led to injury, mechanism of injury, type of injury, days of hospitalization, type of defect reconstruction/treatment, timing of injury, waiting time in ED, timing of the operation,

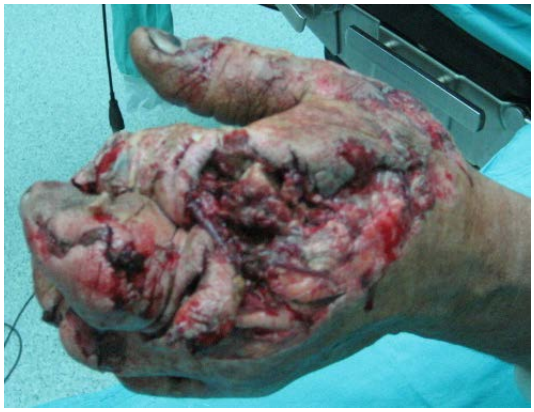


Fig. 1 – Hand injury caused by corn harvester.

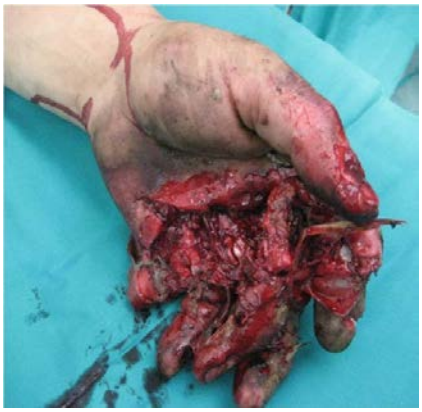


Fig. 2 – Hand injury in agriculture.

perceived cause of the occupational injury, and reasons for operative treatment delay. Following factors were noted as the reasons for operative treatment delay: alcohol abuse, the time elapsed from last food intake, preoperative evaluation of the patient (diagnostic procedures and therapy), bad general health condition or other injuries that postponed operation, operating room (OR) availability, and disposal of specialist medical staff. For every patient, the Modified Hand Injury Severity Score (MHISS) was calculated. All data were analyzed using SPSS 21.0 (IBM Corp. Armonk, NY, USA). For numerical and categorical variables, mean and standard variation were calculated with descriptive analysis and was displayed as such in various graphical manners.

Results

This study included 34,796 patients with hand injuries treated at the University Clinical Center of Vojvodina during the 2012–2018 time period, with 554 (1.6%) hospitalized for treatment. The average mean age of hospitalized cluster was 43.2 [standard deviation (SD) ± 15.58] years. Most of the patients were men, 485 (87.55%), while there were just 69 women (12.45%). Among them, 51% of injuries occurred at home, 15.7% at off-duty work, 14% at on-duty work, 13% in road traffic accidents, and 6.3% during leisure activities.

Injuries that occurred as work-occupational hand injuries (77; 13.9%) were also independently analyzed. The mean age of the patients injured while working was 40.92 ± 15.03 years. The trend of incidence of such injuries is shown in Figure 3.

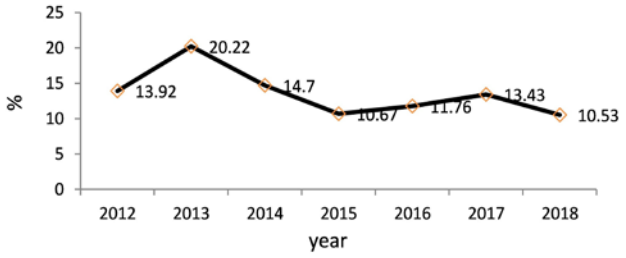


Fig. 3 – Yearly incidence of work-occupational injuries.

As a perceived cause of injury that occurred at work, patients specified the following causes: not being well (6; 7.7%), working faster than usual due to time restraints (29; 37.67%), not being experienced (first time doing something) (6; 7.8%), working overtime (12; 15.6%), not being familiar with the equipment (11; 14.29%), faulty equipment (6; 7.8%), injury caused by other person's actions (4; 5.2%), and being distracted (3; 3.9%).

Most of the patients who were required to wear safety gloves at work according to safety standards did wear protective gloves during injury (40/61; 65.57%).

Most injuries occurred while handling sharp items such as knife blades. The distribution of mechanisms of hand injuries/tools is shown in Figure 4.

The average length of stay in the hospital after a hand injury in a hospitalized group of patients was 4.07 days. Figure 5 presents the length of hospital stay for various mechanisms/tools of injury.

For all hospitalized patients, MHISS was calculated and compared with the mechanism/tool of injury as presented in Figure 6. All patients, according to MHISS, had severe hand injuries, but for groups with glass and blunt injuries, the injuries were categorized as moderate.

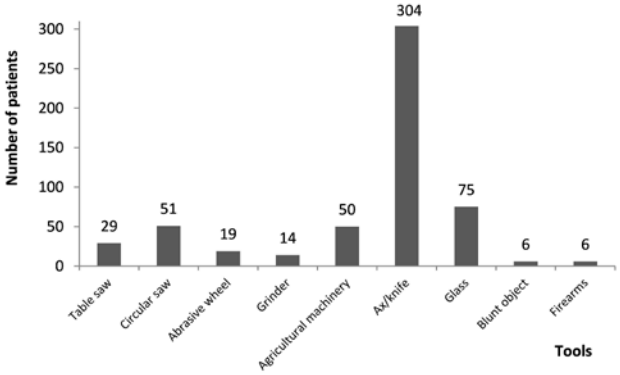


Fig. 4 – Distribution of injury mechanisms/tools.

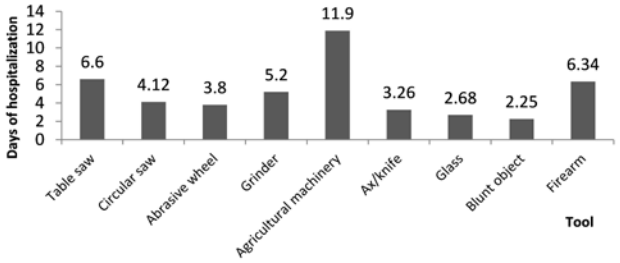


Fig. 5 – Length of hospitalization for every mechanism/tool of injury.

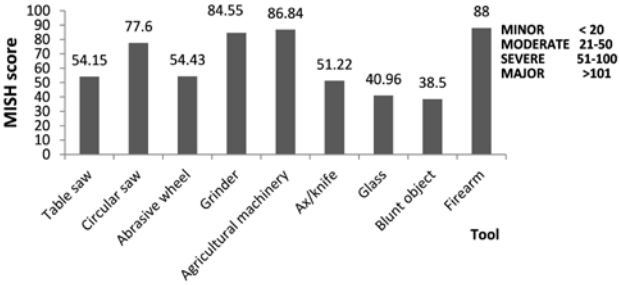


Fig. 6 – Modified Hand Injury Severity Score (MHISS) in correlation with mechanism/tool of injury.

Amputation of one or more fingers was seen in every mechanism/tool of the injury group but blunt, glass and firearm groups. Injuries with circular saw and agricultural machinery had the highest percentages of amputation risk (Figure 7).

All reconstructive techniques were used in the closure of defects after hand injury: direct suture (347.81%), skin graft (32.7%), skin flaps (8.2%), amputation (26.6%), amputation and skin graft (13.3%), amputation and skin flap (4.1%).

The average time from injury to arrival at the University Clinical Center of Vojvodina was 2.5 hours. The data was obtained from patients recalling the time of injury, so it has

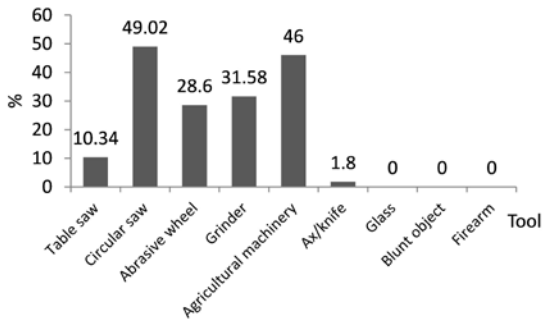


Fig. 7 – Presence of amputation of one or more digits in different mechanisms/tools of injury.

to be taken with caution. Most often, patients reported the following reasons for the delay of arrival at the University Clinical Center of Vojvodina: initial referral to a secondary level hospital, waiting for transportation, underestimating the need for surgical treatment, or being injured far away from the referral center. As reasons for the delay of surgical treatment after arrival to the ED, two group-related causes were identified: patient-related (consumption of alcohol, prior food intake, arrival after midnight, comorbidities, need for additional diagnostic procedures, associated injuries that required delay of surgical treatment), and hospital related (occupancy of OR or surgeon). The yearly distribution of cause-related delay by groups is shown in Figure 8.

A yearly decreasing trend in waiting time between arrival to the ED and operative treatment was observed (Figure 9).

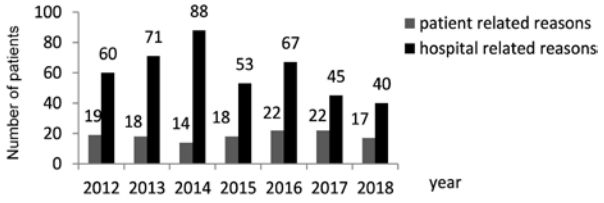


Fig. 8 – Reasons for the delay of operative treatment.

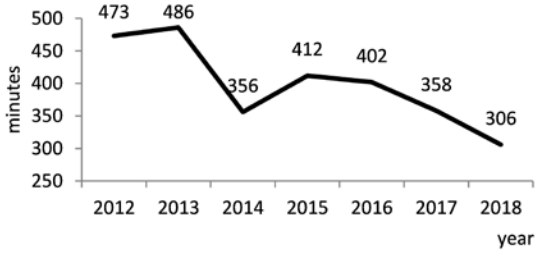


Fig. 9 – Yearly trend of waiting time between emergency department arrival and operative treatment.

In general cluster, most of the patients were injured during day shifts; 6–12 hrs (180; 32.49%), 12–18 hrs (184; 33.21%), 18–24 hrs (131; 23.65%), 24–06 hrs (59; 10.65%).

Special attention was paid to the reasons for patient-related operative treatment delay, such as alcohol abuse, as one of the preventable factors. It was present in 11% of the entire cluster, and 40% of patients who abused alcohol had arrived after midnight. Daily quartered distribution of patients who abused alcohol upon admission was as follows: 40.91% in 24–06 hrs time interval, 21.15% in 18–24 hrs, 3.68% in 12–18 hrs, and 2.74% in 6–12 hrs time interval.

The average elapsed time while waiting from arrival to operative intervention in 2018 was 5 hours. Factors that emerged as risk factors for longer waiting were the time of arrival, age of the patient, need for more than one specialist, occupancy of ORs, and need for additional diagnostic procedures.

Discussion

Hand injuries often present a multilevel impact on society in general. Costs of medical treatment, rehabilitation, absence from work, health insurance reimbursements, and costs of prequalification are just some of the problems that have to be taken into consideration.

Our study reveals that just 1.6% of all patients who suffered hand injuries and were referred to the ED required hospitalization. One must acknowledge that this does not mean that injuries managed under local or regional anesthesia in the outpatient department did not result in invalidity or produce considerable final costs. In our study, we focused on patients whose injuries required hospitalization. All of them, according to the MHISS score, were classified as severe (MHISS > 50) or moderate (MHISS 21–50), as represented in Figure 6. Most of the injured patients were men (70–92% depending on the calendar year examined), fully capable of working, and around 40 years old (43.20; SD ± 15.58). Larsen et al. [5] presented results similar to ours, where most of the injured were males, with females being dominant only in the group of assault victims older than 65. They also found that 1 out of every 55 Dutch and 1 out of every 28 Danish people presented to ED with hand injuries, thus confirming the importance of adequate management and good primary surgical treatment of these injuries. In our study, men were dominant in all age groups. In the group of patients older than 65, women presented just 9.2% of the entire cluster.

It is also interesting to analyze occupational hand injuries presented in different studies. Occupational acute hand injuries were responsible for 13.9% of all hand injuries in our sample of patients. The average age of those patients was 40.92 ± 15.03 years which is significantly higher than the data presented in a review article by Sorock et al. [6], where young workers under 24 years of age were at the highest risk of hand trauma. This significantly younger age of injured compared with other groups of patients with hand injuries could be attributed to a lack of experience in work or underestimation of the importance of safety measures. This was confirmed in a multicenter study on occupational hand injuries by Wu et al. [7] in Foshan, PR China. The study included 2,186 patients, with most injuries occurring due to occupational hazards. The patients were mostly young men that lacked safety training. This makes young men an especially vulnerable group that has to be addressed in security briefings and education plans made by occupational management. Our study showed no significant age difference between the

people injured at work, at home, or in other activities, but most of our patients with occupational hand injuries also confirmed not having any special safety training. One can safely assume that the working population in Serbia is exposed to more difficult working conditions than in the USA, meaning that risk factors should differ. Authors cannot overrate working conditions in Serbia, where older machinery, sometimes outdated technology, and the economic situation pushes people to work longer hours or more jobs simultaneously. When asked about circumstances leading to injury, it was interesting to see that patients mentioned the perceived reasons as the most important for injury occurring. Most of them, 37.67%, said they were in a hurry to finish the job or that they were working overtime (15.6%). In the Wu et al. [7] study, distraction was most often seen as patients' idea of the injury cause. Authors cannot claim that it was a lack of experience in our sample, as most of the injured were over 40 years old, but it looks like people in Vojvodina underestimate the importance of safety measures and standards which, in combination with outdated machinery, puts them at higher risk for accidents. Close studies of these patients and analysis of circumstances before the moment of injury could provide useful information for National Health Service and labor departments and consecutively lead to the creation of targeted strategies that would make a safer working environment.

Usually, in the region of Vojvodina, most severe injuries happened due to hand or finger conquassation, which occurred in the agricultural industry while working with heavy types of machinery such as corn snappers or harvesters (Figures 1 and 2). Those injuries are characterized by "T triad" as in excess Time until treatment, Thresh/wound contamination, and big Trauma and often require more operative procedures, have more complications, and longer hospitalization in general [8, 9]. In this study, patients injured by agricultural machinery had the longest hospitalization, an average of 11.9 days, which is significantly longer than the average of 4 days for all injury mechanisms in general.

As this survey reveals, men are often injured while working at home with circular saw and table saw as part of their do-it-yourself (DIY) activity. Women are also more likely to suffer an injury at home but usually suffer minor cuts, small burns, and lacerations that can be treated without hospital admission. Working during off hours is also a category presented as a place/circumstance of injury (15.7%). As seen in previous studies, illegal or off-license work often puts workers in a position to work without proper protection, in unsafe conditions, with prolonged working hours, and without adequate training and education for that particular job. All of these factors are known to facilitate the occurrence of injuries.

A wide palette of reconstructive procedures (skin graft, local flaps, direct sutures, amputations) is being done in order to treat hand injuries adequately. Most of the hospitalized patients had good skin coverage that did not require skin grafts or skin flaps in order to close the wound, but despite that had to be hospitalized as complex reconstructions of tendons, nerves, and bone fractures are usually done in general anesthesia.

The golden standard/window for wound closure is within 6 hrs from the moment of injury. This means that the best results and the lowest risk of infection can be expected if primary wound care is done in the above-mentioned time window. In practice, it is very difficult to arrange all the necessary stages of treatment in such a short period, especially if a large area of one medical center is the referral hospital for a vast area of the region. Many factors contribute to operative treatment delay. In our study, patients needed an average of 2.5 hours just to arrive at the ED. This data is uncertain as patients were recalling the time of injury and were sometimes unsure about it. The average waiting time in ED for operative treatment in these seven years was 6 hrs and 39 min, but this time is getting significantly shorter throughout the years, which suggests that changes made in organizational structure have been giving good results. Shorter time from admission to definite treatment and, thus, improved medical care was achieved by the better organization of the triage system, employment of more specialists in the ED, implementation of a new information system that covers all patient steps through ED service, and, for sure, by a continuous struggle to continuously educate doctors and nurses. In 2018, last year analyzed, the time to definite treatment was around 5 hrs which is considered very good compared with more developed countries. This is a common problem seen in all EDs worldwide. ED setting is specific and complex. Numerous attempts have been made to improve ED care services around the world [10–12]. Reviewing literature that addresses this issue, the authors came across many models that have been proposed in different ED settings: various systems of patient grouping (Emergency Severity Index Triage System – ESI, tree-level triage evaluation system, etc.), "fast track" models, senior doctor assessment at triage instead of nurse triage model, are only some of the possible solutions to a problem in which one may achieve better results within available resources [10, 13]. Ajami et al. [14] presented results that demonstrate that in recent years patient waiting time in the ED has increased in many countries, mostly due to the rising number of patient referrals to EDs. The same study found that waiting time for medical examination in EDs in England was increased to 4 hrs, and in Canada to 2 hrs. We have to keep in mind that this is just waiting time for medical examination with more elapsed time when adding time from arrival to ED to surgical intervention. Horwitz et al. [15] revealed that fewer than half of the hospital centers in their study, which included 364 non-federal US hospital EDs, admitted their ED patients within 6 hours. Besides a higher inflow of patients, there is also a problem of inexperienced interns, residents, and young specialists who have multi-tasks in several places, different wards, operation theaters, triage rooms, etc. Treating more than one patient at the same time is difficult and requires more experience. Lack of experience in the decision-making process can lead to requesting more investigations and tests in order to make a decision and prolonging the waiting time before operative treatment. The University Clinical Center of Vojvodina is the only tertiary health center and University Hospital in the whole province of Vojvodina. This means that, on a daily basis, 1.5 million people are ori-

ented to this Center in case of complex hand injuries, as most of the hospitals in the region do not have plastic/reconstructive surgeons available on call. Complex hand injuries sometimes require the teamwork of more surgeons like neurosurgeons, orthopedic and vascular surgeons, and others that have to be available at the same time for the same surgical procedure. The reasons for the late onset of surgical interventions are the following: time of arrival, severe comorbidities usually associated with an aging population, need for more than one specialist, consumption of alcohol or food before arrival to the hospital, occupancy of ORs or surgeons, need for additional diagnostic procedures and other associated injuries that required delay. In most cases (78.84%), occupancy of the OR or surgeon was the reason for intervention delay. In the previous years, a higher number of road traffic accidents, usage of more powerful machinery, industrial environment, easy access to alcohol, increasing violence on the street, and immense workload on trauma centers both locally and worldwide all led to prolonged waiting times. It is most important to shorten the time from injury to arrival in ED, as upon arrival, primary wound care is done, with wounds temporarily dressed in sterile conditions. While waiting for intervention, preoperative antibiotics, pain therapy, and, if needed, blood transfusion is administered. This means that patient is under constant medical supervision. As it was already underlined, loss of time before surgery is a big problem in cases such as injuries in agriculture, which are unfortunately often the most violent ones, as an injured patient is somewhere in the field, far from the nearest local ambulance, and usually alone. Those people take more time to reach the hospital and medical help than the people working near regional health centers or those at home. Besides direct costs of medical treatment and time of work absence, one has to keep in mind that permanent disability often requires prequalification or even early retirement, so these injuries may impose a significant burden on society, as presented in de Putter et al. [16] study. The economic impact of hand injuries is substantial, so prevention strategies should be created and targeted at the most expensive injuries in order to control and lower resource spending. Proper epidemiological analysis of injuries in ED should provide directions for training priorities for the medical crew.

The limitation of this study is certainly the inclusion of patients from a single center. Even though the University Clinical Center of Vojvodina is the largest hospital in the region and is the only tertiary level center in Vojvodina, hand injuries are also treated in local hospitals within 100 kilometers' reach. Problems that are dominant in those health centers could be different and, at the same time, interesting for evaluation. Another limitation is patient-related: false data recalling, such as time of injury or concealing the truth (usually concerning the place and circumstances of the injury, use of protective gloves, etc.). Occupational injuries are usually followed by insurance company compensations and employment problems, so injured employee tends to be under pressure, not to mention the circumstances of the injury, and thus give false information to medical staff.

Conclusion

Hand injuries present a complex problem that is sometimes underestimated by both patients and general practitioners, usually regarded as something unimportant and easily treatable. Need for highly specialized surgeons (plastic, orthopedic, vascular, or hand surgeons where available), sometimes special equipment (e.g., microscopes), multiple operations, prolonged rehabilitation, possible invalidity, and high costs of treatment are putting this medical problem at the pinnacle of our attention. A more comprehensive and detailed study could give us better insight into this problem and allow us to draw more relevant conclusions. One can argue that different medical care levels (primary/secondary/tertiary) have different dominant problems and thus require individual approaches and special logistic plans for health care improvement. Additionally, closer insight into the circumstances of occupational hand injuries can result in a better approach to safety management and further safety training for specific work-related risks.

REFERENCES

1. *Murphy GR, Gardiner MD, Glass GE, Kreis IA, Jain A, Hettiaratchy S*. Meta-analysis of antibiotics for simple hand injuries requiring surgery. Br J Surg 2016; 103(5): 487–92.
2. U.S. Department of Labor, Bureau of Labor Statistics. Survey of Occupational Injuries and Illnesses. 2018. Available from: https://www.bls.gov/iif/oshwc/osh/case/cd_r6_2018.htm [accessed on 2020 March 7].
3. The Republic of Serbia, Ministry of Labour, Employment, veteran and social affairs, The Administration for the Safety and Health at Work report for 2017. Available from: https://www.minrzs.gov.rs/sites/default/files/2018-11/izvestaj_o_radu_uprave_za_2017.pdf [accessed on 2020 March 7]. (Serbian)
4. *Ootes D, Lambers KT, Ring DC*. The Epidemiology of Upper Extremity Injuries Presenting to the Emergency Department in the United States. Hand (N Y) 2012; 7(1): 18–22.
5. *Larsen CF*, *Mulder S*, *Johansen AM*, *Stam C*. The epidemiology of hand injuries in The Netherlands and Denmark. Eur J Epidemiol 2004; 19(4): 323–7.
6. *Sorock GS, Lombardi DA, Courtney TK, Cotnam JP, Mittleman MA*. Epidemiology of occupational acute traumatic hand injuries: a literature review. Saf Sci 2001; 38(3): 241–56.
7. *Wu Z, Guo Y, Gao J, Zhou J, Li S, Wang Z*, et al. The epidemiology of acute occupational hand injuries treated in emergency departments in Foshan City, South China. Ulus Travma Acil Cerrahi Derg 2018; 24(4): 303–10.
8. *Nikolić J, Janjić Z*. Burn injuries in agriculture. Trakt i pog maš 2009; 14(4): 122–8.
9. *Nikolić J, Janjić Z*. Specifities of hand trauma in agriculture. Vojnosanit Pregl 2009; 66(10): 802–6. (Serbian)
10. *Ashour OM, Okudan Kremer GE*. Dynamic patient grouping and prioritization: a new approach to emergency department flow improvement. Health Care Manag Sci 2016; 19(2): 192–205.
11. *Ortíz-Barrios MA, Alfaro-Saíz JJ*. Methodological Approaches to Support Process Improvement in Emergency Departments: A Systematic Review. Int J Environ Res Public Health 2020; 17(8): 2664.

12. *Kaushal A, Zhao Y, Peng Q, Strome T, Weldon E, Zhang M,* et al. Evaluation of fast track strategies using agent-based simulation modeling to reduce waiting time in a hospital emergency department. Soc Econ Plann Sci 2015; 50: 18–31
13. *Abdulwahid MA*, *Booth A*, *Kuczawski M*, *Mason SM*. The impact of senior doctor assessment at triage on emergency department performance measures: systematic review and meta-analysis of comparative studies. Emerg Med J 2016; 33(7): 504–13.
14. *Ajami S, Ketabi S, Yarmohammadian MH, Bagherian H*. Wait Time in Emergency Department (ED) Process. Med Arh 2012; 66(1): 53–7.
15. *Horwitz LI, Green J, Bradley EH*. US emergency department performance on wait time and length of visit. Ann Emerg Med 2010; 55(2): 133–41.
16. *de Putter CE*, *Selles RW*, *Polinder S*, *Panneman MJ*, *Hovius SE*, *van Beeck EF*. Economic impact of hand and wrist injuries: healthcare costs and productivity costs in a population-based study. J Bone Joint Surg Am 2012; 94(9): e56.

Received on March 8, 2020
Revised on February 13, 2021
Accepted on March 29, 2021
Online First April 2021

ORIGINAL ARTICLE
UDC: 618.19-006-08-092.9
DOI: https://doi.org/10.2298/VSP210126039J

Anti-PD-1 therapy activates tumoricidic properties of NKT cells and contributes to the overall deceleration of tumor progression in a model of murine mammary carcinoma

Anti-PD-1 terapija aktivira tumoricidna svojstva NKT ćelija i doprinosi ukupnom usporavanju progresije tumora u modelu mišjeg karcinoma dojke

Marina Jovanović*, Nevena Gajović*, Milena Jurišević†, Sofija Sekulić‡, Nebojša Arsenijević*, Midrag Jocić§, Milan Jovanović‖, Ružica Lukić¶, Ivan Jovanović*, Dragče Radovanović**

University of Kragujevac, Faculty of Medical Sciences, *Center for Molecular Medicine and Stem Cell Research, †Department of Pharmacy, ‡Department of Infectious Diseases, **Department of Surgery, Kragujevac, Serbia; Military Medical Academy, §Institute for Transfusiology and Haemobiology, ‖Clinic for Abdominal Surgery, Belgrade, Serbia; ¶University of East Sarajevo, Faculty of Medicine, Department of Microbiology, Foča, Bosnia and Herzegovina

Abstract

Background/Aim. Immune checkpoint therapy is a well-established therapeutic approach in the treatment of malignant diseases and is thought to be mostly based on facilitating the adaptive immune response. However, the cells of the innate immune response, such as natural killer T (NKT) cells, might also be important for a successful anti-programmed cell death protein-1 (anti-PD-1) therapy, as they initiate the antitumor immune response. The aim of this study was to investigate the influence of anti-PD-1 therapy on the immune response against tumors. **Methods.** For tumor induction, 4T1 cells synergic to BALB/c background were used, after which mice underwent anti-PD-1 treatment. After the mice were sacrificed, NKT cells, dendritic cells (DCs), and macrophages derived from spleen and primary tumor tissue were analyzed using flow cytometry. **Results.** Anti-PD-1 therapy enhanced the expression of activating molecules CD69, NKp46, and NKG2D in NKT cells of the tumor and spleen. This therapy activated NKT cells directly and indirectly via DCs. Activated NKT cells acquired tumoricidic properties directly, by secreting perforin, and indirectly by stimulating M1 macrophages polarization. **Conclusion.** Anti-PD-1 therapy activates changes in DCs and macrophages of primary tumor tissue towards protumoricidic activity. Since anti-PD-1 therapy induces significant changes in NKT cells, DCs, and macrophages, the efficacy of the overall antitumor response is increased and has significantly decelerated tumor growth.

Key words:
antineoplastic agents; breast neoplasms; immunomodulation; killer cell, natural; macrophages; mice.

Apstrakt

Uvod/Cilj. Imunoterapija je danas dobro poznat terapijski pristup u lečenju malignih bolesti koji se temelji na stimulisanju stečenog imunskog odgovora. Međutim, ćelije urođenog imuskog odgovora, kao što su prirodne T ćelije ubice – *naturall killer T cells* (NKT), takođe mogu biti bitne za uspešnu terapiju i započinjanje antitumorskog imunskog odgovora delovanjem na protein 1 programirane ćelijske smrti (PD-1). Cilj rada bio je da se ispita uticaj anti-PD-1 terapije na antitumorski imunski odgovor. **Metode.** Za indukciju tumora korišćene su 4T1 ćelije, singene za BALB/c miševe, nakon čega su miševi tretirani anti-PD-1 antitelom. Nakon žrtvovanja miševa, NKT ćelije, dendritske ćelije (DC) i makrofagi iz slezine i primarnog tumora analizirani su uz pomoć protočne citometrije. **Rezultati.** Anti-PD-1 terapija je povećala ekspresiju aktivirajućih molekula CD69, NKp46, i NKG2D u NKT ćelijama slezine i tumora. Ova terapija aktivira NKT ćelije direktno i indirektno, preko DC. Aktivirane NKT ćelije nakon anti-PD-1 terapije stiču tumoricidna svojstva direktno, preko povećanog stvaranja perforina, i indirektno, putem polarizacije makrofaga u pravcu M1 fenotipa. **Zaključak.** Anti-PD-1 terapija je podstakla promene fenotipa DC i makrofaga u primarnom tumorskom tkivu u pravcu antitumorske aktivnosti. Kako

Correspondence to: Miodrag Jocić, Military Medical Academy, Institute for Transfusiology and Haemobiology, Crnotravska 17, 11 000 Belgrade, Serbia; E-mail: jocicmiodrag@gmail.com

anti-PD-1 terapija indukuje značajne promene u NKT ćelijama, DC i makrofagima, efikasnost sveukupnog antitumorskog odgovora je veća i značajno je usporila rast tumora.

Ključne reči:
antineoplastici; dojka, neoplazme; imunomodulacija; ćelije ubice, prirodne; makrofagi; miševi.

Introduction

Immunotherapy is an emerging approach to the treatment of many cancers nowadays [1]. Since their discovery, immune checkpoint inhibitors (anti-programmed cell death protein 1 (anti-PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibodies have been utilized in various diseases, such as autoimmune or even infectious diseases. However, they are predominantly used in malignant diseases, with evolving strategies in the management of the diseases [2].

The underlying mechanism of anti-PD-1 therapy is blockage of the programmed death ligand (PDL)/PD-1 axis. Under physiological circumstances, PDL is found on many epithelial, endothelial, and immune cells, such as dendritic cells (DCs) and macrophages [3]. The main role of this ligand is to limit over-reactive immune response, therefore restricting tissue damage due to unrestrained immune response, since the activation of the PDL/PD-1 axis potently hinders T-cell receptor activation [4, 5]. However, during a malignant disease, PDL is often found on cancer cells. PD-1 molecule is mainly expressed on effector immune cells, such as T lymphocytes, natural killer (NK) cells, and natural killer T (NKT) cells [6]. Given its expression on cancer cells and effector cells of the immune response, the activation of the PDL/PD-1 axis in these terms subsequently leads to a deteriorating immune response to malignant diseases [7]. Bearing in mind these assets of a PDL/PD-1 axis, it is clear that its inhibition is important for treating many diseases, especially cancers. Until now, anti-PD-1 therapy has been approved for many types of solid cancers, such as metastatic melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancer, and triple-negative breast cancer with high PDL expression [8–10].

Although therapeutic PDL/PD-1 blockage is thought to be mainly carried through blockage on T lymphocytes, there is emerging evidence that other effector cells, such as NK and NKT cells, take part in the beneficial effects of PDL/PD-1 axis blockage [11]. Until now, it has been well known that in some malignant diseases, the PD-1 molecule is more expressed in NK cells, which suggests damaged NK cell function [12]. Since it is well known that anti-PD-1 therapy increases cytokine production, especially in T lymphocytes, it remains unclear whether anti-PD-1 therapy acts directly on NK cells or indirectly via secretion of activating molecules, such as interferon (IFN) -γ [13]. Data are very modest when it comes to NKT cells and anti-PD-1 therapy. These cells play an important role in the interplay between innate and acquired immune responses [14]. Moreover, it is known that NKT cells produce cytokines that can activate macrophages and DCs and, therefore, coordinate immune response [15]. However, the effect of anti-PD-1 therapy on NKT cells is yet to be elucidated. Our data imply that NKT cells might also be important for more effective anti-PD-1 therapy in malignancies and might contribute to the overall effective immune response to mammary carcinoma, as anti-PD-1 therapy induces phenotype changes in NKT cells.

Methods

Mice

Female, six to eight weeks old, BALB/C wild type (WT) mice were used in all experiments. Experiments were conducted at the Center for Molecular Medicine and Stem Cell Research of the Faculty of Medical Sciences, University of Kragujevac, Serbia. The mice were housed under standard laboratory conditions (22 ± 2 °C, relative humidity 51 ± 5%, and a 12-hour light-dark cycle) throughout the whole experiment. All experiments were approved by the Animal Ethics Board of the Faculty of Medical Sciences, University of Kragujevac, Serbia (01-12188). Mice were divided into two experimental groups, each group consisting of six mice per group: 1) wild type (WT) BALB/C untreated mice and 2) WT anti-PD-1 treated mice – treated with the anti-PD-1 antibody on the third, sixth, ninth, and eleventh day after tumor induction.

Induction of tumor

Murine mammary carcinoma-4T1, syngenic to the BALB/c background, was purchased from the American Type Culture Collection (ATCC, USA). 4T1 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mmol/L L-glutamine, 1 mmol/L penicillin-streptomycin, and 1 mmol/L mixed nonessential amino acids (Sigma-Aldrich). Cultured 4T1 cells were harvested by brief treatment with 0.25% trypsin and 0.02% EDTA in phosphate-buffered saline (PBS, PAA Laboratories GmbH, Etobicoke, Canada) and washed three times in serum-free PBS before use in all *in vivo* and *in vitro* experiments. The viable cell number was determined by trypan blue exclusion. Suspensions only with > 95% viable cells were used in experiments. Each mouse was inoculated with 5×10^3 4T1 cells into the 4th mammary fat pad. The dosage of 5×10^3 4T1 cells per mouse was determined based on our preceding experiments.

Administration of anti-PD-1 antibody

Murine anti-PD-1 antibody was purchased from BioXcell. Antibody was administered intraperitoneally to mice on

the third, sixth, ninth, and eleventh day, beginning from the day of tumor induction, at 150 μg per mouse of anti-PD-1 antibody dissolved in 150 μL of PBS, as previously described by Qin et al. [16] and Shimizu et al. [17]. WT mice that did not receive anti-PD-1 therapy were injected with 150 μL of PBS only, on the same days, according to the model from the study by Vo et al. [18].

Evaluation of tumor growth

The appearance of the primary tumor was monitored daily after the induction by palpation. After tumor appearance, the diameter of the primary tumor was measured three times per week using a caliper. On the 40th day after tumor induction, mice were sacrificed; the primary tumor and spleen were surgically removed.

Flow cytometric analysis of splenocytes and tumor-infiltrating leukocytes

We analyzed the spleen for the assessment of systemic antitumor immune response as our previous results illustrated that phenotype changes in splenocytes are more likely to resemble phenotype changes occurring in tumor-infiltrating leukocytes [19]. Single-cell suspensions of the spleen were obtained by mechanical dispersion, while single-cell suspensions of primary tumors were obtained by enzymatic digestion. Primary 4T1 tumors were minced and placed in 5 mL of DMEM containing 1 mg/mL collagenase I, 1 mM EDTA, and 2% FBS (all from Sigma-Aldrich) for enzymatic digestion. After incubation for 2 hrs at 37 °C, 10 mL of 0.25% trypsin was added and incubated for 3 min, followed by DNase I (Sigma-Aldrich) solution for 1 min, and the digests were filtered through a 40 mm nylon cell strainer (BD Biosciences).

Fluorochrome-labeled anti-mouse mAbs specific for CD3 (145-2C11), CD49b (HMa2), NKp46 (29A1.4), CD69 (H1.2F3), CD11c (N418), F4/80 (T45-2342), NKG2D (CX5), KLRG-1 (2F1), or isotype-matched controls (BD Pharmingen, New Jersey (NJ)/Invitrogen, Carlsbad, California (CA)) were used. For intracellular staining, cells were stimulated with phorbol 12-myristate 13-acetate (50 ng/mL, Sigma-Aldrich), ionomycin (500 ng/mL, Sigma-Aldrich), and GolgyStop (BD Pharmingen, NJ) for 4 hrs and stained with fluorochrome-labeled anti-mouse mAbs specific for perforin (eBio0MAK-D), granzyme (16g6; NGZB), Foxp3 (MF23), IFN-γ (XMG1.2), interleukin (IL)-10 (JES5-16E3), tumor necrosis factor (TNF)-α (MP6-XT22), (Pharmingen/BioLegend/eBiosciences). For the purpose of flow cytometry (FACS) analysis, 20,000 to 50,000 cells were acquired. Flow cytometry was conducted on FACSCalibur Flow Cytometer (BD Biosciences, San Jose, CA), and the data were analyzed using FlowJo (Tree Star).

Statistical analysis

The data were analyzed using commercially available software (SPSS version 23.0). All results were analyzed using the Student's *t*-test, Mann-Whitney *U* test, ANOVA, or Kruskal-Wallis test where appropriate. Data are presented as mean ± SEM. Statistical significance was set at $p < 0.05$.

Results

Anti-PD-1 therapy activates splenic NKT cells and skews its phenotype towards a more tumoricidic one

Administration of anti-PD-1 therapy significantly decelerated tumor growth compared to untreated WT mice. The significant difference between tumor diameters was detected on the 14th day after tumor induction (WT vs. WT + anti-PD-1: 1.57 mm vs. 0.50 mm; $p < 0.05$) and remained until the 40th day when mice were sacrificed (WT vs. WT + anti-PD-1: 11.93 mm vs. 9.37 mm; $p < 0.05$). Further, we analyzed NKT cells in the spleen of tumor-bearing WT mice and WT mice treated with anti-PD-1 antibodies. There was no difference in the percentage of $CD3^+CD49b^+$ NKT cells between the experimental and control groups (Figure 1A). Expression of the activation marker CD69 was significantly elevated in WT anti-PD-1 treated mice compared to WT untreated mice ($p < 0.05$; Figure 1B). The percentage of IFN-γ^+ and perforin$^+$ $CD3^+CD49b^+$ NKT cells was significantly higher, while percentage of $FoxP3^+$ $CD3^+CD49b^+$ NKT cells was significantly lower in WT anti-PD-1 treated mice in comparison to WT untreated mice ($p < 0.05$; Figures 1C–E). There were no significant changes in percentage and phenotype changes in macrophages and DC in the spleen (data not shown).

Enhanced accumulation and alteration toward tumoricidal phenotype of NKT cells in the tumor microenvironment

Within primary tumor tissue, the percentage of $CD3^+CD49b^+$ NKT cells was significantly higher in anti-PD-1 treated mice compared to the untreated group ($p < 0.05$; Figure 2A). Percentage of $NKp46^+$ ($p < 0.05$; Figure 2B) and $NKG2D^+$ ($p < 0.05$; Figure 2C), as well as IFN-γ^+ $CD3^+CD49b^+$ NKT cells ($p < 0.01$; Figure 2D) was significantly higher in WT anti-PD-1 treated mice. Percentage of $FoxP3^+$ ($p < 0.05$; Figure 2E) and $KLRG1^+$ $CD3^+CD49b^+$ NKT cells ($p < 0.05$; Figure 2F) was significantly lower in WT anti-PD-1 treated mice in comparison to WT untreated mice.

Anti-PD-1 therapy facilitates the accumulation and polarization of macrophages in the tumor microenvironment

Anti-PD-1 treatment significantly increased the percentage of $F4/80^+$ cells within primary tumor tissue compared to WT untreated mice ($p < 0.05$; Figure 3A). In addition, the expression of TNF-α in $F4/80^+$ cells was significantly higher in anti-PD-1 treated mice compared to untreated mice ($p < 0.05$; Figure 3B).

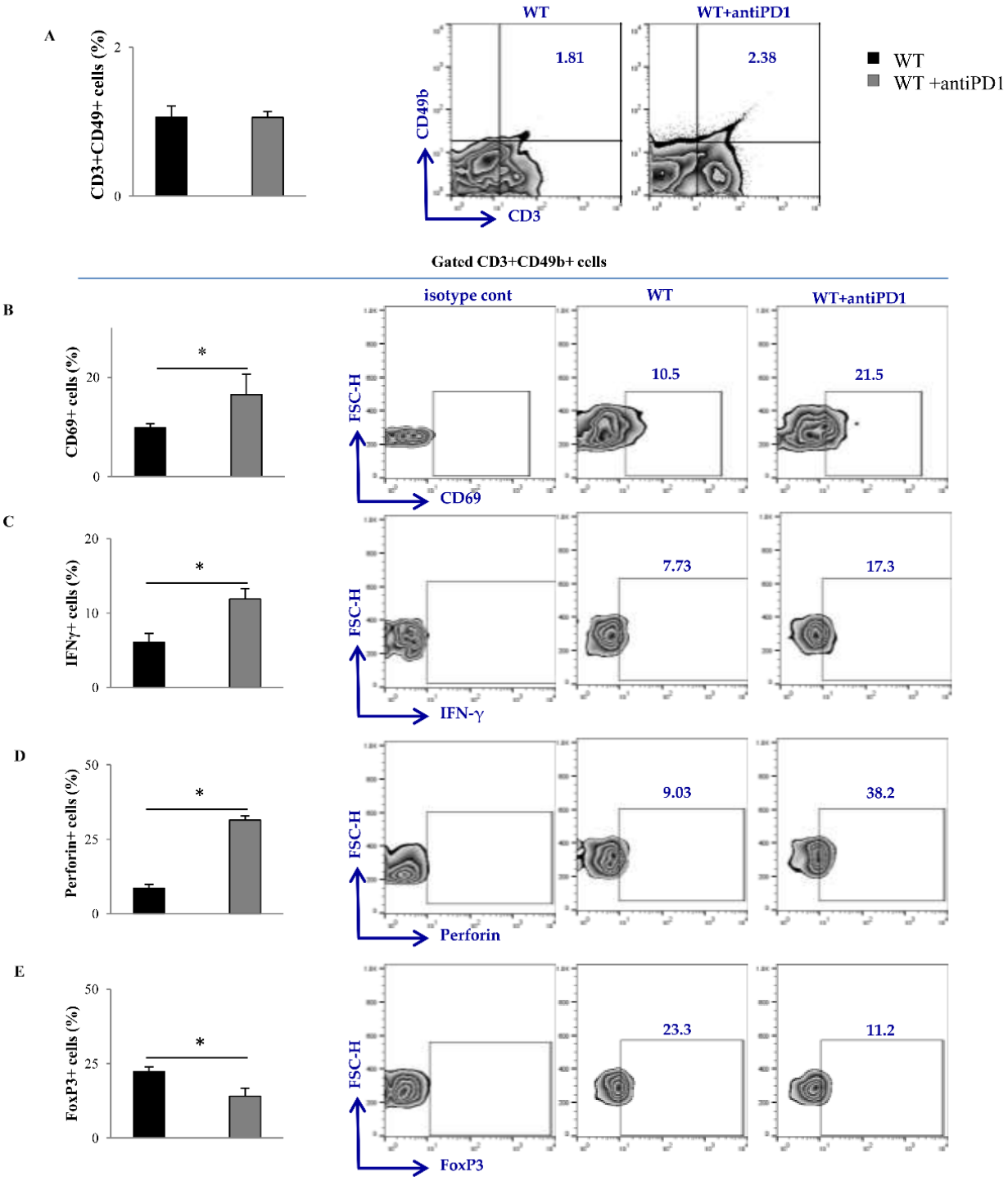


Fig. 1 – Altered phenotype of splenic natural killer T (NKT) cells in anti-programmed cell death protein-1 (anti-PD-1) treated mice. The graphs and representative flow cytometry (FACS) plots display the percentage of $CD3^+CD49b^+$ cells (A), $CD69^+$ (B), interferon (IFN) γ^+ (C), $perforin^+$ (D), and $FoxP3^+$ (E) $CD3^+CD49b^+$ NKT in spleens of wild type (WT) and WT anti-PD-1 treated mice, acquired by FACS. Data are shown as mean ± SEM of six mice per group and are representative of three separate experiments. Statistical significance was tested by the Mann-Whitney Rank Sum test or Student's unpaired *t*-test, where appropriate.

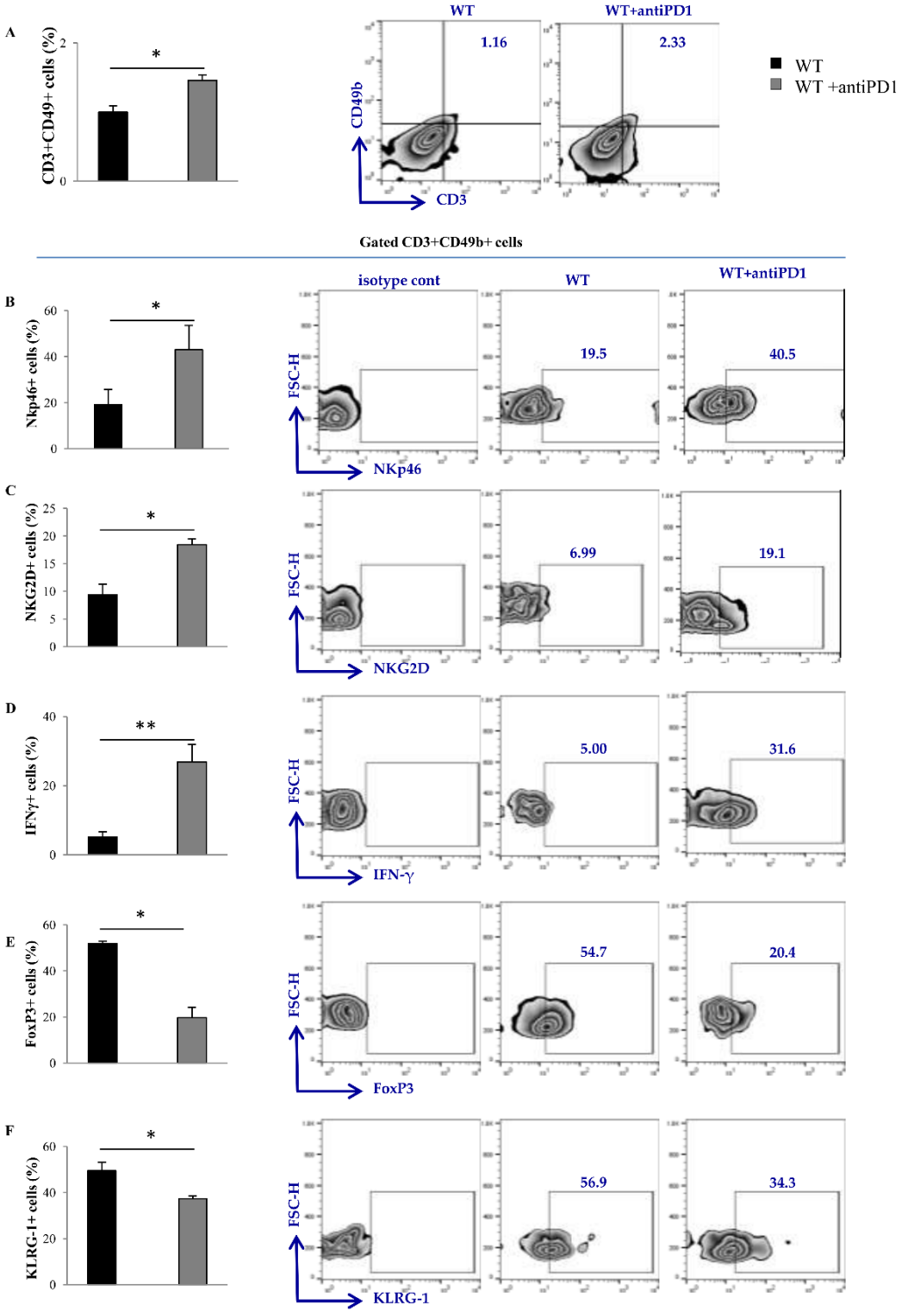


Fig. 2 – Enhanced tumoricidic phenotype of natural killer T (NKT) cells in the tumor microenvironment. The graphs and representative flow cytometry (FACS) plots display the percentage of $CD3^+CD49b^+$ cells (A), $NKp46^+$ (B), $NKG2D^+$(C), interferon (IFN) γ^+ (D), $FoxP3^+$ (E) and $KLRG1^+$ (F) $CD3^+CD49b^+$ NKT in primary tumor of wild type (WT) and WT anti-PD-1 treated mice, acquired by FACS. Data are shown as mean ± SEM of six mice per group and are representative of three separate experiments. Statistical significance was tested by the Mann-Whitney Rank Sum test or Student's unpaired *t*-test, where appropriate.

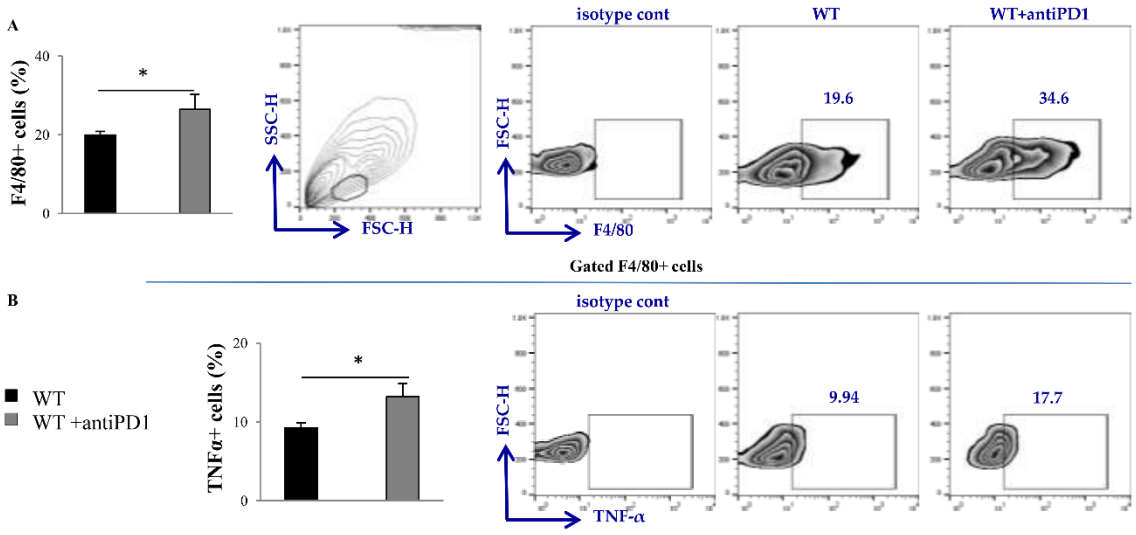


Fig. 3 – Macrophage activation in primary tumor tissue of anti-programmed cell death protein-1 (anti-PD-1) treated mice. The graphs and representative flow cytometry (FACS) plots display the percentage of F4/80$^+$ cells (A) as well as the percentage of tumor necrosis factor (TNF) α$^+$ F4/80$^+$ cells (B) in primary tumor tissue. Data are shown as mean ± SEM of six mice per group and are representative of three separate experiments. Statistical significance was tested by the Mann-Whitney Rank Sum test or Student's unpaired *t*-test, where appropriate.

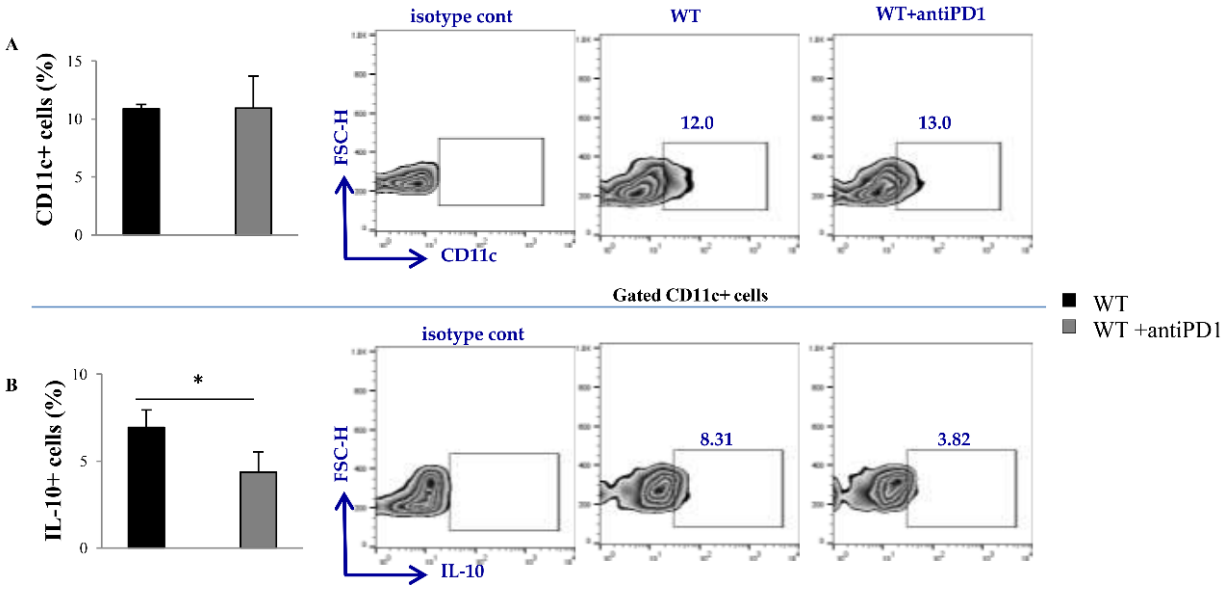


Fig. 4 – Altered phenotype of dendritic cells in the tumor microenvironment of anti-programmed cell death protein-1 (anti-PD-1) treated mice. The graphs and representative flow cytometry (FACS) plots display the percentage of CD11c$^+$ cells (A) as well as the percentage of interleukin (IL) -10$^+$ CD11c$^+$ cells (B) in primary tumor tissue. Data are shown as mean ± SEM of six mice per group and are representative of three separate experiments. Statistical significance was tested by the Mann-Whitney Rank Sum test or Student's unpaired *t*-test, where appropriate.

Anti-PD-1 therapy diminishes the expression of immunosuppressive molecules in dendritic cells within the primary tumor

There was no statistical difference in the percentage of CD11c$^+$ cells in the tumor microenvironment between groups (Figure 4A). However, the percentage of IL-10 producing CD11c$^+$ cells was significantly lower in the tumor microenvironment of anti-PD-1 treated mice ($p < 0.05$; Figure 4B).

Presumed mechanism of action of anti-PD-1 therapy on NKT, dendritic cells, and macrophages is given in Figure 5.

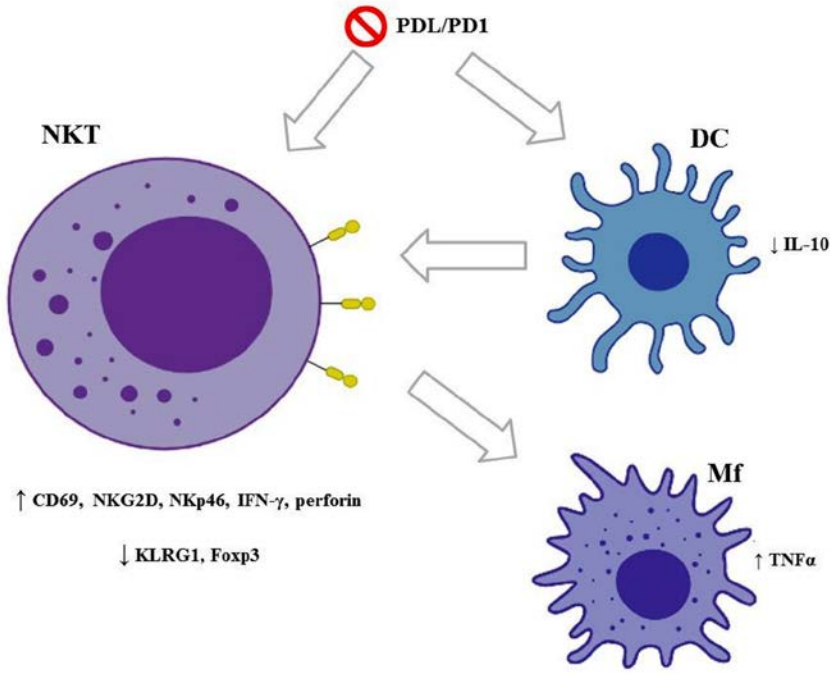


Fig. 5 – Effects of anti-programmed cell death protein-1 (anti-PD-1) therapy on natural killer T (NKT), dendritic cells (DC), and macrophages (Mf). Anti-PD-1 therapy acts directly on NKT cells by facilitating their pro-tumoricidic phenotype, which further polarizes Mf towards the M1 phenotype via augmented interferon (IFN) γ secretion. In addition, anti-PD-1 therapy lowers interleukin (IL)-10 production in DC, making them less tolerogenic and more efficient in activating NKT cells.
PDL – programmed death ligand.

Discussion

As it is well known, checkpoint inhibitors are currently taking an important role in the management of malignant diseases [20, 21]. More specifically, anti-PD-1 antibody has been and is yet to be investigated in numerous oncological diseases, such as melanoma, lung, head and neck, and genitourinary cancers [11, 22]. As it prolongs the half-life of effector immune cells, anti-PD-1 therapy efficiently modulates and stimulates a more efficient immune response [23]. Many studies have shown beneficial effects on T lymphocytes. It has been shown that anti-PD-1 therapy efficiently increases the percentage of cytotoxic T lymphocytes (CTL) within tumor tissue. Moreover, there is some evidence that anti-PD-1 therapy elevates the percentage of $CD4^+$ cells in the peripheral blood of patients undergoing anti-PD-1 therapy [24–28]. Even though T lymphocytes are rather important for compliant anti-PD-1 therapy, other cells, such as NK and NKT cells might contribute to more potent effects of anti-PD-1 therapy. Until now, the anti-PD-1 therapy has been thoroughly studied in terms of adaptive immune response [25, 27], but is yet to be studied in innate immunity during an antitumor immune response, especially regarding NKT cells. It is of great significance to elucidate the effects of immune checkpoint therapy on NK and NKT cells, as these cells might be the key to initiating successful anti-PD-1 therapy when the function of T lymphocytes is impaired [6, 29]. NKT cells have an important role in antitumor immunity. As these cells possess the tremendous capacity to rapidly secrete IFN-γ, IL-2, TNF-α, and IL-4 after antigen stimulation on the one hand and the possibility of specific recognition of antigens on the other, NKT cells might be one of the first cells to instigate antitumor immune response [30]. Additionally, malignancies have the potential to disrupt the metabolism of fatty acids and use them as a source for tumor expansion, while NKT cells that are mostly targeted to lipid antigens might suppress tumor progression by being aimed at altered lipid antigens [31]. In addition, the interaction of NKT cells with other innate immunity cells, such as antigen-presenting cells, stimulates antitumor immune response altogether [32–34].

As NKT cells present an important player in antitumor immunity, we focused our research on the effects of anti-PD-1 therapy on NKT cells, DCs, and macrophages in a model of murine mammary carcinoma. Initially, prior to analysis of immune cells phenotype, tumor growth and progression were significantly slower in anti-PD-1 treated mice when compared to untreated mice. This finding is in line with many previous clinical trials revealing the beneficial effect of anti-PD-1 therapy on decelerating tumor growth and progression, including lung cancer, renal cancer, and especially melanoma [3, 10, 11]. Further on, we analyzed the phenotype of NKT cells in the spleen and tumor microenvironment. Although the percentage of $CD3^+CD49^+$ NKT cells in spleens of anti-PD-1 treated mice remains unchanged (Figure 1A), the phenotype of $CD3^+CD49^+$ NKT cells is remolded towards a more active one. There was a significantly higher percentage of $CD69^+CD3^+CD49^+$ cells in

anti-PD-1 treated mice, which implies that therapy might enforce activation of $CD3^+CD49^+$ cells in the spleen (Figure 1B). In line with this finding, it has been shown that in highly immunosuppressive tumors, such as head and neck carcinomas, ligands for PD-1 in tumor tissue potentially inhibit expression of CD69 and consequently dampen down activation of immune cells [35]. Anti-PD-1 therapy also significantly raised the percentage of IFN-γ^+ $CD3^+CD49^+$ cells in the spleen (Figure 2C). Available data suggest that anti-PD-1 therapy increases the expression of IFN-γ and inhibits the progression of aggressive tumors such as NK/T lymphomas [36]. Furthermore, it has been shown that augmented IFN-γ production in NKT cells stimulates CTL- mediated antitumor immunity in a model of highly immunogenic T cell lymphoma [37]. On the other hand, anti-PD-1 therapy also enhanced the production of perforin in $CD3^+CD49^+$ cells, suggesting that anti-PD-1 therapy can directly enhance the cytotoxic potential of NKT cells (Figure 2D). In addition, the expression of immunosuppressive marker FoxP3 was significantly lower in $CD3^+CD49^+$ cells of anti-PD-1 treated mice (Figure 2E). This indicates that anti-PD-1 therapy, besides directly enhancing IFN-γ production, simultaneously weakens immunosuppressive assets of NKT cells, therefore contributing to the more tumoricidic phenotype of NKT cells altogether.

When it comes to the tumor microenvironment, the percentage of $CD3^+CD49^+$ cells was significantly higher in anti-PD-1 treated mice (Figure 2A), implicating intensive accumulation of NKT cells in primary tumor tissue due to anti-PD-1 therapy. As it is already known, the presence of NKT cells within primary tumor tissue modifies the tumor microenvironment by secreting IFN-γ that activates effector cells and suppresses immunosuppressive populations, therefore enabling a more fluent antitumor immune response [38–40]. Our results imply that anti-PD-1 therapy might stimulate these beneficial properties of NKT cells. Moreover, the percentage of $NKp46^+$ and $NKG2D^+$ cells was also significantly increased in anti-PD-1 treated mice (Figures 2B and 2C), which reflects a more dexterous phenotype of NKT cells in the tumor microenvironment. Similarly, as in the spleen, anti-PD-1 therapy also raised the percentage of IFN-γ-producing $CD3^+CD49^+$ cells within primary tumor tissue (Figure 2D). Furthermore, the expression of FoxP3 and KLRG-1 markers in $CD3^+CD49^+$ NKT cells was significantly diminished in the tumor microenvironment, which is indicative of the NKT cell phenotype that is less prone to anergy [38].

As NKT cells are known to interact with many immune cells, such as T cells, DCs, and macrophages [41, 42], and the fact that 4T1 mammary carcinoma presents a low immunogenic tumor, we further analyzed DCs and macrophages within the primary tumor. Tumor-associated macrophages (TAMs) are one of the most abundant cells within the primary tissue of the tumor [43]. Unfortunately, TAMs that reside in the tumor microenvironment are mostly those of an immunosuppressive M2 phenotype, thus allowing the immune escape of the tumor [44]. Given the vast range of macrophage immunomodulatory properties, facilitating these cells might be of great significance when it comes to revealing more potent therapeutic strategies in malignancy. As it is known, TAMs might stimulate an antitumor immune response by secreting TNF-α and also suppress the antitumor immune response by secreting IL-10 inducing overall immunosuppression [45, 46]. Our results showed that anti-PD-1 therapy significantly enhanced the percentage of $F4/80^+$ macrophages within the tumor microenvironment (Figure 3A) and, in addition, significantly increased the production of TNF-α in $F4/80^+$ macrophages (Figure 3B), which is a hallmark of the M1 phenotype [47]. When it comes to DCs, anti-PD-1 therapy did not alter the percentage of resident $CD11c^+$ DCs within the tumor microenvironment (Figure 4A). Yet, the percentage of IL-10 producing $CD11c^+$ DCs was significantly decreased in anti-PD-1 treated group (Figure 4B). As professional antigen-presenting cells, DCs are constantly circulating throughout the tumor microenvironment, where they are continuously exposed to immunosuppressive molecules produced by cancer cells [48]. As such, DCs might become tolerogenic and stimulate further immunosuppression by secreting molecules such as IL-10 [49]. According to their role in immune responses, DCs are traditionally divided into two groups: conventional or classical DCs and plasmacytoid DCs [50]. Conventional DCs express high levels of major histocompatibility complex (MHC) molecules, thus stimulating antitumor immunity, while plasmacytoid DCs are mainly involved in interferon secretion. Apart from classification, during an antitumor immune response, DCs can switch between tolerogenic and effector phenotypes [49, 51]. As our results showed markedly lowered expression of IL-10 in DCs, we believe that anti-PD-1 therapy, at least in part, might abrogate polarization of DCs towards tolerogenic phenotype. DCs, as it is known, interact with NKT cells via direct contact or indirectly by expressing and secreting modulating molecules, such as CD40, type I and II interferons, IL-10, and TNF-α [52]. Given our result, that DCs of anti-PD-1 treated mice have a more immunogenic phenotype than those in untreated mice, DCs of anti-PD-1 treated mice might be even more potent in triggering NKT cell activation besides the already shown direct activation of NKT cells by anti-PD-1 therapy. As mentioned before, upon activation, NKT cells rapidly secrete activating molecules that stimulate other immune cells, such as macrophages. Since our results imply enhanced secretion of IFN-γ in NKT cells upon anti-PD-1 therapy, and, on the other hand, IFN-γ strongly facilitates macrophages towards the M1 phenotype [53], we speculate that anti-PD-1 driven NKT cells polarize macrophages towards the antitumorigenic, M1 phenotype (Figure 5).

Conclusion

Anti-PD-1 therapy activates NKT cells directly and indirectly via DCs. Activated NKT cells provide tumoricidic properties directly by secreting perforin and indirectly by polarizing macrophages towards the M1 phenotype. Further studies are needed to clarify the interplay between NKT cells and other immune cells in the context of anti-PD-1 therapy, shedding new light on various beneficial aspects of immune checkpoint therapy.

R E F E R E N C E S

1. *Farkona S, Diamandis EP, Blasutig IM*. Cancer immunotherapy: the beginning of the end of cancer? BMC Med 2016; 14: 73.
2. *Li B, Chan HL, Chen P*. Immune Checkpoint Inhibitors: Basics and Challenges. Curr Med Chem 2019; 26(17): 3009–25.
3. *Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK*, et al. PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome. Front Pharmacol 2017; 8: 561.
4. *Zamani MR, Aslani S, Salmaninejad A, Javan MR, Rezaei N*. PD-1/PD-L and autoimmunity: A growing relationship. Cell Immunol 2016; 310: 27–41.
5. *Habib S, El Andaloussi A, Elmasry K, Handoussa A, Azab M, Elsawey A*, et al. PDL-1 Blockade Prevents T Cell Exhaustion, Inhibits Autophagy, and Promotes Clearance of Leishmania-adonovani. Infect Immun 2018; 86(6): e00019-18.
6. *Mazzaschi G, Facchinetti F, Missale G, Canetti D, Madeddu D, Zecca A,* et al. The circulating pool of functionally competent NK and CD8+ cells predicts the outcome of anti-PD1 treatment in advanced NSCLC. Lung Cancer 2019; 127: 153–63.
7. *Oyer JL, Gitto SB, Altomare DA, Copik AJ*. PD-L1 blockade enhances anti-tumor efficacy of NK cells. Oncoimmunology 2018; 7(11): e1509819.
8. *Patel SP, Kurzrock R*. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Mol Cancer Ther 2015; 14(4): 847–56.
9. *Rouanne M, Roumiguié M, Houédé N, Masson-Lecomte A, Colin P, Pignot G,* et al. Development of immunotherapy in bladder cancer: present and future on targeting PD(L)1 and CTLA-4 pathways. World J Urol 2018; 36(11): 1727–40.
10. *Kwok G, Yau TC, Chiu JW, Tse E, Kwong YL*. Pembrolizumab (Keytruda). Hum Vaccin Immunother 2016; 12(11): 2777–89.
11. *Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB*. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. Cancers (Basel) 2020; 12(3): 738.
12. *Dong W, Wu X, Ma S, Wang Y, Nalin AP, Zhu Z,* et al. The Mechanism of Anti-PD-L1 Antibody Efficacy against PD-L1-Negative Tumors Identifies NK Cells Expressing PD-L1 as a Cytolytic Effector. Cancer Discov 2019; 9(10): 1422–37.
13. *Zhang C, Liu Y*. Targeting NK Cell Checkpoint Receptors or Molecules for Cancer Immunotherapy. Front Immunol 2020; 11: 1295.
14. *Das R, Verma R, Sznol M, Boddupalli CS, Gettinger SN, Kluger H*, et al. Combination therapy with anti-CTLA-4 and anti-PD-1 leads to distinct immunologic changes in vivo. J Immunol 2015; 194(3): 950–9.
15. *Exley MA, Wilson SB, Balk SP*. Isolation and Functional Use of Human NKT Cells. Curr Protoc Immunol 2017; 119: 14.11.1–14.11.20.
16. *Qin L, Dominguez D, Chen S, Fan J, Long A, Zhang M*, et al. Exogenous IL-33 overcomes T cell tolerance in murine acute myeloid leukemia. Oncotarget 2016; 7(38): 61069–80.
17. *Shimizu T, Fuchimoto Y, Fukuda K, Okita H, Kitagawa Y, Kuroda T*. The effect of immune checkpoint inhibitors on lung metastases of osteosarcoma. J Pediatr Surg 2017; 52(12): 2047–50.
18. *Vo MC, Jung SH, Chu TH, Lee HJ, Lakshmi TJ, Park HS,* et al. Lenalidomide and Programmed Death-1 Blockade Synergistically Enhances the Effects of Dendritic Cell Vaccination in a Model of Murine Myeloma. Front Immunol 2018; 9: 1370.
19. *Jovanovic I, Radosavljevic G, Mitrovic M, Juranic VL, McKenzie AN, Arsenijevic N,* et al. ST2 deletion enhances innate and acquired immunity to murine mammary carcinoma. Eur J Immunol 2011; 41(7): 1902–12.
20. *Taniguchi M, Harada M, Dashtsoodol N, Kojo S*. Discovery of NKT cells and development of NKT cell-targeted anti-tumor immunotherapy. Proc Jpn Acad Ser B Phys Biol Sci 2015; 91(7): 292–304.
21. *Ribeiro Gomes J, Schmerling RA, Haddad CK, Racy DJ, Ferrigno R, Gil E,* et al. Analysis of the Abscopal Effect With Anti-PD1 Therapy in Patients With Metastatic Solid Tumors. J Immunother 2016; 39(9): 367–72.
22. *Khan M, Lin J, Liao G, Tian Y, Liang Y, Li R,* et al. Comparative analysis of immune checkpoint inhibitors and chemotherapy in the treatment of advanced non-small cell lung cancer: A meta-analysis of randomized controlled trials. Medicine (Baltimore) 2018; 97(33): e11936.
23. *Carbognin L, Pilotto S, Milella M, Vaccaro V, Brunelli M, Caliò A,* et al. Differential Activity of Nivolumab, Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity Analysis of Trials in Melanoma, Lung and Genitourinary Cancers. PLoS One 2015; 10(6): e0130142.
24. *Iwai Y, Hamanishi J, Chamoto K, Honjo T*. Cancer immunotherapies targeting the PD-1 signaling pathway. J Biomed Sci 2017; 24(1): 26.
25. *Ngiow SF, Young A, Jacquelot N, Yamazaki T, Enot D, Zitvogel L,* et al. A Threshold Level of Intratumor CD8+ T-cell PD1 Expression Dictates Therapeutic Response to Anti-PD1. Cancer Res 2015; 75(18): 3800–11.
26. *Sun C, Mezzadra R, Schumacher TN*. Regulation and Function of the PD-L1 Checkpoint. Immunity 2018; 48(3): 434–52.
27. *Antonia SJ, Vansteenkiste JF, Moon E*. Immunotherapy: Beyond Anti-PD-1 and Anti-PD-L1 Therapies. Am Soc Clin Oncol Educ Book 2016; 35: e450–8.
28. *Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA,* et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science 2017; 355(6332): 1428–33.
29. *Kagamu H, Kitano S, Yamaguchi O, Yoshimura K, Horimoto K, Kitazawa M,* et al. CD4+ T-cell Immunity in the Peripheral Blood Correlates with Response to Anti-PD-1 Therapy. Cancer Immunol Res 2020; 8(3): 334–44.
30. *Bae EA, Seo H, Kim BS, Choi J, Jeon I, Shin KS,* et al. Activation of NKT Cells in an Anti-PD-1-Resistant Tumor Model Enhances Antitumor Immunity by Reinvigorating Exhausted CD8 T Cells. Cancer Res 2018; 78(18): 5315–26.
31. *Terabe M, Berzofsky JA*. Tissue-Specific Roles of NKT Cells in Tumor Immunity. Front Immunol 2018; 9: 1838.
32. *Tiwary S, Berzofsky JA, Terabe M*. Altered Lipid Tumor Environment and Its Potential Effects on NKT Cell Function in Tumor Immunity. Front Immunol 2019; 10: 2187.
33. *Kim N, Kim HS*. Targeting Checkpoint Receptors and Molecules for Therapeutic Modulation of Natural Killer Cells. Front Immunol 2018; 9: 2041.
34. *Teyton L*. New Directions for Natural Killer T Cells in the Immunotherapy of Cancer. Front Immunol 2017; 8: 1480.
35. *Sivori S, Pende D, Quatrini L, Pietra G, Della Chiesa M, Vacca P*, et al. NK cells and ILCs in tumor immunotherapy. Mol Aspects Med 2020; 100870.
36. *Theodoraki MN, Yerneni SS, Hoffmann TK, Gooding WE, Whiteside TL*. Clinical Significance of PD-L1+ Exosomes in Plasma of Head and Neck Cancer Patients. Clin Cancer Res 2018; 24(4): 896–905.
37. *Xue W, Li W, Zhang T, Li Z, Wang Y, Qiu Y,* et al. Anti-PD1 up-regulates PD-L1 expression and inhibits T-cell lymphoma progression: possible involvement of an IFN-γ-associated JAK-STAT pathway. Onco Targets Ther 2019; 12: 2079–88.
38. *Qin Y, Oh S, Lim S, Shin JH, Yoon MS, Park SH*. Invariant NKT cells facilitate cytotoxic T-cell activation via direct recognition of CD1d on T cells. Exp Mol Med 2019; 51(10): 1–9.

39. *Krijgsman D, Hokland M, Kuppen PJK.* The role of natural killer T cells in cancer-A phenotypical and functional approach. Front Immunol 2018; 9: 367.
40. *Bedard M, Salio M, Cerundolo V.* Harnessing the power of invariant natural killer T cells in cancer immunotherapy. Front Immunol 2017; 8: 1829.
41. *Díaz-Basabe A, Strati F, Facciotti F.* License to Kill: When iNKT Cells Are Granted the Use of Lethal Cytotoxicity. Int J Mol Sci 2020; 21(11): 3909.
42. *Fallarini S, Paoletti T, Orsi Battaglini N, Lombardi G.* Invariant NKT cells increase drug-induced osteosarcoma cell death. Br J Pharmacol 2012; 167(7): 1533–49.
43. *Hix LM, Shi YH, Brutkiewicz RR, Stein PL, Wang CR, Zhang M.* CD1d-expressing breast cancer cells modulate NKT cell-mediated antitumor immunity in a murine model of breast cancer metastasis. PLoS One 2011; 6(6): e20702.
44. *Tan B, Shi X, Zhang J, Qin J, Zhang N, Ren H,* et al. Inhibition of Rspo-Lgr4 Facilitates Checkpoint Blockade Therapy by Switching Macrophage Polarization. Cancer Res 2018; 78(17): 4929–42.
45. *Xiang W, Shi R, Kang X, Zhang X, Chen P, Zhang L,* et al. Monoacylglycerol lipase regulates cannabinoid receptor 2-dependent macrophage activation and cancer progression. Nat Commun 2018; 9(1): 2574.
46. *Najafi M, Hashemi Goradel N, Farhood B, Salehi E, Nashtaei MS, Khanlarkhani N,* et al. Macrophage polarity in cancer: A review. J Cell Biochem 2019; 120(3): 2756–65.
47. *Li TF, Li K, Wang C, Liu X, Wen Y, Xu YH,* et al. Harnessing the cross-talk between tumor cells and tumor-associated macrophages with a nano-drug for modulation of glioblastoma immune microenvironment. J Control Release 2017; 268: 128–46.
48. *Donzelli S, Milano E, Pruszko M, Sacconi A, Masciarelli S, Iosue I,* et al. Expression of ID4 protein in breast cancer cells induces reprogramming of tumour-associated macrophages. Breast Cancer Res 2018; 20(1): 59.
49. *Sumpter TL, Dangi A, Matta BM, Huang C, Stolz DB, Vodovotz Y,* et al. Hepatic stellate cells undermine the allostimulatory function of liver myeloid dendritic cells via STAT3-dependent induction of IDO. J Immunol 2012; 189(8): 3848–58.
50. *Motta JM, Rumjanek VM.* Sensitivity of Dendritic Cells to Microenvironment Signals. J Immunol Res 2016; 2016: 4753607.
51. *Balan S, Saxena M, Bhardwaj N.* Dendritic cell subsets and locations. Int Rev Cell Mol Biol 2019; 348: 1–68.
52. *Fujii SI, Shimizu K.* Exploiting Antitumor Immunotherapeutic Novel Strategies by Deciphering the Cross Talk between Invariant NKT Cells and Dendritic Cells. Front Immunol 2017; 8: 886.
53. *Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F,* et al. Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol 2018; 233(9): 6425–40.

Received on January 26, 2021
Revised on March 28, 2021
Accepted on March 30, 2021
Online First April 2021

ORIGINAL ARTICLE

UDC: 616-006:616.24-006-08
DOI: https://doi.org/10.2298/VSP210102038R

Clinical outcome and side effects of concomitant chemoradiotherapy in the treatment of locally advanced inoperable non-small cell lung cancer: our experiences

Klinički ishod i neželjena dejstva primene istovremene hemioradioterapije u lečenju lokalno uznapredovalog inoperabilnog nemikrocelularnog karcinoma pluća: naša iskustva

Bojan Radojičić*†, Marija Radojičić*‡, Miroslav Mišović*, Dejan Kostić*§

***Military Medical Academy, Institute of Radiology, Department of Radiotherapy, Belgrade, Serbia; †Center for Military Medical Institutions, Department of Radiology, Belgrade, Serbia; ‡Health Center "Simo Milošević", Department of Radiology, Belgrade, Serbia; §University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia**

Abstract

Background/Aim. About 1.8 million new lung cancer cases are diagnosed worldwide every year, and about 1.6 million cases have a fatal outcome. Despite improvements in treatment in the previous decades, the survival of patients with lung cancer is still poor. The five-year survival rate is about 50% for patients with localized disease, 20% for patients with regionally advanced disease, 2% for patients with metastatic disease, and about 14% for all stages. The median survival of patients with untreated non-small cell lung carcinoma (NSCLC) in the advanced stage is four to five months, and the annual survival rate is only 10%. The aim of the study was to determine the results of treatment with concomitant chemoradiotherapy (CHRT) in terms of efficacy and toxicity in selected patients with advanced inoperable NSCLC. **Methods**. The study included data analysis of 31 patients of both sexes who were diagnosed and histopathologically verified with NSCLC in inoperable stage III and were referred by the Council for Malignant Lung Diseases to the Radiotherapy Department of the Military Medical Academy in Belgrade, Serbia for concomitant CHRT treatment. Upon expiry of the three months from the performed radiation treatment (RT), the tumor resonance was assessed based on multislice computed tomography (MSCT) examination of the chest and upper abdomen according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. According to the same criteria, progression-free survival (PFS), as well as overall survival (OS), was assessed every three months during the first two years, then every 6 months or until the onset of disease symptoms. **Results.** The median PFS was 13 months, and the median OS was 20 months. During and immediately after RT, 9 (29%) patients had a grade 2 or higher adverse events. **Conclusion.** The use of concomitant CHRT in patients in the third stage of locally advanced inoperable NSCLC provides a good opportunity for a favorable therapeutic outcome with an acceptable degree of acute and late toxicity and represents the standard therapeutic approach for selected patients in this stage of the disease.

Key words:
carcinoma, non-small-cell lung; disease progression; drug-related side effects and adverse reactions; chemoradiotherapy; survival.

Apstrakt

Uvod/Cilj. Godišnje se u svetu dijagnostikuje oko 1,8 miliona novoobolelih, a umre oko 1,6 miliona obolelih od karcinoma pluća. Uprkos poboljšanjima u lečenju tokom prethodnih decenija, preživljavanje bolesnika sa karcinomom pluća je i dalje loše. Petogodišnja stopa preživljavanja je oko 50% za bolesnike sa lokalizovanom bolešću, 20% za bolesnike sa regionalno uznapredovalom bolešću, 2% za bolesnike sa metastatskom bolešću, a za sve stadijume oko 14%. Srednje preživljavanje bolesnika sa nelečenim nesitnoćelijskim karcinomom pluća (NSCLC) u odmaklom stadijumu bolesti je četiri do pet meseci, a na godišnjem nivou stopa preživljavanje iznosi svega 10%. Cilj rada bio je da se utvrdi efikasnost i toksičnost istovremene hemioradioterapije (CHRT) kod odabranih bolesnika sa uznapredovalim inoperabilnim NSCLC. **Metode.** Studija je obuhvatila analizu podataka 31 bolesnika oba pola, koje je

Correspondence to: Bojan Radojičić, Military Medical Academy, Institute of Radiology, Department of Radiotherapy, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: radioterapija.rs@gmail.com

uputio Konzilijum za maligne bolesti pluća na Odeljenje radioterapije Vojnomedicinske akademije u Beogradu, kod kojih je dijagnostikovan i patohistološki verifikovan NSCLC u III inoperabilnom stadijumu radi sprovođenja konkomitantne CHRT. Po isteku tri meseca od sprovedene terapije zračenjem (RT) vršena je procena odgovora tumora na RT primenom multislajsne kompjuterizovane tomografije (MSCT) grudnog koša i gornjeg abdomena po kriterijumu *Response Evaluation Criteria in Solid Tumors* (RECIST) verzija 1.1. Po istom kriterijumu vršena je i procena preživljavanja do progresije bolesti (PFS) i ukupno preživljavanje bolesnika (OS) svaka tri meseca tokom prve dve godine, zatim na 6 meseci ili do pojave simptoma bolesti. **Rezultati.** Medijana PFS iznosila je 13 meseci, a medijana OS 20 meseci. Tokom i neposredno nakon RT, neželjeni događaj gradusa 2 ili većeg imalo je 9 (29%) bolesnika. **Zaključak.** Primena istovremene CHRT kod bolesnika koji su u trećem stadijumu lokalno uznapredovalog inoperabilnog NSCLC daje dobru mogućnost za povoljan terapijski ishod, uz prihvatljiv stepen akutne i kasne toksičnosti i predstavlja standardni terapijski pristup za odabrane bolesnike u tom stadijumu bolesti.

Ključne reči:
pluća, nesitnoćelijski karcinom; bolest, progresija; lekovi, neželjeni efekti i neželjene reakcije; radiohemioterapija; preživljavanje.

Introduction

Lung cancer is a significant health problem globally due to its frequency and the fact that it belongs to the group of the most deadly forms of cancer. According to the results of GLOBOCAN from 2018, about 1.8 million new patients are diagnosed worldwide every year, and about 1.6 million die from lung cancer [1, 2]. Despite improvements in treatment in previous decades, the survival of patients with lung cancer is still poor. The five-year survival rate is about 50% for patients with localized disease, 20% for patients with regionally advanced disease, 2% for patients with metastatic disease, and about 14% cumulatively for all stages [3]. The median survival of patients with untreated non-small cell lung carcinoma (NSCLC) in the advanced stage of the disease is four to five months, and the annual survival rate is only 10% [4].

The primary goal in treating all cancers is healing, which unfortunately is not possible in a large number of cases. The secondary goal is to stop the further progression of this chronic disease and improve patients' quality of life.

The main problem in the treatment of lung cancer is that a large number of patients are detected only when the symptoms and/or signs appear, i.e., when the disease has already progressed, when the chances of being cured are much lower, and the therapeutic approach is more complex. The main types of lung cancer therapy are surgery, radiotherapy (RT), chemotherapy (CHT), and target therapy.

The therapeutic approach depends on the type of tumor, the stage of the disease, the general condition of the patient, and the patient's motivation to accept a certain type of treatment. In patients with disease limited to the lung parenchyma, the optimal approach is resection of the affected lobe and mediastinal lymph nodes [5, 6]. At the same time, in patients with advanced disease, the best way to ensure optimal treatment is a multidisciplinary approach by surgeons, medical oncologists, and radiation oncologists who make joint decisions regarding treatment based on current guides for the treatment of cancer patients [7–10]. Locally advanced NSCLC refers to a heterogeneous group of patients in stage III of the disease. It includes patients with tumor spread to extrapulmonary structures (T3-4) or mediastinal lymph nodes (N2-3) without distant metastases (M0). In locally advanced diseases, surgery is less commonly used, especially as the primary approach [11, 12]. The multimodal approach is the basic therapeutic principle (CHT and/or RT, possibly surgery), and the decision on the modality of treatment largely depends primarily on the precisely determined stage of the disease. The status of mediastinal lymph nodes (N2) is especially important when deciding on the therapeutic approach from the aspect of the malignant cells invasion, localization, the number of affected nodes, and the time of their pathohistological diagnosis (before, peri- or postoperative). Patients with N2 are between patients with resectable and unresectable diseases and thus represent a group with the most complex treatment. Patient selection affects not only the response to therapy but also how well the patient will tolerate therapy, i.e., whether possible acute complications will affect the course of therapy and cause temporary or permanent interruption or lead to serious impairment of the patient's health and even death. The treatment of locally advanced NSCLC is very challenging and must be individualized.

Methods

This research was conducted at the Radiotherapy Department of the Institute of Radiology of the Military Medical Academy in Belgrade, Serbia and has had a retrospective-prospective character. The study included data analysis of 31 patients of both sexes who were diagnosed and pathohistologically verified with NSCLC in inoperable stage III and were referred by the Council for Malignant Lung Diseases to the Radiotherapy Department of the Military Medical Academy for concomitant chemoradiotherapy (CHRT) treatment.

Determination of the stage of the disease was performed based on VIII revision of the tumor, node and metastasis (TNM) classification for malignant tumors of the lungs and pleura [13]. It also included insight into medical history and clinical examination of a patient, chest multislice computed tomography (MSCT) not older than one month, abdominal ultrasound, bronchoscopy with an endoscopic evaluation of tumors, and nodal status.

Inclusion criteria were as follows: histopathologically diagnosed NSCLC [14], inoperable stage III disease, a decision indicated by the council on combined CHRT, performance status (PS) 0 or 1, complete blood count laboratory values, liver

enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, hemoglobin, leukocytes, erythrocytes within the reference values, and the patient's consent to therapeutic procedures. Exclusion criteria were as follows: performance status 2 or higher, previously diagnosed and/or treated malignant diseases of the chest, and previously applied RT of the chest.

In all selected patients, treatment was based on concomitant CHRT, with CHT administered according to the cisplatin/etoposide protocol in two cycles (cisplatin 50 mg/m^2 for 1, 8, 29, and 36 days, etoposide 50 mg/m^2 for 1–5 and 29–33 days), and on RT performed according to 3DCRT protocol in a standard fractionation regimen of 2 Gy per day in total TD 60Gy for 6 weeks [15, 16]. Standardized recommendations of the International Commission on Radiation Units and Measurements – ICRU 50 and ICRU 62, were used to delineate air volumes – gross tumor volume (GTV), clinical target volume (CTV), planning target volume (PTV), as well as organ at risk (OAR) [17].

Upon expiry of the three-month period from the performed RT, the response to the therapy was assessed based on MSCT examination of the chest and upper abdomen according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [18]. According to the same criteria, the assessment for progression-free survival (PFS) was executed, which was routinely performed every three months during the first two years, then every 6 months, or until the onset of symptoms of the disease.

Assessment of the degree of toxicity of concomitant CHRT was performed according to the criteria of National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0 [19]: grade I implies mild symptoms, grade II – moderate symptoms, grade III – significant complications, grade IV – life-threatening complications, grade V – the death of a patient.

The IBM Statistical Package for the Social Sciences (SPSS) Statistics package version 24 was used for statistical data processing. Numerical features were presented through means (median, arithmetic mean) and measures of variability (standard deviation, range of values). Attribute features are shown using frequencies and percentages. The Likelihood Ratio Test was used to test the relationship between the two categorical variables. Mortality rates were calculated, as well as overall survival (OS) and PFS. Cox regression was used to test the influence of individual variables on OS and PFS.

The value of significance level $p \leq 0.05$ was considered statistically significant.

Results

The study included 31 patients of both sexes with histopathologically verified NSCLC in stage III inoperable, with an average age of 65.67 ± 8.28. There were 14 (45.2%) respondents in stage IIIA, while 17 (54.8%) were in stage IIIB. There were no patients in stage IIIC to meet the criteria to enter the study. Fourteen patients had histopathologically verified adenocarcinoma and squamous cell carcinoma, while no histopathological tumor subtype (NSCLC NOS) was specified for three patients. The largest number of patients included in the study, 13 (41.9%), had histological grade (HG) 2, 10 (32.3%) subjects had HG 3, and 8 (25.8%) subjects had HG 1.

Tumor response was assessed based on MSCT of the chest and upper abdomen according to RECIST 1.1. criteria. Evaluation and comparison were carried out between the ini-

Table 1

Progression-free survival in relation to research subgroups

Parameter	Average	SE	95% confidence interval		Median	SE	95% confidence interval		Log Rank (Mantel-Cox)	df	*p*
			lower limit	upper limit			lower limit	upper limit			
Stage											
IIIA	14.143	1.508	11.188	17.098	15.000	0.598	13.829	16.171			
IIIB	11.532	1.107	9.362	13.702	12.000	0.606	10.812	13.188	2.840	1	0.092
Total	12.796	0.940	10.953	14.639	13.000	0.651	11.723	14.277			
HT											
AdenoCa	13.455	1.417	10.677	16.233	13.000	0.833	11.367	14.633			
SCC	11.143	1.374	8.449	13.836	12.000	0.926	10.185	13.815	2.633	2	0.268
NSCLC	17.333	1.856	13.696	20.971	16.000	0.816	14.400	17.600			
Total	12.796	0.940	10.953	14.639	13.000	0.651	11.723	14.277			
HG											
1	11.375	2.645	6.190	16.560	11.000	4.950	0.000	16.702			
2	12.538	1.293	10.005	15.072	13.000	0.540	11.942	14.058	0.401	2	0.818
3	14.175	1.113	11.993	16.357	15.000	1.243	12.564	17.436			
Total	12.796	0.940	10.953	14.639	13.000	0.651	11.723	14.277			
ECOG status											
0	12.659	1.120	10.464	14.854	13.000	0.581	11.862	14.138			
1	13.600	0.927	11.782	15.418	14.000	2.191	9.706	18.294	0.059	1	0.809
Total	12.796	0.940	10.953	14.639	13.000	0.651	11.723	14.277			
Localization											
left	13.467	1.521	10.486	16.448	13.000	1.932	9.213	16.787			
right	12.007	1.072	9.905	14.109	13.000	0.588	11.848	14.152	0.744	1	0.388
Total	12.796	0.940	10.953	14.639	13.000	0.651	11.723	14.277			

SE – standard error; AdenoCa – adenocarcinoma; SCC – squamous cell carcinoma; NSCLC – non-small cell lung carcinoma; HT – histopathological types of cancer; HG – histological gradus; ECOG – Eastern Cooperative Oncology Group.

tial scan (before starting the combined treatment) and the control scan, which was performed three months after the end of the treatment. Six (19.4%) respondents had a complete response (CR), seven (22.6%) had stable disease (SD), and seventeen (54.8%) respondents had a partial response (PR). The disease was progressive (PD) in one subject (3.2%).

If CR, PR, and SD were taken for a "favorable" response to therapy, we concluded that 30 (96.8%) respondents would have had a "favorable" response.

PFS was routinely evaluated with chest and upper abdominal MSCT every three months for the first two years, then every 6 months, or until symptoms of the disease appeared.

The obtained results (Table 1) did not show a statistically significant difference between the median time to disease progression in patients with IIIA and IIIB stage, 15 months vs. 12 months. There was no statistically significant difference between the median PFS in patients with adenocarcinoma, patients with squamous cell carcinoma, and patients with NSCL NOS (13 vs. 12 vs. 16 months, respectively). The PFS median for subjects with HG1 was 11 months, with HG2 was 13 months, and with HG3 was 15 months. For patients in the Eastern Cooperative Oncology Group (ECOG) PS 0, the median PFS was 13 months, and for ECOG PS1, the median was 14 months. PFS median in subjects with tumor localization on the left or right side was the same and amounted to 13 months (Table 2).

The mortality rate in the examined sample was 83.9%. The total number of survivors at the end of the follow-up period was 5 (16.1%), and the average survival time for patients was 20 months. The median of survivors was 28 months (Figure 1).

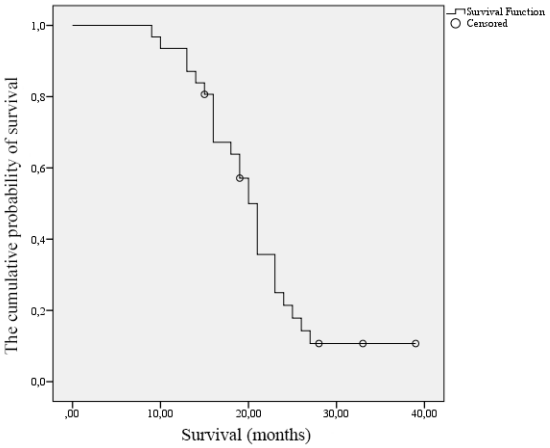


Fig. 1 – Overall survival in patients with non-small cell lung carcinoma.

In order to examine whether OS was similar or different for different study groups, we used the Log Rank test (Table 3).

Table 2

Median time to disease progression for patients with non-small cell lung carcinoma (NSCLC)

Average	SE	95% confidence interval		Median	SE	95% confidence interval	
		lower limit	upper limit			lower limit	upper limit
12.796	0.940	10.953	14.639	13.000	0.651	11.723	14.277

SE – standard error.

Table 3

Median overall survival of patients with non-small cell lung carcinoma (NSCLC) in relation to research subgroups

Parameter	Average	SE	95% confidence interval		Median	SE	95% confidence interval		Log Rank (Mantel-Cox)	Average	SE
			lower limit	upper limit			lower limit	upper limit			
Stage											
IIIA	23.461	2.181	19.185	27.737	23.000	1.304	20.443	25.557			
IIIB	18.892	1.395	16.158	21.627	18.000	1.372	15.311	20.689	2.407	1	0.121
Total	21.129	1.401	18.383	23.875	20.000	0.858	18.318	21.682			
PH											
AdenoCa	24.085	2.746	18.703	29.468	21.000	4.014	13.132	28.868			
SCC	18.000	1.301	15.450	20.550	19.000	1.871	15.333	22.667	5.002	2	0.082
NSCLC NOS	24.000	2.517	19.067	28.933	26,000	5.715	14.798	37.202			
Total	21.129	1.401	18.383	23.875	20.000	0.858	18.318	21.682			
HG											
1	18.875	2.997	13.001	24.749	18.000	3.536	9.070	22.930			
2	20.846	1.865	17.191	24.501	21.000	2.516	16.068	25.932	1.298	2	0.523
3	21.756	1.324	19.160	24.351	21.000	1.304	18.444	23.556			
Total	21.129	1.401	18.383	23.875	20.000	0.858	18.318	21.682			
ECOG status											
0	20.657	1.484	17.747	23.566	20.000	0.929	18.179	21.821			
1	21.200	1.927	17.424	24.976	21,000	2.191	16.706	25.294	0.240	1	0.624
Total	21.129	1.401	18.383	23.875	20.000	0.858	18.318	21.682			
Localization											
left	21.687	2.394	16.994	26.380	23.000	4.112	14.940	31.060			
right	20.167	1.183	17.849	22.485	20.000	0.614	18.797	21.203	0.554	1	0.457
Total	21.129	1.401	18.383	23.875	20.000	0.858	18.318	21.682			

SE – standard error; PH – pathohistological types of cancer; AdenoCa – adenocarcinoma; SCC – squamous cell carcinoma; HG – histological gradus; ECOG – Eastern Cooperative Oncology Group; NOS –not otherwise specified.

The obtained results did not show a statistically significant difference between the median OS of patients with stage IIIA and IIIB (23 vs. 18 months, respectively). There was no statistically significant difference between median OS in patients with adenocarcinoma and patients with squamous cell carcinoma (21 vs. 19 months, respectively). There was a statistically significant difference compared to NSCLC NOS, 26 months, but due to the small sample, these results should be interpreted with caution. The median survival of subjects with HG1 was 18 months, and for HG2 and HG3, it was 21 months. The median OS of patients with NSCLC is shown in Table 4.

Out of the total number of patients (n = 31) during RT treatment, adverse events (AEs) – grade 2 and more were found in 9 (29%) patients, while patients with AEs – grade 1 were not recorded, because their condition did not require any medical treatment (Table 5). Each of the 9 patients had exactly one AE. In 5 patients, there was a break in the RT treatment, but not longer than two weeks. AEs did not cause discontinuation of therapy in any patient.

Table 4

Median overall survival of patients with non-small cell lung carcinoma (NSCLC)

Average	SE	95% confidence interval		Median	SE	95% confidence interval	
		lower limit	upper limit			lower limit	upper limit
21.129	1.401	18.383	23.875	20.000	0.858	18.318	21.682

SE – standard error.

Table 5

Adverse events during radiotherapy

Adverse events	Frequency	Percentage (%)
Radioesophagitis grade 2	5	16.1
Radioesophagitis grade 3	2	6.5
Pneumonitis grade 3	1	3.2
Radiodermatitis grade 2	1	3.2
No higher stage complications	22	71.0
Total	31	100.0

Discussion

The main controversies in the treatment of NSCLC are related to its third stage, where, due to the heterogeneity of this group, all three therapeutic disciplines – surgery, CHT, and RT – can be applied. Numerous studies have tried to answer which method of treatment is the most effective and for which subgroup within the third stage of the disease. Phase III RTOG 94-10 study [20] and the Japanese study [21] deal with the difference in the effectiveness of bimodal treatment between concomitant and sequential CHRT, where the results in terms of OS and PFS were unequivocally in favor of concomitant CHRT.

Phase III RTOG 93-09 study [22] yielded results on efficacy and toxicity between treatments using trimodal (induction CHRT and surgery) and definitive concomitant CHRT in stage III patients, with an emphasis on the thoracic surgeon. Depending on his/her assessments of potential resectability, the appropriate treatment model will be applied, but it should be noted that lobectomy imposed itself as a more effective therapeutic approach, while pulmectomy has no benefit in PFS and OS compared to definitive CHRT [22]. Most guidelines for the treatment of locally advanced NSCLC are based on the results of these studies. New studies, primarily Pacific Trial [23], have shown the benefit of using immunotherapy after concomitant CHRT in PFS and OS, and its inclusion in routine practice is expected.

The results of OS of patients with NSCLC in our study demonstrate that the median survival is 20 months. Out of the total number of survivors, 75% of patients had a survival of 16 months, 50% of patients had 20 months, and 25% of patients had a survival of 23 months. The results of PFS of patients with NSCLC demonstrate a median of 13 months.

If we compare our results with the results obtained in countries with a similar health care system, we can see that the results are approximately the same. In a study conducted by Liu et al. [24] at the Radiation Oncology Department in Beijing, 251 patients with concomitant CHRT were treated; an average PFS of 10 months and an average OS of 21 months were obtained. The study conducted by Yilmaz et al. [25] at the Department of Pulmonology at the University Clinic in Bolu, Turkey, examined the efficacy and safety of concomitant CHRT in inoperable stage III NSCLC. Eighty-two patients were treated with concomitant CHRT (two cycles of cisplatin etoposide and RT 1.8-2 Gy *per* fraction in TTD 60-66Gy); an average PFS of 9 months and an average OS of 20 months were obtained. Toxicity of therapy in grades 2-3 was diagnosed in 19.2% of patients as radiation pneumonitis and in 8.5% as radiation esophagitis. The study conducted by Crvenkova [26] at the University Clinic for Radiotherapy and Oncology in Skopje, North Macedonia examined the average survival and sequential side effects of concomitant CHRT in inoperable stage III NSCLC. The results demonstrate average survival in concomitant CHRT of 19 months and in sequential 13 months, PFS 16 months in concomitant and 9 months in sequential CHRT. Grade 3 radiation esophagitis occurred only with concomitant therapy and was the result of RT discontinuation, but no longer than 7 days. The conclu-

sion was that concomitant CHRT (RT according to the 3DCRT protocol) is the optimal therapeutic choice for patients with locally advanced inoperable NSCLC, with an acceptable level of acute complications. Moreover, the RTOG studies 94-10, 91-06 [27] and the SWOG 90-19 [28] study gave similar results.

In studies PROCLAIM [29] and SWOG 95-04 [30], the results are somewhat better, primarily due to the diagnostic use of positron emission tomography/computed tomography (PET/CT), which achieves a more precise selection of patients in stage III inoperable compared to stage IV occult, more precisely applied RT and the use of consolidation chemotherapy. With the introduction of PET/CT in our routine practice in diagnosing and planning radiation therapy, the results are expected to be better.

Compared to RTOG trials and studies of the surrounding countries, we obtained approximate results in the form of AEs. Acute radiation-induced esophagitis is the most common AE of concomitant CHRT in the treatment of lung cancer [31]. Although a competitive use of CHRT and higher doses in RT treatment have been associated with the development of esophagitis, advances in RT techniques (3D planned radiation) have reduced the frequency and severity of complications. Five patients had radiation esophagitis grade 2 [5/31 (16.1%)]; in one patient, RT treatment was paused for 7 days. Radioesophagitis grade 3 was present in two patients [2/31 (6.5%)], leading to a two-week RT treatment break. Radiation pneumonitis occurred in one patient and led to a two-week break in RT treatment.

Based on the results, we noticed that at the beginning of ECOG treatment, 64.5% of subjects had PS 0, and 35.5% had PS 1. The initial difference in PS did not lead to a statistically significant difference in "tumor response" between these two groups, nor in PFS and OS. At the end of therapy, 17 (54.8%) patients had PS 0 or 1, and 14 (45.2%) patients had PS 2 or higher. These results speak in favor of the high toxicity of CHRT and confirm the reason that for this type of treatment, patients must initially be in good general condition. Five patients are alive, and two still have no disease progression. The median follow-up of all patients was 20 months, the median of survivors was 28 months, and the median without progression was 33.5 months.

Conclusion

This paper demonstrates the feasibility of combined RT and CHT in our population in patients with inoperable stage III NSCLC, with a median OS of 20 months and a median PFS of 13 months, with an acceptable number of AEs during treatment. Proper patient selection for combined CHRT implies a conciliatory-indicated decision referring to a patient diagnosed with locally advanced inoperable NSCLC in the presence of an experienced thoracic oncologist (who will rule out resectability), provided the patient is in good general condition (PS 0 or 1), with less than 5% of body weight loss, that the basic laboratory values are within the reference values, that the cardiopulmonary reserve is preserved, and that the patient is motivated for this type of treatment. Combined CHRT provides the greatest opportunity for patients in stage III locally advanced inoperable NSCLC, for a favorable therapeutic outcome, with an acceptable degree of acute and late toxicity, and represents the standard therapeutic approach for selected patients in this stage of the disease.

R E F E R E N C E S

1. *Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68(6): 394–424.
2. World Health Organization. European Mortality Database (MDB). Copenhagen, Denmark: WHO Regional Office for Europe; 2019. Available from: http://www.who.int/healthinfo/mortality_data/en/
3. *Fossella FV, Komaki R, Putnam JB.* Lung cancer. New York: Springer-Verlag; 2003.
4. *Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J,* et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346(2): 92–8.
5. *Kadri MA, Dussek JE.* Survival and prognosis following resection of primary non-small cell bronchogenic carcinoma. Eur J Cardiothorac Surg 1991; 5(3): 132–6.
6. *Shields TW.* Surgical therapy for carcinoma of the lung. Clin Chest Med 1993; 14(1): 121–47.
7. Guidelines: Lung and Chest Tumours. ESMO-MCBS Pocket Guidelines. 2018. Available from: https://www.esmo.org › guidelines
8. *Rodrigues G, Choy H, Bradley J, Rosenzweig KE, Bogart J, Curran WJ Jr,* et al. Definitive radiation therapy in locally advanced non-small cell lung cancer: Executive summary of an American Society for Radiation Oncology (ASTRO) evidence-based clinical practice guideline. Pract Radiat Oncol 2015; 5(3): 141–8.
9. NCCN Guidelines with Evidence Blocks - Non-Small Cell Lung Cancer Version 4. 2019. Available from: https://www.nccn.org › guidelines
10. Republican Expert Commission for the Development and Implementation of Good Clinical Practice Guidelines, Ministry of Health of the Republic of Serbia. National guideline of good clinical practice for the diagnosis and treatment of lung cancer. Belgrade: Agency for Accreditation of Health Institutions; 2012. Available from: http://www.zdravlje.gov.rs/downloads/2011/Decembar/Vodici/Vodic%20za%20dijagnostikovanje%20i%20lecenje%20karcinoma%20pluca.pdf
11. *Berghmans T, Paesmans M, Meert AP, Mascaux C, Lothaire P, Lafitte JJ,* et al. Survival improvement in resectable non-small cell lung cancer with (neo)adjuvant chemotherapy: results of a meta-analysis of the literature. Lung Cancer 2005; 49(1): 13–23.
12. *Burdett S, Stewart L, Rydzewska L.* A systematic review and meta-analysis of the literature: chemotherapy and surgery versus surgery alone in non-small cell lung cancer. J Thoracic Oncology 2006; 1(7): 611–21.
13. *Detterbeck F, Boffa D, Kim A, Tanoue L.* The eighth edition lung cancer stage classification. Chest 2017; 151(1):193–203.
14. *Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB,* et al. The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Ra-

diologic Advances since the 2004 Classification. J Thorac Oncol 2015; 10(9): 1243–60.
15. *Dobbs J, Barrett A.* Practical Radiotherapy Planning: Royal Marsden Hospital Practice. 4th ed. London: Hodder Arnold; 2009.
16. *Peter H.* Radiotherapy in practice: External beam therapy. 2nd ed. Oxford: Oxford University Press; 2012.
17. ICRU report 62 (supplement to ICRU 50): Prescribing, recording and reporting photon beam therapy. Bethesda, MD, USA: International Commission on Radiation Units and Measurements; 1999.
18. *Schwartz LH, Litière S, de Vries E, Ford R, Gwyther S, Mandrekar S,* et al. RECIST 1.1-Update and clarification: From the RECIST committee. Eur J Cancer 2016; 62: 132–7.
19. Nacional Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Bethesda, Maryland: National Institutes of Health; 2017.
20. *Curran WJ Jr, Paulus R, Langer CJ, Komaki R, Lee JS, Hauser S*, et al. Sequential vs. concurrent chemoradiation for stage III non-small cell lung cancer: randomized phase III trial RTOG 9410. J Natl Cancer Inst 2011; 103(19): 1452–60.
21. *Furuse K, Fukuoka M, Kawahara M, Nishikawa H, Takada Y, Kudoh S,* et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. J Clin Oncol 1999; 17(9): 2692–9.
22. *Albain KS, Swann RS, Rusch VR, Turrisi AT, Shepherd FA, Smith CJ,* et al. Phase III study of concurrent chemotherapy and radiotherapy (CT/RT) vs. CT/RT followed by surgical resection for stage IIIA(pN2) non-small cell lung cancer: Outcomes update of North American Intergroup 0139 (RTOG-9309). J Clin Oncol 2005; 23: 624s.
23. *Gray JE, Villegas A, Daniel D, Vicente D, Murakami S, Hui R*, et al. Three-Year Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC-Update from PACIFIC. J Thorac Oncol 2020; 15(2): 288–93.
24. *Liu L, Wang X, Ji Z, Wang J, Bi N, Hui Z*, et al. Outcome of concurrent chemoradiotherapy in locally advanced non-small-cell lung cancer patients. Zhonghua Zhong Liu Za Zhi 2015; 37(11): 863–7. (Chinese)
25. *Yilmaz U, Yilmaz U, Yasar Z, Kirakli EK, Ulger S, Ozdogan Y,* et al. Definitive chemoradiotherapy in Stage III nonsmall cell lung cancer: Turkey experience. J Cancer Res Ther 2016; 12(1): 334–9.
26. *Crvenkova S*. Prognostic Factors and Survival in Non-Small Cell Lung Cancer Patients Treated with Chemoradiotherapy. Open Access Maced J Med Sci 2015; 3(1): 75–9.
27. *Lee JS, Scott C, Komaki R, Fossella FV, Dundas GS, McDonald S,* et al. Concurrent chemoradiation therapy with oral etoposide and cisplatin for locally advanced inoperable non-small-cell lung cancer: radiation therapy oncology group protocol 91-06. J Clin Oncol 1996; 14(4): 1055–64.
28. *Albain KS, Crowley JJ, Turrisi AT 3rd, Gandara DR, Farrar WB, Clark JI,* et al. Concurrent cisplatin, etoposide, and chest radiotherapy in pathologic stage IIIB non-small-cell lung cancer: a Southwest Oncology Group phase II study, SWOG 9019. J Clin Oncol 2002; 20(16): 3454–60.
29. *Senan S, Brade A, Wang LH, Vansteenkiste J, Dakhil S, Biesma B,* et al. PROCLAIM: Randomized Phase III Trial of Pemetrexed-Cisplatin or Etoposide-Cisplatin Plus Thoracic Radiation Therapy Followed by Consolidation Chemotherapy in Locally Advanced Nonsquamous Non-Small-Cell Lung Cancer. J Clin Oncol 2016; 34(9): 953–62.
30. *Gandara DR, Chanky, Albain KS, Gaspar LE, Lara PN Jr, Kelly K,* et al. Long-term survival with concurrent chemoradiation therapy followed by consolidation docetaxel in stage IIIB non-small-cell lung cancer: a phase II Southwest Oncology Group Study (S9504). Clin Lung Cancer 2006; 8(2): 116–21.
31. *Wei X, Liu HH, Tucker SL, Liao Z, Hu C, Mohan R,* et al. Risk factors for acute esophagitis in non-small-cell lung cancer patients treated with concurrent chemotherapy and three-dimensional conformal radiotherapy. Int J Radiat Oncol Biol Phys 2006; 66(1): 100–7.

Received on January 2, 2021
Revised on April 2, 2021
Accepted on April 2, 2021
Online First April 2021

ORIGINAL ARTICLE

UDC: 617.547-036.86-02
DOI: https://doi.org/10.2298/VSP210328049P

Pain and functional disability after lumbar microdiscectomy and their correlations with gender, depression, and recovery expectations

Bol i funkcionalna onesposobljenost posle lumbalne mikrodiskektomije i njihova povezanost sa polom, depresijom i očekivanjima oporavka

Slobodan Pantelinac*†, Mirko Grajić‡§, Aleksandar Knežević*†, Dejan Nikolić‡‖, Snežana Tomašević Todorović*†

***University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; †University Clinical Center of Vojvodina, Medical Rehabilitation Clinic, Novi Sad, Serbia; ‡University of Belgrade, Faculty of Medicine, Belgrade, Serbia; §University Clinical Center of Serbia, Center for Physical Medicine and Rehabilitation, Belgrade, Serbia; ‖University Children's Hospital, Physical Medicine and Rehabilitation Department, Belgrade, Serbia**

Abstract

Background/Aim. Among the various factors that can influence continued postoperative back pain and/or leg pain, and functional disability after lumbar microdiscectomy are gender, depression, and pessimism. The aim of this study was to determine the correlations between these factors. **Methods.** The research was conducted after microdiscectomy on 198 patients (95 men and 103 women), with a mean age of 50.20 ± 10.26 years. The following questionnaires were used for examinations: for assessment of pain and its intensity and character – PainDETECT Test; for functional disability – Oswestry Low Back Pain Disability Questionnaire; for the presence and degree of depression – Beck Depression Inventory-II; and questionnaire for the assessment of personal expectations (pessimistic/optimistic) about the treatment results. These assessments were carried out after microdiscectomy – just before rehabilitation treatment, one month later, and then 3 and 6 months after a microdiscectomy. **Results.** Depression ($p < 0.01$) and pessimism ($p < 0.01$) had significant negative influences on the pain and functional disability. The subjective sensation of pain was significantly higher in women than in men ($p < 0.01$), while men had a greater degree of functional disability ($p < 0.01$) than women. **Conclusion.** Pain and functional disability of the patients after lumbar microdiscectomy are significantly interconnected with gender, depression, and pessimism. The sensation of pain was higher in women, while men had a greater degree of functional disability. Globally, the intensity of pain and functional disability were significantly greater in patients with a higher degree of depression and pessimism, and, by registering mentioned factors, it is possible to predict the postoperative results.

Key words:
disability evaluation; intervertebral disc displacement; lumbosacral region; neurosurgical procedures; pain; postoperative complications; postoperative period; risk factors.

Apstrakt

Uvod/Cilj. Među raznim činiocima koji mogu uticati na kontinuirani postoperativni bol u leđima i/ili bol u nozi bolesnika nakon lumbalne mikrodiskektomije, kao i na njihovu funkcionalnu onesposobljenost, nalaze se pol i prisustvo depresije i pesimizma. Cilj rada bio je da se utvrdi povezanost između ovih činilaca. **Metode.** Istraživanjem je obuhvaćeno 198 pacijenata (95 muškaraca i 103 žena), prosečne starosti 50,20 ± 10,26 godina, kojima je urađena mikrodiskektomija. Za ispitivanja su korišćeni sledeći upitnici: Upitnik za procenu bola i njegovog intenziteta i karaktera – *PainDETECT Test*; Upitnik za funkcionalnu onesposobljenost – *Oswestry Low Back Pain Disability Questionnaire*; Upitnik za prisustvo i stepen depresije – Bekova skala depresije II; Upitnik za procenu ličnih očekivanja (pesimističnih/optimističnih) rezultata tretmana. Ispitivanja su izvedena nakon mikrodiskektomije u terminima: neposredno pre rehabilitacionog tretmana, mesec dana kasnije, a zatim tri i šest meseci nakon mikrodiskektomije. **Rezultati.** Značajne negativne uticaje na bol i funkcionalnu onesposobljenost imali su depresija ($p < 0,01$) i pesimizam ($p < 0,01$). Subjektivni osećaj bola bio je veći kod žena nego kod muškaraca ($p < 0,01$), dok su muškarci imali veći stepen funkcionalne onesposobljenosti nego žene ($p < 0,01$). **Zaključak.** Bol i funkcionalna onesposobljenost bolesnika nakon lumbalne mikrodiskektomije su značajno uzajamno povezani sa polom, depresijom i pesimizmom. Senzacija bola bila je veća kod žena, dok su muškarci imali viši stepen funkcionalne onesposobljenosti. Globalno, intenzitet bola i

Correspondence to: Slobodan Pantelinac, University Clinical Center of Vojvodina, Medical Rehabilitation Clinic, Hajduk Veljkova 1-7, 21 000 Novi Sad, Serbia; E-mail: slobodan.pantelinac@mf.uns.ac.rs

funkcionalna onesposobljenost su značajno veći kod bolesnika sa višim stepenom depresije i pesimizma i njihovim registrovanjem je moguće predvideti postoperativne terapijske rezultate.

Ključne reči:
sposobnost, ocena; hernija diskusa; lumbosakralni predeo; neurohirurške procedure; bol; postoperativne komplikacije; postoperativni period; faktori rizika.

Introduction

Microdiscectomy is one of the modern surgical methods for treating low back pain (LBP) and radiculopathy caused by a herniated intervertebral lumbar disc.

Continued postoperative back pain and/or leg pain after lumbar decompression back surgery interventions is commonly called failed back surgery syndrome (FBSS). However, there are other terms for the same disorder, such as postlumbar surgery syndrome, failed back syndrome, persistent postoperative syndrome, etc. [1, 2]. Patients with FBSS describe persistent back, back/leg, or leg pain, with functional insufficiency, with or without sciatica, in 10–40% of cases after all spinal surgeries [3–5]. FBSS is a chronic disorder that has a huge impact on the patients, their disability and quality of life, and health care systems. FBSS can be caused by various mechanical, biological, psychological, and social factors [3, 4]. They were the subject of a number of investigations that often confirmed the mutual interconnectivity of these factors. Among these factors, Bordoni and Marelli [3] and Epker and Black [5] found that psychosocial factors such as depression, poor coping, anxiety, somatization, and hypochondriasis have been associated with the development of FBSS.

Moreover, other authors confirmed that intense and long-lasting pain, particularly neuropathic pain, can cause functional disability associated with various psychological disorders such as fear, anxiety, depression, pessimism, and fear/avoidance beliefs of physical activity, work, quality of life, and social problems [6–11].

FBSS may be associated with severe pain, disability, and higher depression scores. Therefore, this group of patients should be subjected to a clinical examination, evaluated psychiatrically, and treated using a multidisciplinary approach, including surgical interventions, rehabilitation, and, if necessary, psychotherapy [4, 5, 8].

Male gender is also among other risk factors for FBSS and poor recovery, which may be associated with heavy physical work and more intensive smoking in this population [10].

Using the appropriate questionnaires and registering mentioned factors, it is possible to predict the functional recovery and, if necessary, implement additional diagnostic and therapeutic procedures in time to improve the postoperative results [11].

The aim of the study was to find the presence of FBSS and functional disability in our patients after lumbar microdiscectomy and determine their association with gender, depression, and negative attitudes and beliefs, i.e., the pessimism of patients about their recovery.

Methods

The research was conducted on 198 patients (95 men and 103 women), of various professions and with a mean age of 50.20 ± 10.26 years (range 29–69 years). The study involved patients who had undergone surgical treatment of disc herniation by lumbar microdiscectomy, transferred from the Clinic for Neurosurgery to the Medical Rehabilitation Clinic at the University Clinical Center of Vojvodina, Novi Sad, Serbia, in order to perform physical therapy and rehabilitation. People with diabetes mellitus, cerebrovascular insult, and alcohol addiction were not included in the study. Patients received standard physical therapy, adjusted exercises, and instructions on correct posture and ergonomic principles in daily activities.

The examinations were in concordance with the Helsinki Declaration and with the approval of the local Ethics Committee, from April 05, 2011 (00-01/171). The patients gave their written consent before entering the research.

The following questionnaires were used for the examinations: PainDETECT Test for assessment of pain and its intensity and character; Oswestry Low Back Pain Disability Questionnaire for functional disability; Beck Depression Inventory-II (BDI-II) for the presence and severity of depression; and Questionnaire for the Assessment of Personal Opinions of the patients (pessimism and optimism) about the results and the degree of own recovery after surgery. Numeric Pain Rating Scale (NPRS) was used for a pain assessment.

These assessments were carried out after microdiscectomy – just before the rehabilitation treatment, one month later, and then 3 and 6 months after a microdiscectomy.

As indicators of basic data in a statistical analysis, the following terms were used: arithmetic mean, median, mode, mode frequency, minimum and maximum values, standard deviation, and confidence interval. In addition to standard statistical methods and Student's t-test, techniques of mixed model ANOVA with the use of software package STATISTICS 12, serial number AXA 302C271408AR-B, were used. Values of $p < 0.05$ were regarded as statistically significant, and $p < 0.01$ as statistically highly significant.

Results

The number, gender, and age of the examined patients are shown in Table 1.

The mean age of examined persons was 50.2 years, without significant differences between men and women.

Table 1

Baseline characteristics of the study patients after microdiscectomy

Parameter	Mean	Median	Mode	Mode frequency	Min	Max	SD
Patients (n = 198)	50.2	52.0	45	20	29	69	10.26
women (n = 103)	49.2	50.5	45	18	29	69	8.95
men (n = 95)	51.3	52.0	54	12	29	68	11.46

All values refer to the age of patients.
SD – standard deviation.

The current pain intensity in the monitored periods

Current intensities of pain expressed by the NPRS with a range from 0 to 10, estimated at the start and after 1, 3, and 6 months, are shown in Table 2.

The decrease in pain intensity during the observed period, compared with the value at the start, was highly significant ($p < 0.01$), as can be seen in Table 2.

The presence of pain and its neuropathic component was evaluated by Pain DETECT Test. According to the score results, the patients with pain were divided into three categories: a neuropathic pain component is unlikely (probability less than 15%); the result is ambiguous, but a neuropathic pain component can be present; a neuropathic pain component is likely (probability greater than 90%).

The results during the examined period are shown in Table 3.

After relocating patients from the Clinic for Neurosurgery to the Medical Rehabilitation Clinic (0th month), all 198 (100%) patients had pain. Among them, 125 (63.1%) patients had ambiguous results (possible neuropathic pain) and 73 (36.9%) had likely neuropathic pain (probability greater than 90%). After three months, the pain was present in 15 (7.6%) patients, of whom only 1 (6.7%) was with neuropathic pain. After 6 months, only 8 (4.0%) patients had pain, but none of them had neuropathic pain. These results can be seen in Table 3.

Table 2

The current pain intensity in the monitored periods

Monitored periods	Mean	Median	Mode	Mode frequency	Min	Max	SD	*p*	95% CI
At the start	4.64	5	5	79	3	7	0.90	-	4.52–4.77
1 month	2.69	3	3	93	2	4	0.67	< 0.01	2.60–2.79
3 months	1.79	2	1	90	1	3	0.81	< 0.01	1.68–1.90
6 months	0.95	1	0	80	0	3	0.91	< 0.01	0.83–1.08

SD – standard deviation; CI – confidence interval.

Table 3

The presence of pain, its character, and neuropathic components in patients (n = 198) during the examined period

Parameters	Patients, n (%)
0th month (at the start)	
present pain	198 (100.0)
unlikely neuropathic pain	0 (0)
ambiguous result	125 (63.1)
likely neuropathic pain	73 (36.9)
1st month	
present pain	39 (19.7)
unlikely neuropathic pain	16 (41.0)
ambiguous result	20 (51.3)
likely neuropathic pain	3 (7.7)
3rd month	
present pain	15 (7.6)
unlikely neuropathic pain	10 (66.7)
ambiguous result	4 (26.7)
likely neuropathic pain	1 (6.7)
6th month	
present pain	8 (4.0)
unlikely neuropathic pain	7 (87.5)
ambiguous result	1 (12.5)
likely neuropathic pain	0 (0.0)

Functional disability during the examined period

The values of functional disability expressed by the Oswestry Disability Index (ODI) are shown in Table 4.

Mean ODI values during the testing period were highly significantly decreased after microdiscectomy ($p < 0.01$) compared with the value at the start (Table 4).

Depression during the examined period

The degree of depression was assessed by the BDI-II scale and their values during the study period are shown in Table 5. BDI-II values during the period of the research were significantly decreased compared with the values at the start. The score up to 20 of the Beck scale indicated practically a state without depression or with low depression, a score of 21 to 30 pointed to moderate depression, and a score above 30 marked the presence of clinically severe depression.

Over time, the numbers and percentages of patients with low, moderate, and high levels of depression changed. During the testing period, the number of patients with moderate and high levels of depression gradually decreased (Table 6).

The intensity of the pain in men and women

Current pain intensity, expressed by NPRS, was registered among women and men at the beginning of physical therapy (0th month), then after 1 month, and 3 and 6 months after a microdiscectomy. The results are shown in Figure 1.

Global reduction of pain intensity in the 1st, 3rd, and 6th month was very significant compared to the initial state, but the pain was more intensive in women than in men all the time.

Functional disability in men and women

Results of the Oswestry Disability Questionnaire expressed as ODI for men and women during the examined period are shown in Figure 2.

Global ODI values significantly decreased over time, but all ODI values during the monitored period were lower in women than in men, as can be seen in Figure 2.

The degree of depression and the intensity of the current pain

Classification of the degree of depression in the appropriate class was performed according to the values score of the Beck scale (BDI-II). In each of these classes of depression, the current pain intensity was evaluated using the Numerical Rating Scale at a certain time (at the beginning, in the 1st, 3rd, and 6th month). The interconnections between depression and the intensity of current pain in the observed periods are shown in Figure 3.

Patients with clinically severe depression had the greatest intensity of pain in all periods of examination; people with moderate depression had lower pain; pain intensity was the lowest among those who had mild, i.e., minimal depression (Figure 3). Anyway, the intensity of pain during the study period was significantly reduced globally in all groups ($p < 0.01$).

Table 4

Oswestry Disability Index (ODI) values during the observed period

Periods of testing	Mean	Median	Mode	Mode frequency	Min–Max	SD	*p*	95% CI
At the start	50.40	51	60	20	26–78	10.29	-	48.97–51.83
1 month	40.05	38	36	30	22–76	8.91	< 0.01	38.81–41.29
3 months	33.07	32	32	29	20–64	7.77	< 0.01	31.99–34.16
6 months	26.74	26	24	40	14–48	7.23	< 0.01	25.73–27.75

SD – standard deviation; CI – confidence interval.

Table 5

Beck Depression Inventory (BDI-II) values in the investigated periods

Time of examination	Mean	Median	SD	*p*	95% CI
At the start	20.95	19	6.93	-	19.99–21.92
1 month	17.04	15	6.46	<0.01	16.14–14.97
3 months	13.58	12	5.69	<0.01	12.79–14.37
6 months	10.83	9	5.75	<0.01	10.03–11.63

SD – standard deviation; CI – confidence interval.

Table 6

Degree of depression among the patients during investigated periods

Degree of depression	Time intervals			
	at beginning	1 month	3 months	6 months
Low	113 (57.1)	141 (71.2)	175 (88.4)	179 (90.5)
Moderate	64 (32.3)	51 (25.8)	21 (10.6)	18 (9.0)
High	21 (10.6)	6 (3.0)	2 (1.0)	1 (0.5)

Results are shown as numbers (%) of patients.

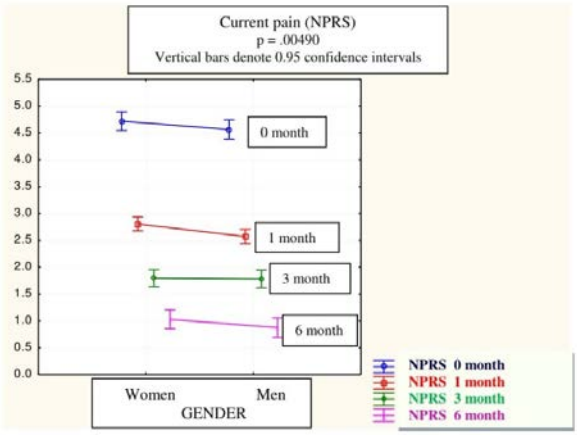


Fig. 1 – Current pain intensity in men and women in the observed periods. NPRS – Numeric Pain Rating Scale.

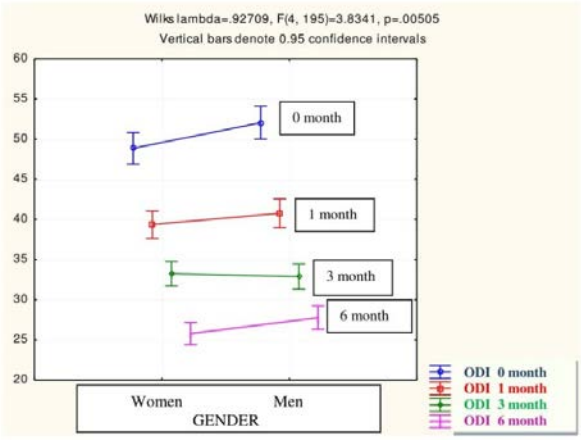


Fig. 2 – Results of Oswestry Disability Index (ODI) for men and women during the examined periods.

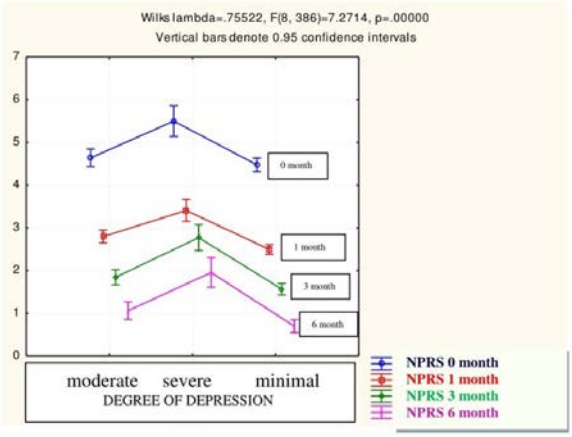


Fig. 3 – The degree of depression and intensity of the current pain in the observed periods. NPRS – Numeric Pain Rating Scale.

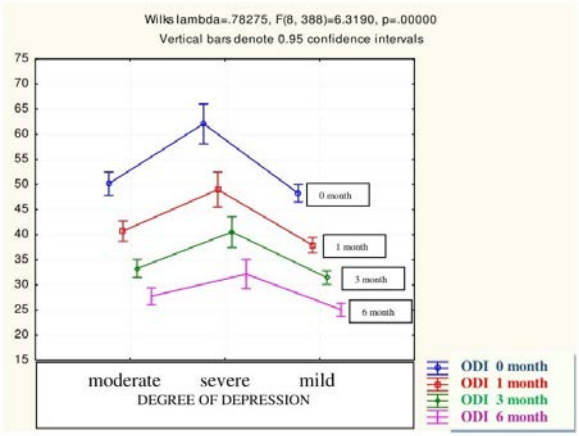


Fig. 4 – The degree of depression and functional disability. ODI – Oswestry Disability Index.

Depression and functional disability

The impact of depression on functional disability is shown in Figure 4. The degree of depression was estimated with BDI-II and the degree of functional disability by using ODI.

The results in Figure 4 show that patients with clinically severe depression had the highest degree of functional disability, those with moderate depression had a lower degree of disability, and the lowest disability showed patients who were practically without depression. The difference between these results was statistically highly significant ($p < 0.01$).

The patient's expectations of the recovery (optimism/pessimism) and the intensity of pain

According to the expectations of patients concerning their recovery after a surgical procedure and performed physical therapy, patients were classified into groups in which then, during the examined period, the intensity of the current pain was estimated. This classification into groups was carried out according to the following patient's expectations of recovery: totally, mainly, partly, a little, I don't know. This classification also showed the degree of optimism or pessimism in the investigated patients.

The link between expectations, i.e., the degree of optimism and pessimism of the patients about their recovery, with the pain intensity after the operation in the monitored period, are shown in Figure 5.

As can be seen in Figure 5, during the postoperative monitoring, the lowest pain intensity was present in patients who were optimistically oriented and expected that treatment would be successful and that they would be totally recovered. On the other hand, greater pain intensity was present in the groups of patients who expected partial or just a little improvement in their health and functional status and patients whose expectations were undetermined.

Expectations regarding the recovery and the degree of disability of patients

The relation of expectations, i.e., the degree of optimism and pessimism of the patients about their recovery, with the ability/disability estimated by ODI after the operation in the monitored period, are shown in Figure 6.

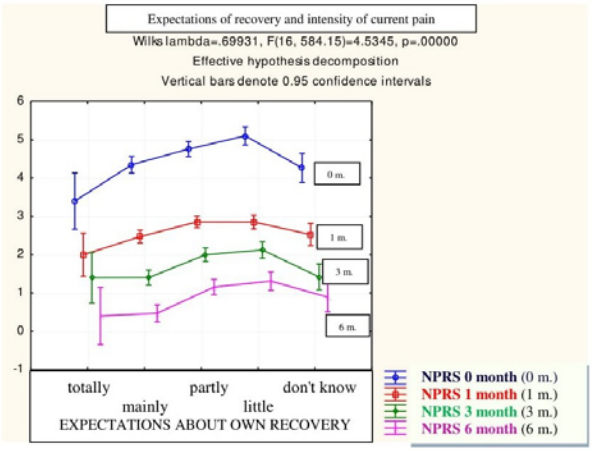


Fig. 5 – Expectations of patients about their recovery and the intensity of pain. NPRS – Numeric pain rating scale.

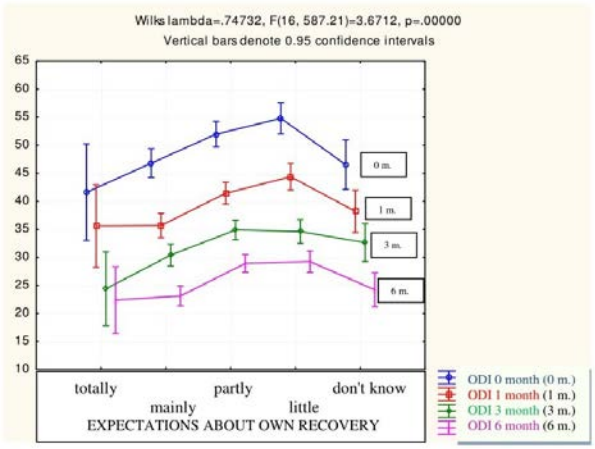


Fig. 6 – The expectations of the patients and the degree of disability. ODI – Oswestry Disability Index.

During the postoperative monitoring, patients who had the lowest ODI were optimistically oriented and expected that treatment would be successful, i.e., that they would be totally recovered. On the other hand, the groups of patients with the greatest disability were those who expected partial or just a little improvement in their health and functional status (Figure 6).

Discussion

FBSS is characterized by persistent back and/or leg pain, with functional insufficiency, after back surgery [1–5].

Chronic pain has a negative impact on the psychological and emotional state, functionality, and quality of life [12–14]. On the other hand, negative emotions and psychological disorders have reverse effects and can increase the intensity of pain perceptions and disability. Mentioned factors in chronic LBP are essentially mutually widely connected and have an interactive relationship. Therefore, these factors should be registered in the diagnostic and included in the treatment procedures because it will enable better success in treatment and faster functional recovery [12–14].

According to published data, patients describe FBSS with persistent uncontrolled back, back/leg, or leg pain, with or without sciatica, with a wide incidence ranging from 10–40% [3–5]. In our study, in the first month of examinations, 39 (19.7%) patients had pain.

For assessing the intensity and character of pain in our study, the PainDETECT questionnaire, simple and proven valuable in practice, was used [15]. The results of this test have shown that during the follow-up period, the number of patients with pain and the number of those with neuropathic or potentially neuropathic pain significantly decreased. These reductions in pain are useful because people with neuropathic pain show higher ratings of pain intensity, depression, anxiety, and functional disability [15, 16].

In our patients, the pain intensity and functional disability were significantly reduced in all subjects during the test period compared with baseline values. However, women constantly had a higher pain intensity than men, while men had a significantly greater degree of functional disability. This could be explained by the presence of greater emotional sensitivity in women and higher mechanical and physical workload of the spine in men due to the nature of their job.

Furthermore, Shi et al. [10] found that FBSS was more common in men than women. They concluded that besides hard physical work, smoking and the duration of preoperative symptoms also significantly influenced clinical outcomes.

Among the various psychological factors, negative influences on postoperative recovery may have depression and pessimism [17–20]. In addition, anxiety, fear, and avoidance beliefs may have similarly negative influences [18, 19].

Most of the research emphasizes the psychological factors and their impact on pain, postoperative recovery, and functionality after a microdiscectomy. However, there are also reversed attitudes implying that the pain reduction after microdiscectomy is the primary and the most important factor that decreases the negative psychological attitudes [19, 20]. For instance, microdiscectomy and nerve decompression reduce pain-associated depression and improve mental well-being and functional status in patients with herniated lumbar disc [19, 20].

Such results were also in the examination of our patients, and the level of depression and functional disability also decreased with the reduction of pain intensity.

Lurie et al. [21], among important psychological and predictive factors, mentioned the patient's positive expectations and optimism for the achievement of better recovery after spinal surgery. They concluded that high expectations of treatment benefits had clinically significant positive associations with outcomes.

The results of our examination confirmed this attitude because the patients with optimistic expectations of their recovery after microdiscectomy had lower pain intensity and better functional status.

In a systematic review, Dorow et al. [22] found that postsurgical back and leg pain was predominantly associated with depression and disability.

On the other hand, Farzanegan et al. [23] found that lumbar discectomy significantly improved depression and disability in patients with herniated discs and chronic low back pain.

It may be noted that the most acceptable attitude could be that all of the above-mentioned factors are in mutual reciprocal connection and that all together have an influence on the recovery and functionality of patients after a microdiscectomy. These conclusions also suggest the results of our examination since the reduction of pain and improvement of psychological state and functionality of the patients during the examined period were parallel. As the treatment of these conditions is complicated, it requires a multidisciplinary approach in many cases.

Conclusion

The intensity of pain and functional disability are significantly associated with depression and pessimism in the patients after lumbar microdiscectomy. The pain sensation was higher in women, while men had a greater degree of functional disability. By registering mentioned factors, it is possible to predict the recovery of the patients after lumbar microdiscectomy.

The mentioned approaches and procedures deserve attention, as well as their application in future patients from our region who will undergo a microdiscectomy for low back pain treatment. It will be the goal of our future activities.

Acknowledgement

The authors would like to thank Dušanka Perišić, PhD, Professor from the Department of Mathematics and Informatics, Faculty of Sciences, University of Novi Sad, for her help in the statistical data processing.

Conflict of interest

None.

Funding

The authors received no financial support for the research and/or authorship of this article.

This work is a part of the doctoral thesis of Slobodan Pantelinac entitled "Influence of biopsychosocial factors on the outcome of rehabilitation in patients after lumbar microdiscectomy".

REFERENCES

1. *Rigoard P, Desai MJ, Taylor RS*. Failed back surgery syndrome: what's in a name? A proposal to replace "FBSS" by "POPS"…. Neurochirurgie 2015; 61(Suppl 1): S16–21.
2. *Daniell JR, Osti OL*. Failed Back Surgery Syndrome: A Review Article. Asian Spine J 2018; 12(2): 372–9.
3. *Bordoni B, Marelli F*. Failed back surgery syndrome: review and new hypotheses. J Pain Res 2016; 9: 17–22.
4. *Sahin N, Sargin S, Atik A*. Failed Back Surgery: A Clinical Review. Int J Orthop 2015; 2(5): 399–404.
5. *Epker J, Block AR*. Psychological Screening Before Spine Surgery: Avoiding Failed Surgery Syndrome. Psychol Inj Law 2014; 7: 317–24.
6. *Cetin A, Gokdemir TM*. Evaluation of pain status and quality of life in patients with lumbar disc hernia who underwent microdiscectomy. Med Sci 2018; 7(4): 745–7.
7. *Strøm J, Bjerrum MB, Nielsen CV, Thisted CN, Nielsen TL, Laursen M,* et al. Anxiety and depression in spine surgery-a systematic integrative review. Spine J 2018; 18(7): 1272–85.
8. *Yalbuzdag SA, Erol AM, Sengul I, Celik C, Solum S, Adilay HU, Gungor B*. Temperament and Character Profile in Failed Back Surgery Syndrome: A Cross-Sectional Clinical Study. Turk Neurosurg 2016; 26(6): 912–7.
9. *Campbell P, Hope K, Dunn KM*. The pain, depression, disability pathway in those with low back pain: a moderation analysis of health locus of control. J Pain Res 2017; 10: 2331–9.
10. *Shi J, Wang Y, Zhou F, Zhang H, Yang H*. Long-term clinical outcomes in patients undergoing lumbar discectomy by fenestration. J Int Med Res 2012; 40(6): 2355–61.
11. *Hegarty D, Shorten G*. Multivariate prognostic modeling of persistent pain following lumbar discectomy. Pain Physician 2012; 15(5): 421–34.
12. *Srivastava S, Yadav P, Panchal NB, Vala UA, Ratnani I, Khania P*. Association of depression and chronic lower-back pain. Arch Psychiatr Psychother 2018; 4: 37–46.
13. *Pinheiro MB, Ferreira ML, Refshauge K, Maher CG, Ordonana JR, Andrade TB,* et al. Symptoms of depression as a prognostic factor for low back pain: a systematic review. Spine J 2016; 16(1): 105–16.
14. *Nijs J, Apeldoorn A, Hallegraeff H, Clark J, Smeets R, Malfliet A*, et al. Low Back Pain: Guidelines for the Clinical Classification of Predominant Neuropathic, Nociceptive, or Central Sensitization Pain. Pain Physician 2015; 18(3): 333–46.
15. *Freynhagen R, Baron R, Gockel U, Tölle TR*. Pain DETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. Curr Med Res Opin 2006; 22(10): 1911–20.
16. *Shamji MF, Shcharinsky A*. Use of neuropathic pain questionnaires in predicting persistent postoperative neuropathic pain following lumbar discectomy for radiculopathy. J Neurosurg Spine 2016; 24(2): 256–62.
17. *Jabłońska R, Ślusarz R, Królikowska A, Haor B, Antczak A, Szewczyk M*. Depression, social factors, and pain perception before and after surgery for lumbar and cervical degenerative vertebral disc disease. J Pain Res 2017; 10: 89–99.
18. *Löbner M, Luppa M, Matschinger H, Konnopka A, Meisel HJ, Günther L*, et al. The course of depression and anxiety in patients undergoing disc surgery: a longitudinal observational study. J Psychosom Res 2012; 72(3): 185–94.
19. *Lebow R, Parker SL, Adogwa O, Reig A, Cheng J, Bydon A,* et al. Microdiscectomy improves pain-associated depression, somatic anxiety, and mental well-being in patients with herniated lumbar disc. Neurosurgery 2012; 70(2): 306–11.
20. *Tharin S, Mayer E, Krishnaney A*. Lumbar microdiscectomy and lumbar decompression improve functional outcomes and depression scores. Evid Based Spine Care J 2012; 3(4): 65–6.
21. *Lurie DJ, Henderson RE, McDonough MC, Berven HS, Scherer AE, Tosteson DT*, et al. The Effect of Expectations on Treatment

Outcome for Lumbar Intervertebral Disc Herniation. Spine (Phila Pa 1976) 2016; 41(9): 803–9.

22. *Dorow M, Löbner M, Stein J, Konnopka A, Meisel HJ, Günther L*, et al. Risk Factors for Postoperative Pain Intensity in Patients Undergoing Lumbar Disc Surgery: A Systematic Review. PLoS ONE 2017; 12(1): e0170303.

23. *Farzanegan G, Alghasi M, Safari S, Ahmadi SA*. Effects of lumbar discectomy on disability and depression in patients with chronic low back pain. Anesth Pain Med 2011; 1(1): 20–4

Received on March 28, 2021
Revised on April 15, 2021
Accepted on April 20, 2021
Online First April 2021

ORIGINAL ARTICLE

UDC: 616.714.1-053.5/.81-071.3::616.31
DOI: https://doi.org/10.2298/VSP201119045D

Harmony concept of craniofacial morphology among the young Serbian population in Kosovo and Metohija

Koncept harmonije kraniofacijalne morfologije mlađe srpske populacije na području Kosova i Metohije

Aleksandrija Djordjević*, Jasna Pavlović*, Vladanka Vukićević*, Amila Vujačić*, Sanja Simić*, Brankica Martinović†

University of Priština/Kosovska Mitrovica, Faculty of Medicine,*Department of Orthodontics, †Department of Pediatric and Preventive Dentistry, Kosovska Mitrovica, Serbia

Abstract

Background/Aim. Analyses of the cephalometric value of profile radiographs are very important for orthodontic diagnosis and planning of therapy. These values differ morphologically depending on ethnic and racial affiliation. The aim of this study was to confirm variations of cephalometric norms and the extent of their value and, according to them, confirm the harmony concept of craniofacial morphology among the young Serbian population in Kosovo and Metohija. **Methods.** This retrospective study comprised 183 profile radiograms of patients from Kosovo and Metohija, Serbia, aged 8–33, from which Class I was ascertained among 82 patients (53 female and 29 male). Five cephalometric angles were measured and analyzed. Pearson's correlation coefficient was used to define the strength of correlation between the five variables. Bivariable linear regression was used to form harmonious combinations of individual values in the insight of craniofacial harmonious form. Multiple regression and standard error were used to form a harmonious schema. **Results.** Linear regression equations were used to define cephalometric floating norms. They helped us form a harmonious box and harmonious schema of craniofacial norms of the participants. The extent of harmonious value for orthognathic profile of our examinees varies for *sella* nasion subspinale (SNA) angle from 78° to 81°, for *sella* nasion supramentale (SNB) angle from 75.1° to 78.1°, for maxillary line – nasion *sella* line (NL-NSL) angle from 11.5° to 5.5°, for nasion *sella* basion (NSBa) angle from 134.7° to 125.8°, and for mandibulary line – nasion *sella* line (ML-NSL) angle from 40.5° to 30.6°. **Conclusion.** Cephalometric floating norms that describe the individual craniofacial pattern among the young Serbian population in Kosovo and Metohija, determined and defined by five cephalometric variables, and presented in the form of a harmonious box and harmonious schema and can accurately determine the craniofacial pattern.

Key words:
cephalometry; ethnicity; radiography; serbia.

Apstrakt

Uvod/Cilj. Analiza kefalometrijskih vrednosti profilnih snimaka veoma je važna za ortodontsku dijagnozu i planiranje terapije. Morfološki se te vrednosti razlikuju u zavisnosti od etničke i rasne pripadnosti. Cilj rada bio je da se utvrde varijacije kefalometrijskih normi i opseg njihovih vrednosti i na osnovu njih utvrdi koncept harmonije kraniofacijalne morfologije mlađeg srpskog stanovništva na području Kosova i Metohije. **Metode.** Retrospektivnom studijom analizirana su 183 profilna telerendgen snimka pacijenata sa područja Kosova i Metohije, uzrasta 8–33 godina od kojih je I klasa utvrđena kod 82 pacijenta (53 ženskog i 29 muškog pola). Ukupno je mereno i analizirano pet kefalometrijskih uglova. Jačina korelacije između pet kefalometrijskih varijabli izračunata je na osnovu *Pearson*-ovog koeficijenta korelacije. Za formiranje harmoničnih kombinacija individualnih vrednosti u vidu harmoničnog kraniofacijalnog obrasca korišćena je bivarijantna linearna regresija. Primenom višestruke regresije i standardne greške napravljena je šema harmoničnih vrednosti. **Rezultati.** Jednačine linearne regresije su primenjene radi definisanja fluktuirajućih kefalometrijskih normi. One su nam omogućile formiranje harmoničnog opsega i harmonične šeme kraniofacijalnih normi ispitanika. Opseg harmoničnih vrednosti za ortognat profil naših ispitanika se kretao za *sella nasion subspinale* (SNA) ugao od 78° do 81°, za *sella nasion supramentale* (SNB) ugao od 75.1° do 78.1°, za *maxillary line – nasion sella line* (NL-NSL) ugao od 11.5° do 5.5°, za *nasion sella basion* (NSBa) ugao od 134.7° do 125.8° i za *mandibulary line – nasion sella line* (ML-NSL) ugao od 40.5°

Correspondence to: Aleksandrija Djordjević, University of Priština/Kosovska Mitrovica, Faculty of Medicine, Department of Orthodontics, Andri Dunana bb, 38 220 Kosovska Mitrovica, Serbia. E-mail: aleksandrija86@yahoo.com

do 30.6°. **Zaključak.** Kefalometrijske fluktuirajuće norme koje opisuju individualni kraniofacijalni obrazac kod mlađe srpske populacije na Kosovu i Metohiji, određene i definisane pomoću pet kefalometrijskih varijabli i predstavljene u obliku harmoničnog okvira i harmonične šeme mogu precizno odrediti kraniofacijalni obrazac populacije.

Ključne reči:
kefalometrija; etničke grupe; radiografija; srbija.

Introduction

Cephalometric analysis of craniofacial morphology represents an important item in orthodontic diagnosis and planning of orthodontic therapy. Analysis of profile cephalometric radiographs enables the therapist to define the skeleton's morphology of the patient and establish the degree of correlation between skeletal and dental factors [1]. The importance of cephalometric radiographs in orthodontic diagnosis has long been established. The purpose of profile radiograph analysis is to define the relationship between maxilla and mandible toward the cranial base just among them in sagittal and vertical line, to define the relationship of teeth to the alveolar bone and the significance and influence of teeth on a patient's profile [2, 3].

In 1931, Broadhent [4] and Hofrath [5] simultaneously published methods that were used to achieve standardized commercial profile radiographs of the head. After the publication of these methods, numerous authors dealt with the same and similar research and defined numerous cephalometric analyses with standardized norms [6–9]. In conventional cephalometric analysis, a patient's cephalometric values are compared with already established norms specific to distinctive ethnic and race groups [10–15]. Solow [16] considered that the analyses were incomplete, and their main lack was the absence of mutual dependence on craniofacial parameters. Within his study, Solow [16] showed usage of those parameters in some isolated, individual form and established a high correlation between individual sagittal and vertical variables, which developed the concept of "Craniofacial pattern". Based on Solow's [16] studies, it has been noticed that every patient's cephalometric values overcome the standard deviation of the population's mean values, and those values can be considered acceptable if a correlation between them exists [10].

In his study, Hasund et al. [17] defined and showed combinations of acceptable values for different types of faces. For orthognathic patients, harmonious combination and mean value variables are *sella* nasion subspinale (SNA) angle 82°, *sella* nasion supramentale (SNB) angle 80°, for maxillary line – nasion *sella* line (NL-NSL) angle 8.5°, nasion *sella* basion (NSBa) angle 130°, and mandibulary line – nasion *sella* line (ML-NSL) angle 32° [17]. Segner [18] and Segner and Hasund [19], in their studies among the adult population in Europe, researched individual craniofacial patterns and constructed floating norms to describe the skeleton's sagittal and vertical relationship [20]. The term "floating norms" is applied to describe individual norms that float according to correlated cephalometric measures. The concept of floating norms research is based on the correlation between five craniofacial variables, SNA, SNB, ML-NSL, NL-NSL, and NSBa [18].

Linear correlation coefficient "r" was used to describe the relationship between two variables [19]. The higher the absolute value of "r", the better the linear correlation between the two variables [17]. Linear regression equations were used to construct the harmonious box and harmonious schema. The harmonious box and harmonious schema were constructed and patented by Segner [18] and Segner and Hasund [19] within their studies modeled after Bergen's cephalometric analysis [17] (Figures 1 and 2). The harmonious box, according to Segner and Hasund [21], represents a very important diagnostic tool in orthodontic diagnosis and planning of orthodontic therapy. This concept represents individual skeletal form or pattern, which shows a sagittal and vertical relationship with the application of appropriate floating norms. The harmonious box is constructed so that it consists of three zones – retrognathic, orthognathic, and prognathic [21]. Within the harmonious box, a single horizontal line is illustrated, which connects the mean values of all five cephalometric variables. If the line is flat, it can be concluded that the patient's face is harmonious. The type of patient's face depends on its place in the harmonious box, apropos of the zone in which the patient's value variables are located [10].

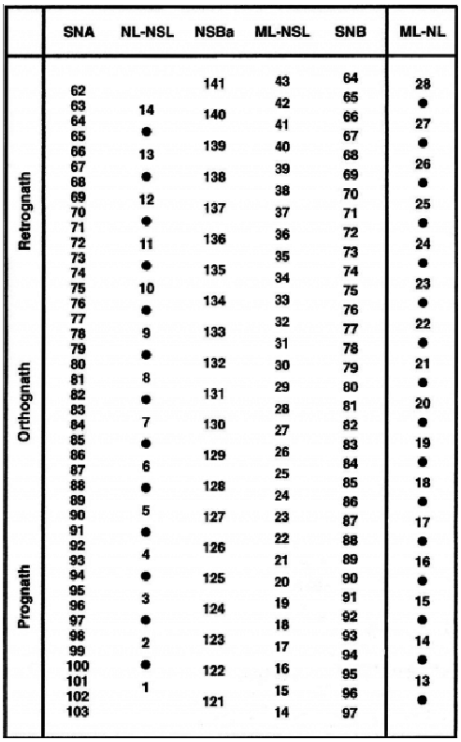


Fig. 1 – Segner-Hasund harmonious box [10]. The harmony box is constructed based on the five cephalometric variables (SNA, SNB, ML-NSL, NL-NSL, and NSBa), which were found to have a certain correlation pattern to one another [18]. For abbreviations, see under Table 1.

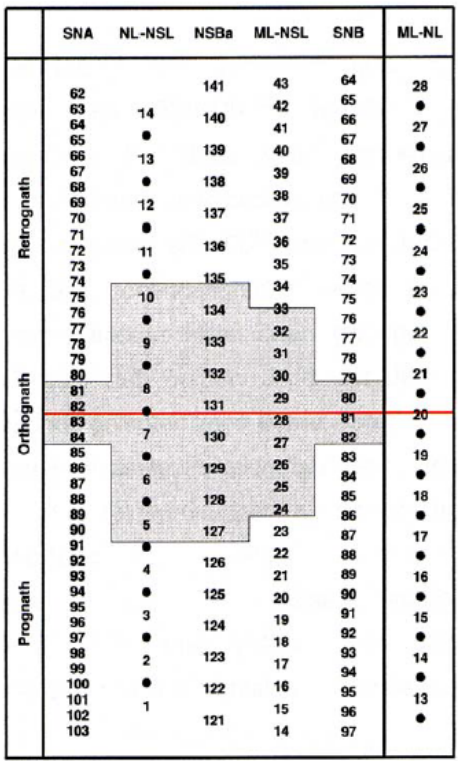


Fig. 2 – Segner-Hasund harmonious schema in the harmonious box [multiple regression analysis, particularly the standard error (SE), was calculated to construct the harmonious schema]. The red horizontal line in the middle of the harmonious schema represents the mean values of the five cephalometric variables. For abbreviations, see under Table 1.

Many researchers wrote and conducted studies on the cephalometric floating norms. Segner [18] was one of the first who defined floating norms for the population of Northern Europe. Other researchers such as Tollaro et al. [22], Franci et al. [20], Ngarmprasertchai [23], and Mahaini [24] researched and defined floating norms for the population of Europe, North America, Thailand, and Syria, respectively. In 2009, Sevilla-Naranjilla and Rudzki-Janson [10] defined and presented floating norms for the population of the Philippines. In 2012, Řeháček et al. [25] defined floating norms for the Czech population.

The aim of our study was to determine and define floating cephalometric norms so as to describe individual cephalometric patterns among the young Serbian population in Kosovo and Metohija, Serbia.

Methods

Within our study, 183 profile cephalometric radiographs of patients, aged 8–33, mean age 16.9, were analyzed. Class I was diagnosed among 82 people (53 female and 29 male). Class I criteria was subspinale nasion supramentale (ANB) angle 2 ± 2°, balanced profile, and without previous orthodontic treatment. This retrospective study was conducted at the Department of Orthodontics, Faculty of Medicine, the University of Priština/Kosovska Mitrovica, Kosovo and Metohija, Serbia.

During the analysis of each profile cephalometric radiograph, five angles were measured, including SNA (maxillary prognathism), SNB (mandibular prognathism), NL-NSL (maxillary inclination), ML-NSL (mandibular inclination), and NSBa (cranial base angle). The mandibular plane – maxillary plane (MP-NP) angle was calculated as the difference between ML-NSL and NL-NSL (Figure 3).

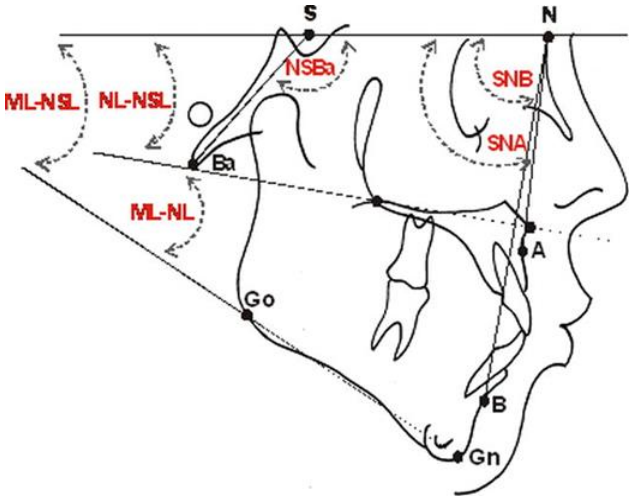


Fig. 3 – Illustration of cephalometric landmarks and correlated angular measurements (SNA, SNB, NSBa, NL-NSL, and ML-NSL) used in this study [10]. For abbreviations, see under Table 1.

Descriptive statistics were calculated for five cephalometric variables. Pearson's coefficient correlation was applied to describe the correlation between five cephalometric variables (SNA, SNB, NSBa, ML-NSL, and NL-NSL) used to form a harmonious box. Bivariable linear regression analysis was used to form and construct a harmonious box. Multiple regression analysis, particularly the statistic error (SE), was used to form the harmonious schema.

A full analysis of data was conducted within the SPSS program, version 21.0.

Results

Descriptive statistics of all five variables are shown in Table 1, which shows mean values among examined parameters.

Table 1

Descriptive statistics (means, SD and ranges) for all five cephalometric variables

Variables	Mean ± SD	Min–Max
SNA	79.50 ± 3.97	70–87
NL-NSL	8.46 ± 3.78	1–17
NSBa	130.24 ± 5.26	119–144
ML-NSL	35.59 ± 6.03	22–49
SNB	76.63 ± 3.94	67–85

SNA – *Sella* nasion subspinale angle; SNB – *Sella* nasion supramentale angle; ML-NSL – mandibular line - nasion *sella* line; NL-NSL – maxillary line - nasion *sella* line; NSBa – nasion *sella* basion angle; SD – standard deviation.

Linear correlation coefficients among SNA, SNB, NL-NSL, ML-NSL, and NSBa variables, apropos of the correlation between prognathism, inclination, and angle of maxilla and mandible, are shown in Table 2. For the maxillary complex, a negative correlation between SNA and NL-NSL variables was found (r = -0.484). This means that a smaller NL-NSL angle is expected with the increase of maxillary prognathism. In the case of the mandible and lower face, a negative correlation between SNB and ML-NSL variables was established (r = -0.496). That means that a mandible's smaller angle of inclination (ML-NSL) follows a bigger mandibular prognathism (SNB). Linear regression equations with appropriate values r^2 and SE are shown in Table 3. They are illustrated in the spectrum of the harmonious box (Figure 4). All five variable combinations are shown, with SNA as an independent variable and the other as dependent variables. Based on the results, the range of analyzed variables for orthognathic, prognathic, and retrognathic zones was obtained. The range of variables for orthognathic zone for SNA was from 78° to 81°, for SNB from 75.1° to 78.1°, for NL-NSL from 11.5° to 5.5°, for NSBa from 134.7° to 125.8°, and for ML-NSL from 40.5° to 30.6°.

Table 2

Linear correlation coefficients (r) between SNA, NL-NSL, NSBa, SNB, and ML-NSL variables (Pearson's correlation coefficients described the high association among variables used in the construction of the harmonious box)

Variables	NL-NSL	NSBa	ML-NSL	SNB
SNA	-0.484*	-0.292*	-0.505*	0.979*
NL-NSL		0.354*	0.342*	-0.517*
NSBa			-0.065 ns	-0.330*
ML-NSL				-0.496*

***p < 0.01; ns – no significant.**
For abbreviations, see under Table 1.

Table 3

Linear regressions with corresponding r^2 and standard error (SE) of the estimate for the young Serbian population (the bivariate linear regression equations are used to construct the harmonious box, with SNA as the independent variable and NL-NSL, NSBa, ML-NSL, and SNB each as dependent variable)

Variables	Regression equations	R^2	SE
NL-NSL	45.146–0.461xSNA	0.234	3.33
NSBa	161.010–0.387xSNA	0.085	5.06
ML-NSL	96.557–0.767xSNA	0.255	5.24
SNB	-0.717+0.973xSNA	0.958	0.81
SNA	108.158–0.220xNSBa	0.085	3.82
SNB	108.812–0.247xNSBa	0.109	3.74
ML-NSL	93.649–0.758xSNB	0.246	5.27

For abbreviations, see under Table 1.

Multiple correlation coefficients R, R^2, and SE were calculated with multiple regression analysis and presented in Table 4 and graphically illustrated in Figure 5. Mean values of all five variables (SNA 79.50°, SNB 76.63°, NL-NSL 8.46°, ML-NSL 35.59°, and NSBa 130.24°) among our participants formed the horizontal line within the harmonious schema. However, one of the combinations of the variables represents the curvy line within the harmonious schema (Figure 6). An example of a harmonious combination of a nineteen-year-old girl's face is shown with a curvy line which is a characteristic of the following values of examined parameters – SNA 80°, SNB 78°, NL-NSL 8°, NSBa 133.7°, and ML-NSL 30.6° – disregarding the individually analyzed angles that are incompatible with the mean value of Class I. All values of

	SNA	NL-NSL	NSBa	SNB	ML-NSL	ML-NL
Retrognathic	64	15.6	136.2	61.5	47.4	31.6
	65	15.2	135.8	62.5	46.7	31.3
	66	14.7	135.4	63.5	45.9	31.0
	67	14.2	135.0	64.4	45.1	30.7
	68	13.8	134.7	65.4	44.4	30.5
	69	13.3	134.3	66.4	43.6	30.2
	70	12.8	133.9	67.4	42.8	29.9
	71	12.4	133.5	68.3	42.1	29.6
	72	11.9	133.1	69.3	41.3	29.3
	73	11.5	132.7	70.3	40.5	29.0
	74	11.0	132.3	71.3	39.8	28.7
	75	10.5	131.9	72.2	39.0	28.4
	76	10.1	131.6	73.2	38.2	28.2
	77	9.6	131.2	74.2	37.5	27.9
	78	9.2	130.8	75.1	36.7	27.6
Orthognathic	79	8.7	130.4	76.1	35.9	27.3
	80	8.2	130.0	77.1	35.1	27.0
	81	7.8	129.6	78.1	34.4	26.7
	82	7.3	129.2	79.0	33.6	26.4
	83	6.8	128.8	80.0	32.9	26.1
	84	6.4	128.5	81.0	32.1	25.9
	85	5.9	128.1	81.9	31.3	25.6
	86	5.5	127.7	82.9	30.6	25.3
	87	5.0	127.3	83.9	29.8	25.0
	88	4.5	126.9	84.9	29.0	24.7
	89	4.1	126.5	85.8	28.2	24.4
	90	3.6	126.1	86.8	27.5	24.1
Prognathic	91	3.2	125.8	87.8	26.7	23.8
	92	2.7	125.4	88.8	25.9	23.6
	93	2.2	125.0	89.7	25.2	23.3
	94	1.8	124.6	90.7	24.4	23.0
	95	1.3	124.2	91.7	23.7	22.7
	96	0.9	123.8	92.7	22.9	22.4
	97	0.4	123.4	93.6	22.1	22.1
	98	0	123	94.6	21.4	21.8

Fig. 4 – Harmonious box among the young Serbian population in Kosovo and Metohija, Serbia.
For abbreviations, see under Table 1.

Table 4

Standard errors (SE) of the estimate when one of the variables SNA, NL-NSL, NSBa, ML-NSL, or SNB is predicted from the other four by means of a multiple regression analysis of the young Serbian population (multiple regression analyses are used to present the degree of variability allowed among the five cephalometric variables in describing a harmonious face)

Variables	R	R^2	SE
SNA	0.979	0.958	0.814
NL-NSL	0.484	0.234	3.159
NSBa	0.501	0.251	4.687
SNB	0.980	0.960	0.789
ML-NSL	0.980	0.960	5.046

For abbreviations, see under Table 1.

variables are located inside the harmonious schema and orthognathic zone, which indicates that the face is of harmonious and orthognathic type (Figures 6 and 7).

The comparison of our results with those from the studies by Sevilla-Naranjilla and Rudzki-Janson [10] and Řeháček et al. [25] is shown in Figure 8, and variables' mean values of Filipino and Czech populations are drawn in our harmonious box and harmonious schema.

	SNA	NL-NSL	NSBa	ML-NSL	SNB	ML-NL
Retrognathic	64	15.6	136.2	47.4	61.5	31.6
	65	15.2	135.8	46.7	62.5	31.3
	66	14.7	135.4	45.9	63.5	31.0
	67	14.2	135.0	45.1	64.4	30.7
	68	13.8	134.7	44.4	65.4	30.5
	69	13.3	134.3	43.6	66.4	30.2
	70	12.8	133.9	42.8	67.4	29.9
	71	12.4	133.5	42.1	68.3	29.6
	72	11.9	133.1	41.3	69.3	29.3
Orthognathic	73	11.5	132.7	40.5	70.3	29.0
	74	11.0	132.3	39.8	71.3	28.7
	75	10.5	131.9	39.0	72.2	28.4
	76	10.1	131.6	38.2	73.2	28.2
	77	9.6	131.2	37.5	74.2	27.9
	78	9.2	130.8	36.7	75.1	27.6
	79	8.7	130.4	35.9	76.1	27.3
	80	8.2	130.0	35.1	77.1	27.0
	81	7.8	129.6	34.4	78.1	26.7
	82	7.3	129.2	33.6	79.0	26.4
	83	6.8	128.8	32.9	80.0	26.1
	84	6.4	128.5	32.1	81.0	25.9
	85	5.9	128.1	31.3	81.9	25.6
	86	5.5	127.7	30.6	82.9	25.3
Prognathic	87	5.0	127.3	29.8	83.9	25.0
	88	4.5	126.9	29.0	84.9	24.7
	89	4.1	126.5	28.2	85.8	24.4
	90	3.6	126.1	27.5	86.8	24.1
	91	3.2	125.8	26.7	87.8	23.8
	92	2.7	125.4	25.9	88.8	23.6
	93	2.2	125.0	25.2	89.7	23.3
	94	1.8	124.6	24.4	90.7	23.0
	95	1.3	124.2	23.7	91.7	22.7
	96	0.9	123.8	22.9	92.7	22.4
	97	0.4	123.4	22.1	93.6	22.1
	98	0	123.0	21.4	94.6	21.8

Fig. 5 – Harmonious schema among the young Serbian population in Kosovo and Metohija, Serbia represents the range of variability among the five cephalometric variables in the harmonious box and is represented by the standard error (SE) of the estimate of the multiple regression analysis. The horizontal line in the middle of the harmonious schema represents the mean values of the five cephalometric variables.
For abbreviations, see under Table 1.

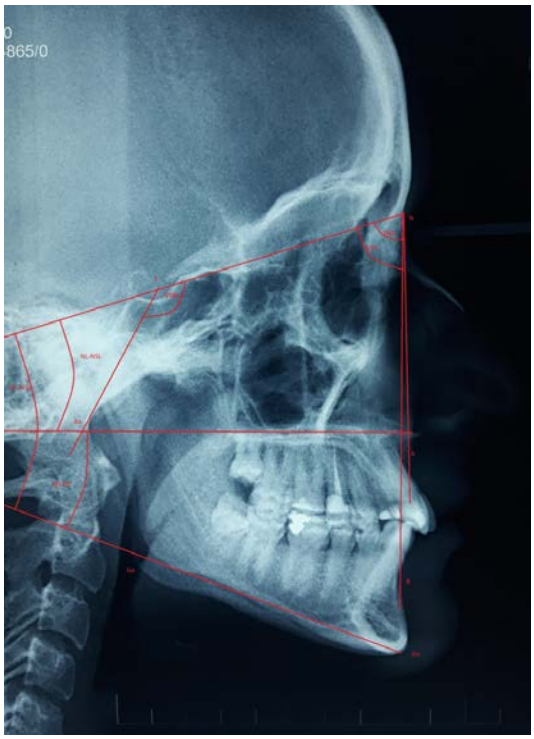


Fig. 7 – Lateral cephalometric radiograph of the patient with a harmonious combination.

	SNA	NL-NSL	NSBa	ML-NSL	SNB	ML-NL
Retrognathic	64	15.6	136.2	47.4	61.5	31.6
	65	15.2	135.8	46.7	62.5	31.3
	66	14.7	135.4	45.9	63.5	31.0
	67	14.2	135.0	45.1	64.4	30.7
	68	13.8	134.7	44.4	65.4	30.5
	69	13.3	134.3	43.6	66.4	30.2
	70	12.8	133.9	42.8	67.4	29.9
	71	12.4	133.5	42.1	68.3	29.6
	72	11.9	133.1	41.3	69.3	29.3
Orthognathic	73	11.5	132.7	40.5	70.3	29.0
	74	11.0	132.3	39.8	71.3	28.7
	75	10.5	131.9	39.0	72.2	28.4
	76	10.1	131.6	38.2	73.2	28.2
	77	9.6	131.2	37.5	74.2	27.9
	78	9.2	130.8	36.7	75.1	27.6
	79	8.7	130.4	35.9	76.1	27.3
	80	8.2	130.0	35.1	77.1	27.0
	81	7.8	129.6	34.4	78.1	26.7
	82	7.3	129.2	33.6	79.0	26.4
	83	6.8	128.8	32.9	80.0	26.1
	84	6.4	128.5	32.1	81.0	25.9
	85	5.9	128.1	31.3	81.9	25.6
	86	5.5	127.7	30.6	82.9	25.3
Prognathic	87	5.0	127.3	29.8	83.9	25.0
	88	4.5	126.9	29.0	84.9	24.7
	89	4.1	126.5	28.2	85.8	24.4
	90	3.6	126.1	27.5	86.8	24.1
	91	3.2	125.8	26.7	87.8	23.8
	92	2.7	125.4	25.9	88.8	23.6
	93	2.2	125.0	25.2	89.7	23.3
	94	1.8	124.6	24.4	90.7	23.0
	95	1.3	124.2	23.7	91.7	22.7
	96	0.9	123.8	22.9	92.7	22.4
	97	0.4	123.4	22.1	93.6	22.1
	98	0	123.0	21.4	94.6	21.8

Fig. 6 – An example of harmonious combinations (red connected line) presented in a harmonious box and harmonious schema. All values of the patient lie inside the harmonious schema and are described as orthognathic and harmonious profiles.
For abbreviations, see under Table 1.

	SNA	NL-NSL	NSBa	ML-NSL	SNB	ML-NL
Retrognathic	64	15.6	136.2	47.4	61.5	31.6
	65	15.2	135.8	46.7	62.5	31.3
	66	14.7	135.4	45.9	63.5	31.0
	67	14.2	135.0	45.1	64.4	30.7
	68	13.8	134.7	44.4	65.4	30.5
	69	13.3	134.3	43.6	66.4	30.2
	70	12.8	133.9	42.8	67.4	29.9
	71	12.4	133.5	42.1	68.3	29.6
	72	11.9	133.1	41.3	69.3	29.3
Orthognathic	73	11.5	132.7	40.5	70.3	29.0
	74	11.0	132.3	39.8	71.3	28.7
	75	10.5	131.9	39.0	72.2	28.4
	76	10.1	131.6	38.2	73.2	28.2
	77	9.6	131.2	37.5	74.2	27.9
	78	9.2	130.8	36.7	75.1	27.6
	79	8.7	130.4	35.9	76.1	27.3
	80	8.2	130.0	35.1	77.1	27.0
	81	7.8	129.6	34.4	78.1	26.7
	82	7.3	129.2	33.6	79.0	26.4
	83	6.8	128.8	32.9	80.0	26.1
	84	6.4	128.5	32.1	81.0	25.9
	85	5.9	128.1	31.3	81.9	25.6
	86	5.5	127.7	30.6	82.9	25.3
Prognathic	87	5.0	127.3	29.8	83.9	25.0
	88	4.5	126.9	29.0	84.9	24.7
	89	4.1	126.5	28.2	85.8	24.4
	90	3.6	126.1	27.5	86.8	24.1
	91	3.2	125.8	26.7	87.8	23.8
	92	2.7	125.4	25.9	88.8	23.6
	93	2.2	125.0	25.2	89.7	23.3
	94	1.8	124.6	24.4	90.7	23.0
	95	1.3	124.2	23.7	91.7	22.7
	96	0.9	123.8	22.9	92.7	22.4
	97	0.4	123.4	22.1	93.6	22.1
	98	0	123.0	21.4	94.6	21.8

Fig. 8 – Comparison of our results (horizontal line) with Czech [25] (connected line) and Filipinos [10] (dashed line).
For abbreviations, see under Table 1.

Discussion

In conventional cephalometric analyses, patients' cephalometric values are compared with already established norms specific to designated ethnic and racial groups. Unlike conventional cephalometric analyses, cephalometric analyses, using floating norms, are defined based upon correlation patterns between the five variables, SNA, SNB, NL-NSL, ML-NSL, and NSBa. Regarding the cephalometric floating norms, it can be said that they are part of initial orthodontic diagnosis and have a significant role in establishing the diagnosis, therapy planning, and estimating the effect of therapy [20, 26, 27]. In our study, the floating norms among the young population in Serbia have been established, and the individual craniofacial pattern has been defined. Patients' skeletal patterns can be considered harmonious as long as a correlation between sagittal and vertical cephalometric values exists and as long as the above-mentioned values lie within the harmonious schema [10].

According to Segner and Hasund [21], a harmonious box represents an important diagnostic tool for diagnosing craniofacial anomalies in orthodontics. It is already mentioned how a harmonious box is formed based on the linear regression, where SNA is represented as an independent variable, while SNB, NL-NSL, ML-NSL, and NSBa as dependent variables. In the upper part of the harmonious box, defined as the retrognathic zone, sagittal values are smaller than mean values. Patients whose values are located in this zone have a retrognathic type of face. Regarding the vertical plane, the type of face is determined based on the inclinations degree of the mandible against the cranial base, ML-NSL angle. Within our study, it was noticed that values of ML-NSL, NL-NSL, and NSBa variables are higher in the retrognathic zone of the harmonious box.

In the middle part of the harmonious box, variable values match with the mean values, and the type of face is represented as orthognathic. In the lower part of the harmonious box, sagittal values are higher than mean values, and the type of face is presented as a prognathic type of face. Vertical values such as ML-NSL, NL-NSL, and NSBa variables are reduced within the prognathic zone of the harmonious box. Among the participants whose SNA angle is enlarged and NL-NSL is reduced, the NSBa is too reduced. Generally, the smaller the cranial angle, the more prognathic and converse the face is [17]. The above-mentioned results are confirmed in our and other studies [10, 25]. If the five variables follow one of the harmonious combinations, the patient's face is considered harmonious and orthognathic. Based on everything mentioned, it can be concluded that the face can be represented as orthognathic and harmonious, then retrognathic and harmonious, and prognathic and harmonious. When the values of some of the five variables are located outside the harmonious schema, the face is considered disharmonious.

Cephalometric analysis should diagnose and locate regions of the skeletal anomaly [28]. The harmonious schema was formed by calculating SE when one of the variables gathered was based upon the rest of the four variables with the help of multiple regression analysis. In our study, multiple regression analysis showed that values of SNA and SNB variables had much higher correlation and fewer standard errors compared to NL-NSL, ML-NSL, and NSBa variables. That means the higher the SE in regression, the higher the variability degree of NL-NSL, ML-NSL, and NSBa variables. On the other hand, sagittal variables such as SNA and SNB have a significantly lower degree of variability. Thus, among harmonious combinations, it is unnecessary that variables' values lie exclusively on the flat horizontal line within the harmonious schema and harmonious box because certain deviations of NL-NSL, ML-NSL, and NSBa variables can be tolerated. The same results are presented by Segner [18] and Sevilla-Naranjilla and Rudzki-Janson [10] in their respective studies. The harmonious schema represents the degree of allowed change between five correlating cephalometric values to describe a harmonious face. The patient whose cephalometric variable values are located within the harmonious schema has a harmonious skeletal pattern [18]. The face is considered harmonious in cases when the patient's value is located within the retrognathic or prognathic zone of the harmonious box [18].

Comparing our results, our harmonious schema, and the harmonious schema of Segner and Hasund [21], we have noticed that they are similar in the aspect of two variables, NL-NSL and NSBa. The NL-NSL variables harmonious box ranges from 11.5° to 5.5°, while in Segner and Hasund [21] study, it ranges from 10.5° to 4.5°. When considering the NSBa variable, it varies from 134.7° to 125.8° among our participants, and from 135° to 127°, within the study by Segner and Hasund [21]. Other variables such as SNA, SNB, and ML-NSL show a smaller degree of variability among our participants compared to SNA, SNB, and ML-NSL in the study by Segner and Hasund [21]. In the study by Segner and Hasund [21], SNA variable values range from 80.5° to 84°, SNB from 79° to 82.5°, and ML-NSL from 33° to 24°. Among our study results, these variables are not showing such a degree of variability; therefore, the values of SNA range from 78° to 81°, SNB from 75.1° to 78.1°, and ML-NSL from 40.5° to 30.6°.

In addition, comparing our results with those from the studies by Sevilla-Naranjilla and Rudzki-Janson [10] and Řeháček et al. [25], the mean value of the variables among Filipinos and Czechs are drawn in our harmonious box and schema. It is concluded that similarities between Filipinos and our participants do not exist because the variables' sagittal values of Filipinos are located outside the harmonious schema. In the case of the Czechs, mainly all the values of the variables are located within the orthognathic zone of the harmonious box. The exception is the SNB angle, whose values are within the prognathic zone of the harmonious box. That means that the problem is sagittal and Czechs show mandibular prognathism.

Graphic illustration of cephalometric values in the form of a harmonious box and schema needs to enable the therapist to diagnose the type of face much easier and establish whether the relationship between the bones is harmonious and balanced.

Furthermore, the therapist with this concept's help needs to be in the state to define which angle values differ the most from harmonious combinations and harmonious boxes and in which direction orthodontic therapy should be implemented [18].

Conclusion

Analysis of individual craniofacial patterns using cephalometric floating norms enables the forming of the concept of harmonious values of determined parameters for a particular population. Cephalometric floating norms of the young population of Serbia, based on five cephalometric variables, determine the harmonious box values and the craniofacial's pattern of this population more precisely. Such an analysis can ease the diagnosis by defining the parameters more responsible for skeletal disharmony. In this way, it can assist in planning orthodontic therapy and assessing therapeutic effects.

R E F E R E N C E S

1. *Munandar S, Snow MD.* Cephalometric analysis of Deutero-Malay Indonesians. Aust Dent J 1995; 40(6): 381–8.
2. *Wahl N.* Orthodontics in 3 millennia. Chapter 8: The cephalometer takes its place in the orthodontic armamentarium. Am J Orthod Dentofacial Orthop 2006; 129(4): 574–80.
3. *Mittal S, Chaela D, Bhullar M, Aggarwal I, Palkit T, Chhatwalia S.* To Establish Cepahalometric Floating Norms as a Guide toward Harmonious Cranial Individual Pattern among North Indian Adults. Dent J Adv Stud 2020; 8(3): 102–8.
4. *Broadbent BH.* A new X-ray technique and its application to orthodontia. Angle Orthod 1931; 1: 45–66.
5. *Hofrath H.* Bedeutung der Ro¨ntgenfern und AbstandsAufnahmefu¨r die Diagnostik der Kieferanomalien. Fortschr der Orthod. 1931; 1: 231–58. (German)
6. *Zeng XL, Forsberg CM, Linder-Aronson S.* Craniofacial morphology in Chinese and Swedish children with Angle Class I and Class II occlusal relations. Aust Orthod J 1998; 15(3): 168–76.
7. *El-Batouti A, Øgaard B, Bishara SE.* Longitudinal cephalometric standards for Norwegians between the ages of 6 and 18 years. Eur J Orthod 1994; 16(6): 501–9.
8. *Park IC, Bowman D, Klapper L.* A cephalometric study of Korean adults. Am J Orthod Dentofacial Orthop 1989; 96(1): 54–9.
9. *Sevilla-Naranjilla MA, Rudzki-Janson I.* Cephalometric features of Filipinos with Angle Class I occlusion according to the Munich analysis. Angle Orthod 2004; 75(1): 63–8.
10. *Sevilla-Naranjilla MA, Rudzki-Janson I.* Cephalometric floating norms as a guide toward a harmonious individual craniofacial pattern among Filipinos. Angle Orthod 2009; 79(6): 1162–8.
11. *Avesh S, Adit S, Chaturvedi TP.* Sof tissue cephalometric norms in a north Indian ethic population. J Orthod Sci 2012; 1(4): 92–7.
12. *Nivedita S, Rajat M, Pritam M, Tushar N, Smruti N, Anand G.* Cephalometric norms for east Indian population using Burstone Legan analysis. J Int Oral Health 2016; 8(12): 1076–81.
13. *Thilander B, Persson M, Adolfsson U.* Roentgemp-cephalometric standards for a Swedish population: A longitudinal study between the ages of 5 and 31 years. Eur J Orthod 2005; 27(4): 370–89.
14. *Carrillo LE, Kubodera IT, Gonzales LB, Bastida MN, Preyra EG.* Cephalometric norms according to the Harvolds analysis. Int J Odontostomat 2009; 3(1): 33–9.
15. *Yadav AO, Walia CS, Borle RM, Chaoji KH, Rajan R, Datarkar AN.* Cephalometric norms for Central Indian population using Burstone and Legan analysis. Indian J Dent Res 2011; 22(1): 28–33.
16. *Solow B.* The pattern of the craniofacial associations: a morphological and methodological correlation and factor analysis study on young adults. Acta Odont Scand 1966; 24(Suppl 46): 174.
17. *Hasund A, Boe OE, Jenatschke F, Nordeval K, Thunoldk DK, Wisth PJ.* Clinical cephalometry for the Bergen-Technique. Orthodontic. Bergen, Norway: Orthodontic Department, Dental Institute, University of Bergen; 1974.
18. *Segner D.* Floating norms as a means to describe individual skeletal patterns. Eur J Orthod 1989; 11(3): 214–20.
19. *Segner D, Hasund A.* Individualisierte Kephalometrie. Aufl Hamburg, Germany: Franklin Printing and Publishing House Ltd; 1994. (German)
20. *Franchi L, Baccetti T, McNamara JA Jr.* Cephalometric floating norms for North American adults. Angle Orthod 1998; 68(6): 497–502.
21. *Segner D, Hasund A.* Individualisierte Kephalometrie. 3rd ed. Hamburg, Germany: SegnerVerlag & Vertrieb; 1998. p. 50–87. (German)
22. *Tollaro I, Tiziano B, Franchi L.* Floating norms for the assessment of craniofacial pattern in the deciduous dentition. Eur J Orthod 1996; 18(4): 359–65.
23. *Ngarmprasertchai S.* Vergleich der dento-kraniofazialen Morphologie zweier ethnischer Gruppen mit eugnathem Gebiss im Fernröntgenseitenbild [dissertation]. Munich, Germany: der Medizinischen Fakultät der Ludwig-Maximilians-Universität zu München; 2002. (German)
24. *Mahaini L.* Kraniofaziale Strukturen syrischer und deutscherProbanden – Eine kephalometrische Studie [dissertation]. Munich, Germany: der Medizinischen Fakultät der Ludwig-Maximilians-Universität zu München; 2005. (German)
25. *Řeháček A, Janega M, Hofmanová P, Dostálová T.* Cephalometric Floating Norms for Czech Adults. Prague Med Rep 2012; 113(4): 271–8.
26. *Aydemit H, Novruzov Z, Toygar-Memikoglu U.* Turskish norms of McNamaras cephalometric analysis. Turskish J Orthod 2014; 27(3): 100–5.
27. *Satinder S, Ashok U, Ashok J.* Cephalometric norms for orthognathic surgery for North Indian population. Contemp Clin Dent 2013; 4(4): 460–6.
28. *Di Paolo RJ, Philip C, Maganzini AL, Hirce JD.* The quadrilateral analysis: an individualized skeletal assessment. Am J Orthod 1983; 83(1): 19–32.

Received on November 19, 2020
Revised on March 22, 2021
Accepted on April 20, 2021
Online First April 2021

ORIGINAL ARTICLE

UDC: 616.348/.351-006-08
DOI: https://doi.org/10.2298/VSP200924053J

Optimal duration of therapy in the first line treatment of metastatic colorectal cancer: single-center study

Optimalno trajanje terapije u prvoj liniji tretmana kod metastatskog kolorektalnog karcinoma

Saša Jungić*†, Biljana Tubić†‡, Jelena Berendika*, Zdenka Gojković*†, Ivanka Rakita*, Milka Vještica*, Dejan Djokanović*, Radoslav Gajanin†

***University Clinical Center of the Republic of Srpska, Oncology Clinic, Banjaluka, Republic of Srpska, Bosnia and Herzegovina; †University of Banjaluka, Faculty of Medicine, Banjaluka, Republic of Srpska, Bosnia and Herzegovina; ‡Agency for Medicines and Medical Devices of Bosnia and Herzegovina, Banjaluka, Republic of Srpska, Bosnia and Herzegovina**

Abstract

Background/Aim. Standard treatment options for the first-line treatment of metastatic colorectal carcinoma (mCRC) are 5-fluorouracil, folinic acid, oxaliplatin (FOLFOX4)/capecitabine (CapOx), plus bevacizumab (bev) and 5-fluorouracil, folinic acid and irinotecan (FOLFIRI) plus bev. The aim of this study was to compare overall response rate (ORR), progression-free survival (PFS), and overall survival (OS) in patients with mCRC who were treated in the first line with FOLFIRI/bev vs. FOLFOX4/bev. At the same time, the aim was also to compare the safety profile in the observed groups of patients and to investigate optimal treatment duration and characteristics of patients who had the best treatment outcomes. **Methods.** The retrospective-prospective study included patients with mCRC treated with chemotherapy protocols for the first line in combination with bev (FOLFOX4/bev, respectively, FOLFIRI/bev). Treatment efficacy was evaluated on the basis of ORR, PFS, and OS, and the safety of treatment was evaluated by monitoring adverse drug reactions (ADR). **Results.** ORR was 70% in the FOLFIRI/bev group and 50% in the FOLFOX4/bev group. Median PFS for FOLFIRI/bev (n = 30) and for FOLFOX4/bev (n = 30) was 15.6 months and 12.1 months, respectively [hazard ratio (HR) 0.85; 95% confidence interval (CI) 0.47–1.53; p = 0.5591]. Median OS for FOLFIRI/bev and for FOLFOX4/bev was 24.7 months and 19.9 months, respectively (HR 0.67; 95% CI 0.37–1.23; p = 0.1552). In both patient groups, the patients who received more than 9 cycles of induction therapy had better treatment response compared with patients who received less than 9 cycles of therapy. In the FOLFOX4/bev group, PFS was 16.9 vs. 9.7 months, and OS was 22.1 vs. 17.6 months, respectively. In the FOLFIRI/bev group, PFS was 9 months for patients who received less than 9 cycles of therapy vs. 18.8 months for patients who received more than 9 cycles, and OS was 18.0 months vs. 27.7 months, respectively. ADR grade 3 and 4 had 7% of the patients in the FOLFIRI/bev group vs. 27% in the FOLFOX4/bev group. **Conclusion.** Patients who received FOLFIRI/bev compared to those treated with FOLFOX4/bev had better ORR (70% vs. 50 %, respectively), PFS (15.6 months vs. 12.1 months, respectively), and OS (24.7 months vs. 19.9 months, respectively). In both patient groups, the patients who received induction therapy for 4–6 months (more than 9 cycles of therapy) had a better treatment response.

Key words:
clinical protocols; colorectal neoplasms; drug-related side effects and adverse reactions; duration of therapy; folfox protocol; ifl protocol; neoplasm metastasis; survival.

Apstrakt

Uvod/Cilj. Standardne opcije u prvoj liniji lečenja metastatskog karcinoma debelog creva (mCRC) su 5-fluorouracil, folinska kiselina i oksaliplatin (FOLFOX4)/kapecitabin, oksaliplatin (CapOx) uz dodatak bevacizumaba (bev) i 5-fluorouracil, folinska kiselina i irinotekan (FOLFIRI) uz dodatak bev. Cilj rada bio je da se uporedi ukupni odgovor (*overall response rate* – ORR), period do progresije bolesti (*progression-free survival* – PFS) i ukupno preživljavanje (*overall survival* – OS) u grupama bolesnika sa mCRC koji su u prvoj liniji primali FOLFIRI/bev vs. FOLFOX4/bev. Takodje, cilj je bio i da se uporedi sigurnosni profil u ovim grupama bolesnika, kao i da se ispita optimalna dužina lečenja i karakteristike bolesnika koji su imali najbolje ishode lečenja. **Metode.** Retrospektivno-prospektivnim ispitivanjem obuhvaćeni su

Correspondence to: Jelena Berendika, University Clinical Center of the Republic of Srpska, Oncology Clinic, St. 12 beba, 78 000 Banjaluka, Republic of Srpska, Bosnia and Herzegovina. E-mail: jberendika@gmail.com

bolesnici sa mCRC, lečeni primenom hemioterapijskog protokola za prvu liniju terapije, u kombinaciji sa bev (FOLFOX4/bev, odnosno, FOLFIRI/bev). Efikasnost lečenja procenjena je na osnovu ORR, PFS i OS, a bezbednost lečenja praćenjem neželjenih reakcija. **Rezultati.** Parametar ORR bio je 70% u FOLFIRI/bev grupi i 50% u FOLFOX4/bev grupi. Medijana PFS za FOLFIRI/bev grupu (n = 30) iznosila je 15,6 meseci, odnosno 12,1 meseci za FOLFOX4/bev grupu (n = 30) [*hazard ratio* (HR) 0,85; 95% interval poverenja (IP) 0,47–1,53; $p = 0,5591$]. Medijana OS iznosila je 24,7 meseci u FOLFIRI/bev grupi i 19,9 meseci u FOLFOX4/bev grupi (HR 0,67; 95% IP 0,37–1,23; $p = 0,1552$). U obe grupe bolesnika bolji terapijski odgovor imali su bolesnici koji su primili više od 9 ciklusa indukcione terapije u poređenju sa bolesnicima koji su primili manje od 9 ciklusa. U FOLFOX4/bev grupi, PFS je iznosio 16,9 meseci, a OS 22, 1 mesec za bolesnike koji su primili više od 9 ciklusa, u odnosu na 9,7 meseci (PFS) i 17,6 meseci (OS) za bolesnike koji su primili manje od devet ciklusa terapije. U FOLFIRI/bev grupi, PFS je iznosio 9 meseci za bolesnike koji su primili manje od devet ciklusa terapije u odnosu na 18,8 meseci za bolesnike koji su primili više od 9 ciklusa, dok je OS iznosio 18,0 meseci u odnosu na 27,7 meseci u tim grupama bolesnika. Neželjenih dejstava gradusa 3 i 4 imalo je 7% bolesnika u FOLFIRI/bev grupi, a u FOLFOX4/bev grupi 27%. **Zaključak.** Bolesnici koji su primili FOLFIRI/bev protocol, u odnosu na one lečene FOLFOX4/bev protokolom, imali su bolji ORR (70% vs. 50%), PFS (15,6 meseci vs. 12,1 meseci) i OS (24,7 meseci vs. 19,9 meseci). U obe grupe bolesnika bolji ishod imali su bolesnici koji su primali indukcionu terapiju 4–6 meseci (9 do 12 ciklusa).

Ključne reči:
protokoli, klinički; kolorektalne neoplazme; lekovi, neželjeni efekti i neželjene reakcije; lečenje, trajanje; protokol, folfox; protokol, ifl; neoplazme, metastaze; preživljavanje.

Introduction

According to Global Cancer Observatory (GLOBOCAN) 2018, the estimated number of new cancer cases in Bosnia and Herzegovina was 14,385 (7,666 men and 6,719 women). The most common cancers in Bosnia and Herzegovina are lung cancer (2,424 new cases or 16.9%), colorectal cancer (CRC) (1,818 new cases or 12.6%), and breast cancer (1,386 new cases or 9.6%). CRC in Bosnia and Herzegovina ranked second in incidence in both men and women: 772 cases or 11.5% in women and 1,046 cases or 13.6% in men. The total number of cancer deaths from rectal cancer in 2018 in Bosnia and Herzegovina was 585 and 489 deaths from colon cancer [1].

At the time of diagnosis, approximately 80% of patients with CRC have resectable disease [2], but 30–50% of patients who undergo curative surgery experience disease recurrence and die of metastatic diseases [3]. The addition of targeted agents to standard chemotherapy has broadened treatment options. This affected the overall survival (OS). Bevacizumab (bev) is a humanized recombinant monoclonal antibody that blocks all isoforms of vascular endothelial growth factor-A (VEGF-A). Bev, in combination with chemotherapy (CHT), improves progression-free survival (PFS) or OS [4–10].

The aim of this study was to compare overall response rate (ORR), PFS, and OS in the groups of patients with metastatic CRC (mCRC), treated in the first line with FOLFIRI (5-fluorouracil, folinic acid, irinotecan) vs. FOLFOX4 (5-fluorouracil, folinic acid, oxaliplatin) both in combination with bev (FOLFIRI/bev vs. FOLFOX4/bev). At the same time, the safety profile and optimal treatment duration in the observed groups of patients were investigated, as well as the characteristics of patients who had the best treatment outcomes.

Methods

In this retrospective-prospective study, 60 patients with mCRC were treated using FOLFIRI/bev or FOLFOX4/bev protocol. All patients were divided into two groups. Male to female ratio was similar. The first group of patients (n = 30) received the FOLFOX4/bev protocol. The second group of patients (n = 30) received the FOLFIRI/bev protocol. All patients had metastatic disease, with primary tumor histologically confirmed and located in the colon or the rectum. Some patients received adjuvant or neoadjuvant CHT that ended 6 months prior to this study. The patient enrollment period was from January 1, 2014, until December 31, 2016, and patients were followed up until June 15, 2018. The study was conducted at the Oncology Clinic of the University Clinical Center of the Republic of Srpska, Banjaluka, Bosnia and Herzegovina and was approved by the local Ethics Committee from December 23, 2013 (No. 01-9-384.2/13).

Induction therapy protocols were FOLFOX4+bev and FOLFIRI+bev. Patients received induction therapy for a minimum of six and a maximum of 12 cycles.

Post-operative adjuvant therapy was capecitabin + oxaliplatin (XELOX) protocol.

After induction therapy, patients received maintenance therapy: capecitabin (monotherapy) or capecitabin + bev (AVAX).

Statistical analysis

Toxicity and safety were assessed in terms of toxicity and evaluated according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE), Version 3.0. Survival analysis (PFS and OS) was estimated by the Kaplan-Meier method using MedCalc software.

Results

Out of the 60 patients enrolled in the study, 6 were still alive in the FOLFIRI/bev group, and 9 were still alive in the FOLFOX4/bev group at the end of the follow-up (Table 1).

Table 1

Baseline patient demographic and clinical characteristics

Characteristics	FOLFIRI/bev (n = 30)	FOLFOX4/bev (n = 30)
Age (years)	51.5 (41–62)	56.0 (39–73)
Sex		
male	20 (67.0)	17 (57.0)
female	10 (33.0)	13 (43.0)
Site of primary tumor		
right colon	6 (20)	5 (16)
left colon	24 (80)	25 (84)
Adjuvant chemotherapy	10 (33.3)	4 (13.3)
capecitabine/oxaliplatin	3 (10.0)	0 (0.0)
capecitabine/oxaliplatin/radiotherapy	2 (6.7)	0 (0.0)
capecitabine	2 (6.7)	2 (6.7)
5-FU/folinic acid	2 (6.7)	2 (6.7)
cisplatina/5-FU	1 (3.3)	0 (0.0)
Number of metastatic sites		
1	18 (60.0)	8 (26.7)
2	8 (26.7)	14 (46.7)
3	4 (13.3)	8 (26.7)
Palliative radiotherapy	1 (3.3)	8 (26.7)
Induction chemotherapy		
6 cycles received	2 (6.7)	4 (13.3)
12 cycles received	15 (50.0)	5 (16.7)
Maintenance therapy	13	13
AVAX	2–30 cycles	2–37 cycles
FOLFOX4	1	0
capecitabine	0	1
Dose reduction	0 (0.0)	5 (16.7)
Therapy delayed	3 (10.0)	5 (16.7)
Therapy stopped	1 (3.3)	1 (3.3)
Resection of primary tumor	28/30 (93.3)	23/30 (76.7)
Second look surgery	10/30 (33.3)	7/30 (23.3)
curative	5 (16.7)	3 (10.0)
palliative	5 (16.7)	4 (13.3)

All values are expressed as number (percentage) of patients or median (range). AVAX – bevacizumab+capecitabine; FOLFIRI/bev – folinic acid, 5-fluorouracil (5-FU), and irinotecan/bevacizumab; FOLFOX4/bev – folinic acid, 5-fluorouracil (5-FU), and oxaliplatin/bevacizumab.

Localization of metastases

It is shown that in the FOLFIRI/bev group, there was a significantly higher number of patients with metastases in the liver alone, as opposed to the patients in the FOLFOX4/bev group (Figure 1).

Second surgical resection

Ten patients underwent a second surgery in the FOLFIRI/bev group. Out of these 10 patients, 5 patients underwent palliative surgery, and 5 patients underwent curative surgery: 4 patients underwent curative liver surgery without

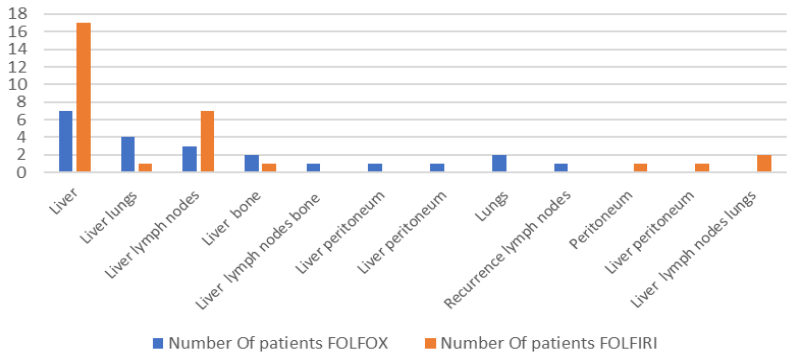


Fig. 1 – Localization of metastases in patients with colorectal cancer treated with FOLFIRI or FOLFOX4 protocol in combination with bevacizumab. FOLFIRI: folinic acid, 5-fluorouracil, and irinotecan; FOLFOX4: folinic acid, 5-fluorouracil, and oxaliplatin.

therapy, and 1 patient underwent curative liver surgery and received 6 cycles of XELOX CHT.

Seven patients underwent a second surgery in the FOLFOX4/bev group. Out of these 7 patients, 4 patients underwent palliative surgery and 3 patients underwent curative surgery: one patient underwent curative liver surgery and received 4 cycles of FOLFOX4/bev chemotherapy, one patient underwent metastasectomy and received XELOX, and one patient underwent curative liver surgery and received 4 cycles of capecitabine.

Evaluation of therapeutic response

Evaluation of response to therapy was performed according to the RECIST criteria, using ultrasonography, hematological and biochemical analyses, computed tomography, magnetic resonance imaging, and tumor markers CEA and CA 19-9. The results are shown in Tables 2 and 3. In both observed groups, the highest number of patients had a partial response to therapy (60.0% in the FOLFIRI/bev group and 46.7% in the FOLFOX4/bev group).

Kaplan-Meier survival estimates of PFS and OS for the FOLFIRI/bev group and FOLFOX4/bev group are presented in Figures 2 and 3, respectively.

PFS and OS were evaluated using: the number of cycles, localization of the primary tumor, and liver-limited dis*ease (LLD).*

Patients who received less than 9 cycles of therapy in both groups were compared to patients who received 9 and more cycles of induction chemotherapy. In both patient groups, significantly higher values of both PFS and OS were observed in patients who received more than 9 cycles of induction CHT with bev. The difference is more noticeable in the FOLFIRI/bev group. Patients with left-sided tumors had better PFS and OS in both patient groups. The difference is more noticeable in the FOLFOX4/bev group. Patients who

Table 2

Response of the patients to the treatment applied

Therapeutic response	FOLFIRI/bev	FOLFOX4/bev
Complete	3 (10.0)	1 (3.3)
Partial	18 (60.0)	14 (46.7)
Stable disease	4 (13.3)	8 (26.7)
Progressive disease	5 (16.7)	7 (23.3)

All values are expressed as number (percentage) of patients.
FOLFIRI/bev – folinic acid, 5-fluorouracil, and irinotecan/bevacizumab;
FOLFOX4/bev – bolnic acid, 5-fluorouracil, and oxaliplatin/bevacizumab.

Table 3

Efficacy parameters of the treatment applied

Therapeutic response	FOLFIRI/bev	FOLFOX4/bev
PFS (months)	15.6 (95% CI: 11.7–19.5)	12.1 (95% CI: 8.9–15,4)
OS (months)	24.7 (95% CI: 20.7–28.7)	19. 9 (95% CI: 15.2–24.5)
ORR (%)	70	50
PFS and OS not reached	6 (4 pts with 37 months)	9 (1 pts with 37 months)

FOLFIRI/bev – folinic acid, 5-fluorouracil, and irinotecan/bevacizumab; FOLFOX4/bev – folnic acid, 5-fluorouracil, and oxaliplatin/bevacizumab; PFS – progression free survival; OS – overall survival; ORR – overall response rate; CI – confidence interval; pts – patients.

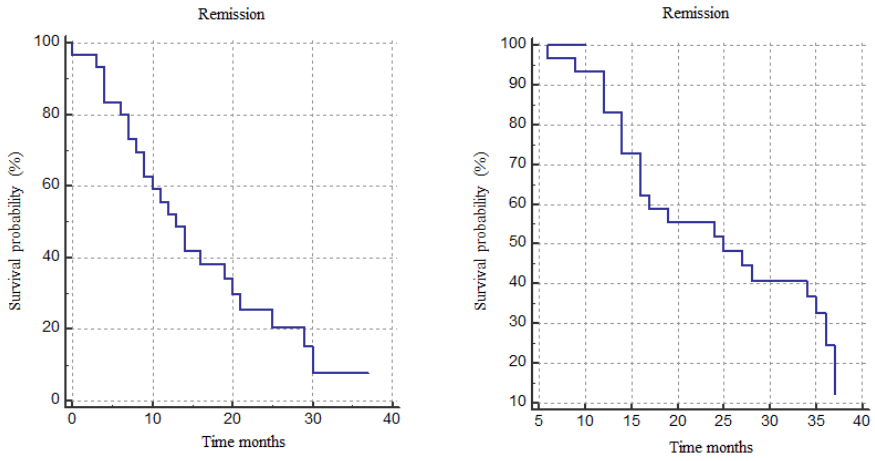


Fig. 2 – Kaplan-Meier survival estimates of PFS (left) and OS (right) for FOLFIRI/bev treatment. PFS – progression free survival; OS – overall survival; FOLFIRI/bev – folinic acid, 5-fluorouracil, and irinotecan/bevacizumab.

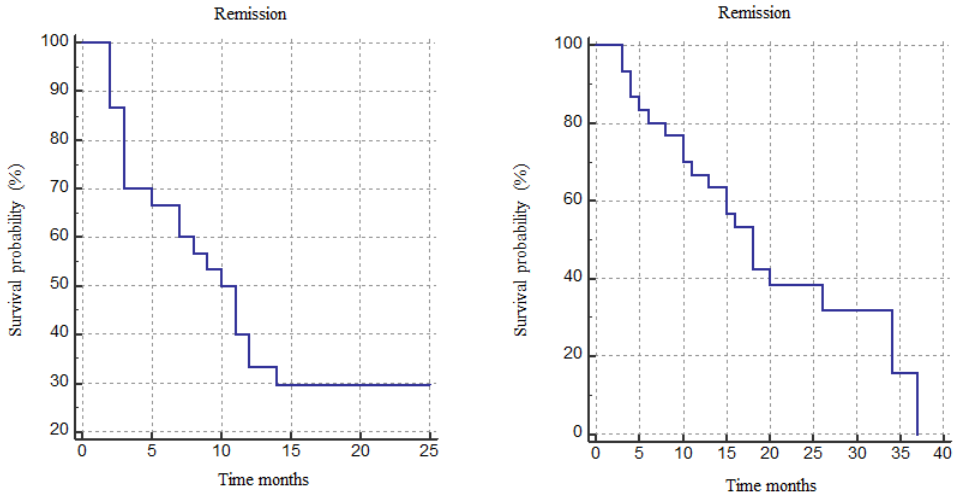


Fig. 3 – Kaplan-Meier survival estimates of PFS (left) and OS (right) for FOLFOX4/bev treatment. PFS – progression free survival; OS – overall survival; FOLFOX4/bev – folinic acid, 5-fluorouracil, and oxaliplatin/bevacizumab;

had LLD were compared to the patients who had metastases in the liver and other organs. Patients who had only liver metastases had better PFS and OS. The difference was more significant in the FOLFOX4/bev group (Table 4).

Delayed therapy

In the FOLFOX4/bev group, therapy was discontinued or delayed in a total of 5 patients due to adverse reactions (ADRs) as described below.

Bev was discontinued due to the occurrence of grade 4 pulmonary embolism. Complete therapy was delayed by 7 days due to the onset of grade 2 leukopenia and pancytopenia. Complete therapy was delayed by 10 days due to the onset of diarrhea, fatigue, and grade 2 pain. Complete therapy was delayed by 7 days due to the onset of grade 2 leukopenia and neutropenia, grade 1 thrombocytopenia, and grade 1 pulmonary embolism. Complete therapy was delayed by 15 days due to the onset of grade 3 leukopenia, grade 2 diarrhea, and grade 2 Hand-Foot Syndrome (HF Sy).

In the FOLFIRI/bev group, therapy was discontinued or delayed in a total of 3 patients. Complete therapy was discontinued in one patient due to grade 4 ileus. Complete therapy was delayed in one patient by 7 days due to the onset of grade 2 neutropenia and by 7 days in one patient due to grade 1 nausea and vomiting.

Adverse drug reactions in a group of patients treated with FOLFOX4/bev

ADRs in the FOLFOX4/bev group are given in Table 5.

The most commonly reported ADRs were hypertension (26.7%), leukopenia (23.3%), neutropenia (16.7%), and proteinuria (16.7%).

Grade 4 of ADRs were leukopenia, fistula, ileus, subileus, leukopenia, neutropenia, and pulmonary thromboembolism. The total percentage of grade 3 and grade 4 ADRs was 27%.

Adverse drug reactions in a group of patients treated with FOLFIRI/bev

ADRs in the FOLFIRI/bev group are given in Table 6.

The most commonly reported ADRs were diarrhea (36.7%), hypertension (30.0%), alopecia (23.3%), and nausea and vomiting (23.3%). Grade 4 ADRs were diarrhea and ileus. The total percentage of ADRs in grades 3 and 4 was 7%.

Table 4

Difference in PFS and OS in patients with mCRC (metastatic colorectal carcinoma) treated with FOLFOX4/bev and FOLFIRI/bev depending on treatment duration, site of primary tumor, and liver metastases

Parameter	FOLFOX4/bev						FOLFIRI/bev					
	PFS	HR	*p*	OS	HR	*p*	PFS	HR	*p*	OS	HR	*p*
Number of cycles												
≤ 9	9.7	0.34 (95% CI 0.14–0.82)	0.0305	17.6	0.51 (95% CI 0.20–0.29)	0.1958	9.0	0.36 (95% CI 0.13–1.03)	0.0084	18.0	0.36 (95% CI 0.13–1.03)	0.0075
> 9	16.9	2.92 (95% CI 1.22–6.96)		22.1	1.95 (95% CI 0.78–4.90)		18.8	2.76 (95% CI 0.97–7.90)		27.7	2.76 (95% CI 0.97–7.83)	
Site of primary tumor												
right	5.8	0.39 (95% CI 0.10–1.54)	0.0432	15.4	0.62 (95% CI 0.19–1.98)	0.3203	10.5	0.57 (95% CI 0.18–1.88)	0.2440	20.3	0.68 (95% CI 0.22–2.07)	0.4113
left	13.4	2.56 (95% CI 0.65–10.05)		21.2	1.62 (95% CI 0.51–5.16)		16.7	1.74 (95% CI 0.53–5.70)		25.8	1.47 (95% CI 0.48–4.50)	
Liver metastases												
yes	11.1	0.77 (95% CI 0.28–2.13)	0.6280	17.3	0.44 (95% CI 0.18–1.07)	0.0946	14.8	0.92 (95% CI 0.41–2.09)	0.8477	23.4	0.95 (95% CI 0.42–2.14)	0.8907
no	13.8	1.29 (95% CI 0.47–3.55)		27.1	2.29 (95% CI 0.94–5.62)		15.9	1.08 (95% CI 0.48–2.45)		25.5	1.06 (95% CI 0.47–2.38)	

FOLFIRI/bev – folinic acid, 5-fluorouracil, and irinotecan/bevacizumab; FOLFOX4/bev – folinic acid, 5-fluorouracil, and oxaliplatin/bevacizumab; PFS – progression free survival; OS – overall survival; HR – hazard ratio; CI – confidence interval; pts – patients.

Table 5

Most frequent treatment-related adverse events per patient in a group of patients treated with FOLFOX4/bev, classified by grades

Adverse events	grade 1	grade 2	grade 3	grade 4	Total
	number of patients				n (%)
Alopecia	1				1 (3.3)
Anemia	2	1			2 (10.0)
Fatigue	2	1			3 (10.0)
Diarrhea	1	2	1		4 (13.3)
Epistaxis	3				3 (10.0)
Fever		1			1 (3.3)
Fistula				1	1 (3.3)
Anorexia	1				1 (3.3)
Hematuria		1			1 (3.3)
HF Sy.	2	2			4 (13.3)
Hypertension	5	3			8 (26.7)
Ileus				3	3 (10.0)
Leucopenia			3	4	7 (23.3)
Nausea and vomiting		1			1 (3.3)
Neuropathy	2				2 (6.7)
Neutropenia		4		1	5 (16.7)
Pancytopenia		1			1 (3.3)
Pulmonary Thromboembolism	1			2	3 (10.0)
Proteinuria	5				5 (16.7)
Rhinitis	1				1 (3.3)
Subileus				1	1 (3.3)
Epiphora		1			1 (3.3)
Thrombocytopenia	2		1		3 (10.0)

FOLFOX4/bev – folnic acid, 5-fluorouracil and oxaliplatin/bevacizumab; HF Sy – Hand-Foot Syndrome.

Table 6

Most frequent treatment-related adverse events per patient in a group of patients treated with FOLFIRI/bev, classified by grades

Adverse events	grade 1	grade 2	grade 3	grade 4	Total
	number of patients				n (%)
Alopecia	7				7 (23.3)
Anemia	1	1			2 (6.7)
Anorexia	1				1 (3.3)
Fatigue	5	1			6 (20.0)
Diarrhea	4	6		1	11 (36.7)
Dyspepsia	1				1 (3.3)
Lower Extremity Embolism	1				1 (3.3)
Epistaxis	5	1			6 (20.0)
HF Sy.		2			2 (6.7)
Hypertension	5	3	1		9 (30.0)
Ileus				1	1 (3.3)
Leucopenia		1			1 (3.3)
Nausea and vomiting	5	2			7 (23.3)
Neutropenia		1	2		3 (10.0)
Obstipation	1				1 (3.3)
Pancytopenia	1				1 (3.3)
Pneumonia		1			1 (3.3)
Proteinuria	3	1			4 (13.3)
Stomatitis	2	1			3 (10.0)
Subileus	1				1 (3.3)
Thrombocytopenia		2			2 (6.7)
Thrombophlebitis	2				2 (6.7)

FOLFIRI/bev – folinic acid, 5-fluorouracil, and irinotecan/bevacizumab; HF Sy – Hand-Foot Syndrome.

Adverse drug reactions depending on the treatment duration

Table 7 shows the number of adverse drug events depending on the number of cycles of therapy classified by grade in patients treated with FOLFOX4/bev.

Table 8 shows the number of the reported ADRs depending on the number of cycles of therapy classified by grade in patients treated with FOLFIRI/bev.

Table 7

Total number of adverse drug events depending on the treatment duration classified by grade in patients treated with FOLFOX4/bev

Number of cycles	grade 1	grade 2	grade 3	grade 4
< 9 (n = 21 patients)	9	14	2	7
≥ 9 (n = 9 patients)	19	7	5	0

FOLFOX4/bev – folnic acid, 5-fluorouracil and oxaliplatin/bevacizumab.

Table 8

Total number of adverse drug events depending on the treatment duration classified by grade in patients treated with FOLFIRI/bev

Number of cycles	grade 1	grade 2	grade 3	grade 4
< 9 (n = 10 patients)	13	9	1	2
≥ 9 (n = 20 patients)	32	14	2	0

FOLFIRI/bev – folinic acid, 5-fluorouracil, and irinotecan/bevacizumab.

Characteristics of the survived patients

In our study, at data cut-off time, 6 patients were alive in the FOLFIRI/bev group and 9 patients in the FOLFOX4/bev group. All these patients had left-sided tumors. Radical surgery of the primary tumor was performed on 10 out of 15 living patients. Two patients in the FOLFIRI/bev group received adjuvant CHT. Ten out of fifteen patients received induction CHT for 9–12 cycles (4–6 months), and 8 of them received subsequent maintenance CHT with bev. Three patients in the FOLFIRI/bev group and 2 patients in the FOLFOX4/bev group underwent curative liver resection (second look surgery). After the liver surgery, both patients in the FOLFOX4/bev group and 1 patient in the FOLFIRI/bev group received CHT.

Discussion

Decisions for the optimal treatment of patients with mCRC should be made by a multidisciplinary team.

Most of the patients have metastatic disease that is not initially resectable. However, it is important to select patients with initially unresectable disease and in whom metastases may become suitable for resection after achieving a good response to combination CHT. The goal of treating this group of patients is to convert initially unresectable mCRC into resectable CRC.

Published results in international journals show the advantage of introducing angiogenesis inhibitors into standard CHT protocols for first-line treatment of mCRC [11].

Patients who received FOLFIRI/bev had better results than the patients treated with FOLFOX4/bev. Potential reasons are that the patients were younger, and the average age was 51. A significantly higher number of patients received adjuvant CHT, and also a higher number of patients had primary tumor resection. More patients in this group received induction CHT for a longer period of time, and a significantly higher number of patients had metastases in the liver alone.

ORR in both observed groups was high and statistically significantly higher in the FOLFIRI/bev group (70%) compared to the FOLFOX4/bev group (50%). ORR in the FOLFIRI/bev group was also higher compared to protocols for oligometastatic disease in the literature [12].

The assumption is that it was directly related to the fact that in both observed groups of patients, there was a large number of patients with left-sided colon tumors and that in the FOLFIRI/bev group, more patients had primary tumor resection and additionally, 56.6% of patients had liver metastases only. The high ORR is also supported by the fact that the average age of patients is lower (FOLFIRI/bev group – 51 years, FOLFOX4/bev group – 56 years). In the Bevacuzimab regimens investigation of treatment effects (BRiTE) study, the OS was 26.0 months in patients under the age of 65 [13].

Right-sided colon tumors are more common in women and have a higher stage at the time of diagnosis. They are mucinous, immunogenic, microsatellite unstable, more commonly RAS and BRAF genes mutated, and as such, have a worse prognosis. Left-sided colon tumors are more likely to have chromosomal instability, epidermal growth factor receptor (EGFR) expression, and higher VEGF-A expression.

In both patient groups, there was a significantly higher number of left-sided tumors (FOLFIRI/bev group 24/30 and FOLFOX4/bev group 25/30). Patients in both groups received bev leading to high ORR. Consequently, both PFS and OS were high.

The impact of localization of the primary tumor as a prognostic factor is known from earlier studies. The importance of localization in the efficacy of bev treatment was pointed out by Jordan et al. [14] in an analysis published in 2018, where 1,080 patients were monitored between 2003 and 2016. Patients with a tumor on the left side were compared with patients with a tumor on the right side, and their response to therapy was analyzed. Patients were divided into two groups: the

group of patients receiving bev and CHT (CHT/bev) and the group of patients receiving CHT only. OS for patients with left-sided tumors in the CHT/bev group was 31.5 months vs. 18.4 months for patients who received CHT only.

In contrast to patients with left-sided tumors, in patients with right-sided tumors, OS was 21.09 months in the CHT/bev group and 18.5 months in the CHT-only group. This indicates that the addition of bev to the treatment of patients with mCRC has a significant effect on the OS in patients with left-sided tumors. That is not the case in patients with right-sided tumors.

A meta-analysis by You et al. [15] published in Frontiers in Oncology showed improved survival when bev was added to chemotherapy in patients with left-sided mCRC.

Analysis by Jordan et al. [14] also showed that there was no benefit when bev was added to the treatment of patients with right-sided tumors regardless of the liver resection. In contrast, the addition of bev to the treatment of patients with left-sided tumors resulted in benefits even in patients who did not undergo resection of the liver metastases. The addition of bev affected the operability of the liver metastases. In the group of patients treated with CHT and bev, 25.3% of patients underwent liver resection in contrast to 18.6% of patients who received CHT only.

The efficacy and safety of bev in combination with CHT vs. CHT alone were analyzed in the meta-analysis published in BMC Cancer 2016 by Botrel et al. [12]. The analysis included 3,914 patients from 9 studies who received first-line treatment for mCRC. Patients who received CHT/bev had better ORR, PFS, and OS. Slightly better outcomes were seen in patients treated with irinotecan-containing protocols. In that meta-analysis, HR for PFS was 0.69 and for OS 0.87.

Median PFS was 15.6 months in the FOLFIRI/bev group (n = 30) and 12.1 months in the FOLFOX4/bev group (n = 30) (HR, 0.85; 95% CI 0.47–1.53; $p = 0.5591$). Median OS was 24.7 months in the FOLFIRI/bev group and 19.9 months in the FOLFOX4/bev group (HR, 0.67; 95% CI 0.37–1.23; $p = 0.1552$).

The Baraniskin et al. [16] meta-analysis published online in the European Journal of Cancer in November 2018 included 7 randomized trials analyzing the addition of bev to CHT in the first-line treatment of mCRC. Patients who received bolus 5-fluorouracil (5-FU) were excluded from the study. The addition of bev affected the prolongation of PFS in all studies except for the group of patients who received only 5-FU continuous infusion with bev. Extension in OS was not observed. This meta-analysis has its limitations in different study designs and objectives as well as different molecular subgroups of patients.

Incidence of ADR, as well as the severity of ADR in both patient groups (FOLFOX4/bev and FOLFIRI/bev), corresponds to the literature data [17, 18].

The difference was observed only for gastrointestinal (GI) perforations (13.3% in the FOLFOX4/bev group and 6.7% in the FOLFIRI/bev group). In the WJOG4407G trial, the incidence of GI perforations was 4% in the FOLFIRI/bev group and 3% in the FOLFOX4/bev group [19].

There were no GI perforations in TRIBE and OLIVIA trials. The reason for this, especially when it comes to the FOLFOX4/bev group, lies in the fact that the resection of the primary tumor was not performed. In the FOLFOX4/bev group, 23 out of 30 patients were operated on, while in the FOLFIRI/bev group, 27 out of 30 patients were operated on. There was less grade 3 and grade 4 ADR in both observed groups compared with the literature data [17, 20].

The incidence of ADR grades 1–3 was higher in the subgroup of patients who received 9 and more FOLFOX4/bev cycles, but the incidence of severe grade 4 ADR was significantly higher in the subgroup of patients who received less than 9 cycles of CHT.

A significant difference in the incidence between the two observed subgroups within the FOLFOX4/bev group was observed for leukopenia (3.3% vs. 20.0%) and proteinuria (3.3% vs.13.3%).

The incidence of grade 1–3 ADRs was higher in the subgroup of patients who received 9 and more FOLFIRI/bev cycles, but the incidence of severe grade 4 ADRs was significantly higher in the subgroup of patients who received less than 9 cycles of CHT.

No ADRs were observed in 5 patients, with 4 patients receiving 12 cycles and 1 receiving 7 cycles of FOLFIRI/bev CHT.

A significant difference in the incidence between the two observed subgroups within the FOLFIRI/bev group was observed for hypertension (0.0% vs. 30.0%), fatigue (0.0% vs. 20.0%), and proteinuria (0.0% vs. 13.3%).

ADRs generally occur within the first three months of treatment. A response to a significantly lower rate of grade 3 and 4 ADRs in both patient groups should be sought in a relatively small sample, as well as in a specific patient population and a significantly younger overall patient population in both groups. Better tolerance in younger patients was confirmed in the previous trials [21].

Patients experienced more nausea and vomiting in the FOLFIRI/bev group, and in the FOLFOX4/bev group, more leukopenia, neuropathies, and more grade 3 and 4 ADRs. In the FOLFIRI/bev group, fatigue, proteinuria, and hypertension occurred only in patients who received 9 to 12 cycles of induction therapy.

About 30% of patients with mCRC have LLD. These patients also die with liver metastases only. Resection of metastases in this subgroup of patients is a significant treatment option. The 5-year survival in patients who underwent resection was 55.2%, compared to 19.5% in patients who were unsuitable for resection. A 10-year survival was reported in 25.0% of patients who underwent resection. In our study, liver resection was performed in 16.7% of patients in the FOLFIRI/bev group and 10% in the FOLFOX4/bev group. Liver resection significantly influenced the increase in ORR in the FOLFIRI/bev group (70.0%).

Following curative resections of liver metastases, clinical studies support the use of bev with CHT (HEPATICA study). In this study, the two-year disease-free period for pa-

tients receiving CAPOX/bev was 70% compared to those receiving CAPOX alone (52%).

In our study, liver metastases alone were present in 17 patients (56.7%) in the FOLFIRI/bev group and in 7 patients (23.3%) in the FOLFOX4/bev group. This difference also resulted in better treatment results in the FOLFIRI/bev group compared to the FOLFOX4/bev group (higher PFS, OS, and ORR).

Limitations of the study

The limitations of this study are relatively small groups of patients. Moreover, the existence of a control group of patients who would receive only chemotherapy would give more information and more comprehensive conclusions. Doing molecular testing of RAS and BRAF genes would be advisable as well.

Conclusion

Patients from both groups received standard first-line CHT with the addition of bev, and the patients who received induction therapy for 4–6 months (9 to 12 cycles of therapy) had better treatment response. Those were the younger patients who had left-sided colon tumors, LLD, and who had their primary tumor resected. The consensus molecular subtypes classification and tumor microenvironment analysis of these patients could give us more information about these results. Those investigations could be the subject of future research.

REFERENCES

1. World Health Organization. The Global Health Observatory. Explore a world of health data. Available from: https://www.who.int/gho/DATABASE/en/
2. *Siegel RL, Miller KD, Jemal A.* Cancer statistics, 2016. CA Cancer J Clin 2016; 66(1): 7–30.
3. *Quasar Collaborative Group. Gray R, Barnwell J, McConkey C, Hills RK, Williams NS, Kerr DJ.* Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet 2007; 370(9604): 2020–9.
4. *Saltz LB, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R,* et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol 2008; 26(13): 2013–9.
5. *Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G,* et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 2003; 21(1): 60–5.
6. *Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W,* et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350(23): 2335–42.
7. *Kabbinavar FF, Schulz J, McCleod M, Patel T, Hamm JT, Hecht JR*, et al. Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. J Clin Oncol 2005; 23(16): 3697–705.
8. *Guan ZZ, Xu JM, Luo RC, Feng FY, Wang LW, Shen L*, et al. Efficacy and safety of bevacizumab plus chemotherapy in Chinese patients with metastatic colorectal cancer: a randomized phase III ARTIST trial. Chin J Cancer 2011; 30(10): 682–9.
9. *Tebbutt NC, Wilson K, Gebski VJ, Cummins MM, Zannino D, van Hazel GA,* et al. Capecitabine, bevacizumab, and mitomycin in first-line treatment of metastatic colorectal cancer: results of the Australasian Gastrointestinal Trials Group Randomized Phase III MAX Study. J Clin Oncol 2010; 28(19): 3191–8.
10. *Cunningham D, Lang I, Marcuello E, Lorusso V, Ocvirk J, Shin DB,* et al. AVEX study investigators. Bevacizumab plus capecitabine versus capecitabine alone in elderly patients with previously untreatedmetastatic colorectal cancer (AVEX): an open-label, randomised phase 3 trial. Lancet Oncol 2013; 14(11): 1077–85.
11. *Welch S, Spithoff K, Rumble R, Maroun J.* Group GCDS. Bevacizumab combined with chemotherapy for patients with advanced colorectal cancer: a systematic review. Ann Oncol 2009; 21(6): 1152–62.
12. *Botrel TEA, Clark LGO, Paladini L, Clark OAC.* Efficacy and safety of bevacizumab plus chemotherapy compared to chemotherapy alone in previously untreated advanced or metastatic colorectal cancer: a systematic review and meta-analysis. BMC Cancer 2016; 16: 677.
13. *Kozloff M, Ulickas Yood M, Berlin J, Flynn PJ, Kabbinavar FF, Purdie DM, et al.* Clinical outcomes associated with bevacizumab-containing treatment of metastatic colorectal cancer: the BRiTE observational cohort study. Oncologist 2009;14(9):862-70.
14. *Jordan F, Grundmann N, Schenkirsch G, Maerkl B, Messmann H, Anthuber M,* et al. Impact of Primary Tumor Localization on the Efficacy of Bevacizumab in Metastatic Colorectal Cancer. Anticancer Res 2018; 38(9): 5539–46.
15. *You XH, Wen C, Xia ZJ, Sun F, Li Y, Wang W,* et al. Primary Tumor Sidedness Predicts Bevacizumab Benefit in Metastatic Colorectal Cancer Patients. Front Oncol 2019; 9: 723.
16. *Baraniskin A, Buchberger B, Pox C, Graeven U, Holch JW, Schmiegel W,* et al. Efficacy of bevacizumab in first-line treatment of metastatic colorectal cancer: A systematic review and meta-analysis. Eur J Cancer 2019; 106: 37–44.
17. *Gruenberger T, Bridgewater J, Chau I, García Alfonso P, Rivoire M, Mudan S,* et al. Bevacizumab plus mFOLFOX-6 or FOLFOXIRI in patients with initially unresectable liver metastases from colorectal cancer: the OLIVIA multinational randomised phase II trial. Ann Oncol 2015; 26(4): 702–8.
18. *Cremolini C, Loupakis F, Antoniotti C, Lupi C, Sensi E, Lonardi S*, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol 2015; 16(13): 1306–15.
19. *Yamazaki K, Nagase M, Tamagawa H, Ueda S, Tamura T, Murata K,* et al . Randomized phase III study of bevacizumab plus FOLFIRI and bevacizumab plus mFOLFOX6 as first-line treatment for patients with metastatic colorectal cancer (WJOG4407G). Ann Oncol 2016; 27(8): 1539–46.
20. *Van Cutsem E, Rivera F, Berry S, Kretzschmar A, Michael M, DiBartolomeo M*, et al. First BEAT investigators. Safety and efficacy of first-line bevacizumab with FOLFOX, XELOX, FOLFIRI and fluoropyrimidines in metastatic colorectal cancer: the BEAT study. Ann Oncol 2009; 20(11): 1842–7.
21. *Lund CM, Vistisen KK, Dehlendorff C, Rønholt F, Johansen JS, Nielsen DL.* Age-dependent differences in first-line chemotherapy in patients with metastatic colorectal cancer: the DISCO study. Acta Oncol 2018; 57(11): 1445–54.

Received on September 24, 2020
Revised on May 14, 2021
Accepted on May 14, 2021
Online First May 2021

ORIGINAL ARTICLE

UDC: 616.311.2-071.3::616.31-089
DOI: https://doi.org/10.2298/VSP210318056D

Gingival biotype – comparative analysis of different evaluation methods

Biotip gingive – komparativna analiza različitih metoda ispitivanja

Filip Djordjević*, Dejan Dubovina*, Marija Bubalo†, Radivoje Radosavljević*, Zoran Bukumirić‡

***University in Priština/Kosovska Mitrovica, Faculty of Medicine, Department of Oral Surgery, Kosovska Mitrovica, Serbia; †Military Medical Academy, Clinic of Stomatology, Belgrade, Serbia; ‡University of Belgrade, Faculty of Medicine, Belgrade, Serbia**

Abstract

Background/Aim. Gingival biotype can have a significant impact on the outcome of the periodontal therapeutic procedures and the predictability of their aesthetic outcome. There is a strong correlation between the types of biotype and the potential gingival recession after restorative, periodontal, and implant surgical procedures. Therefore, accurate identification of gingival biotypes before initiating these procedures is one of the significant predictive factors for their success. The aim of this study was to evaluate the reliability of accurate gingival biotype determination with the use of the visual method, periodontal, and trans-gingival probing compared to the direct measurement method. **Methods.** This prospective study involved 33 patients indicated for apical root resection in the intercanine sector of the upper jaw. Gingival biotype identification was performed in all patients using the following techniques: 1) visual method; 2) periodontal probe technique; 3) trans-gingival probing; and 4) direct measurement after flap elevation. Statistical analysis of the obtained data was performed to assess the diagnostic accuracy of the visual method, periodontal probing method, and trans-gingival probing method in relation to the direct measurement method, used as a gold standard, to discriminate the gingival thickness biotype (thin *versus* thick). **Results.** The overall accuracy of the tested diagnostic procedures compared to direct gingival biotype measurement was 66.7% for the visual method, 78.8% for periodontal probing, and 97.0% for trans-mucosal probing. **Conclusion.** The periodontal probing method can be recommended for gingival biotype determination as a routine method since its sensitivity and overall accuracy are higher compared to the visual method. The trans-gingival method, in terms of sensitivity and comprehensive accuracy, almost completely coincides with the direct method, but it is more invasive compared to the periodontal probing method, and it has to be conducted in local anesthesia.

Key words:
evaluation study; gingiva; methods; periodontium; phenotype; surgery, oral.

Apstrakt

Uvod/Cilj. Biotip gingive može imati značajan uticaj na ishod parodontalnih terapijskih postupaka i predvidljivost njihovog estetskog ishoda. Postoji visoka korelacija između biotipa i potencijalne recesije gingive nakon restaurativnih, parodontalnih i implantoloških hirurških zahvata. Stoga je tačna identifikacija biotipa gingive, pre započinjanja ovih postupaka, jedan od značajnih prediktivnih faktora njihovog uspeha. Cilj rada bio je da se proceni pouzdanost određivanja biotipa gingive primenom vizuelne metode i metoda parodontalnog i transgingivalnog sondiranja u odnosu na direktnu metodu merenja. **Metode.** Prospektivnom studijom obuhvaćena su 33 pacijenta kod kojih je bila indikovana resekcija vrha korena zuba u interkaninom sektoru gornje vilice. Identifikacija gingivalnog biotipa izvršena je kod svih pacijenata primenom: 1) vizuelne metode; 2) tehnike parodontalnog sondiranja; 3) tehnike transgingivalnog sondiranja i 4) direktnog merenja nakon odizanja režnja. Statistička analiza dobijenih podataka izvršena je radi procene dijagnostičke tačnosti vizuelne metode, parodontalnog sondiranja i transgingivalnog sondiranja u odnosu na direktnu metodu, koja se koristi kao zlatni standard u cilju evaluacije biotipa gingive (tanak nasuprot debelom). **Rezultati**. Ukupna tačnost testiranih dijagnostičkih postupaka u određivanju biotipa gingive, u poređenju sa metodom direktnog merenja, bila je: vizuelna metoda – 66,7%; parodontalno sondiranje – 78,8%; transmukozno sondiranje – 97,0%. **Zaključak.** Parodontalna metoda

Correspondence to: Filip Djordjević, University in Priština/Kosovska Mitrovica, Faculty of Medicine, Department of Oral Surgery, Anri Dinana bb, 38 220 Kosovska Mitrovica, Serbia. E-mail: filip85dj@gmail.com

sondiranja može se preporučiti za određivanje biotipa gingive kao rutinska metoda, s obzirom da je njena senzitivnost i ukupna tačnost veća u odnosu na vizuelnu metodu. U pogledu senzitivnosti i sveobuhvatne tačnosti, transgingivalna metoda se gotovo u potpunosti poklapa sa direktnom metodom, ali je invazivnija u poređenju sa metodom parodontalnog sondiranja i mora se sprovesti uz prethodnu primenu lokalne anestezije.

Ključne reči:
procena, istraživanja; gingiva; metodi; periodoncijum; fenotip; hirurgija, oralna.

Introduction

In recent years, the characteristics of the oral mucosa, especially gingival thickness, have become the subject of interest for both implantologists and periodontists, epidemiologists, and many others. The term “gingival biotype” has been used to describe the thickness of gingiva in vestibulo-oral direction [1–3]. The first analysis of gingival anatomy in this sense was given in 1969 by Ochsenbein and Ross [4], who described two main types of gingival morphology: flat and thick, and thin and scalloped gingiva. They have indicated a connection between the gingival contour and the contour of the underlying alveolar bone. Based on this classification, Seibert and Lindhe [5] later introduced the term "periodontal biotype", which further categorized gingiva into thick-flat and thin-scalloped biotypes.

After observing different variations of keratinized tissue and with the increasing use of dental implants, in 1997, Müller and Eger [6] joined the term gingival and periodontal biotype into a soft tissue biotype, which includes both tooth tissue and tissue around implants.

In general, it can be said that a gingival thickness of ≤ 1 mm is defined as a thin biotype and a gingival thickness of ≥ 1 mm as a thick biotype [7]. A thick biotype exists in about 85% of cases; it is characterized by thick gingival tissue and is usually associated with good periodontal health. It has a sufficient width of the attached gingiva, is more resistant to trauma and thus to recessions, and is much easier to be manipulated during surgical procedures. This is explained by the presence of a high percentage of extracellular matrix and collagen that allows tissue contraction as well as good vascularization. The thin biotype is present in the remaining 15% of cases. It is usually transparent and has a small attachment zone. It is usually characterized by bone defects, such as dehiscence and fenestration underneath, and is less resistant to inflammation and trauma [8, 9].

Numerous studies [8, 10–14] have shown that gingival biotype can have a significant impact on the outcome of the therapeutic procedures and the predictability of the aesthetic outcome. There is a strong correlation between gingival biotype and possible gingival recession after restorative, periodontal, and implant surgical procedures. Therefore, accurate identification of gingival biotype before initiating these procedures is one of the essential predictive factors for their success. In that sense, there is a number of methods for determining the gingival biotype: the visual method [2, 10], biotype identification method with the use of periodontal probe [11], direct measurement of the gingival thickness [15], trans-gingival probing [16], ultrasonic measurement and cone-beam computed tomography (CBCT) radiographic examination [17–21].

The aim of this study was to evaluate the reliability of the gingival biotype determination by using the visual method, periodontal probe, and trans-gingival probing in relation to the direct measurement method.

Methods

This prospective clinical study was performed at the Department of Oral Surgery of the Clinic of Dental Medicine, Faculty of Medicine, University in Priština/Kosovska Mitrovica, Kosovska Mitrovica, Serbia, and the private dental practice "Radix" in Kruševac, Serbia. The selection of patients who participated in the study was done according to pre-established criteria. All patients were older than 18 years, had good oral hygiene, and were previously indicated for apical surgery in the intercanine sector of the upper jaw due to chronic periapical lesions that could not be treated endodontically. In addition, an important parameter was the existing indication for the use of a flap design with a horizontal intrasulcular incision. Additional parameters were the presence of attached gingiva > 5 mm wide, as well as a negative history of previous interventions in the intercanine sector of the upper jaw, such as soft tissue augmentation, treatment for gingival recessions, or esthetic extension of the clinical tooth crowns. Patients with fixed prosthetic works, marginal gingiva inflammation, systemic diseases, and bad habits that could compromise the results, such as smoking, alcoholism, or oral breathing due to airway obstruction, were excluded from the study. Systemic therapy with medications that might affect the oral and gingival condition also represented an exclusive factor.

The study included 33 patients (20 males and 13 females) aged 18–72 years. Gingival biotype identification was performed in the lateral incisor zone in 17 patients, the central in 11 patients, and the canine in 5 patients. The evaluation was performed first by visual method and then by periodontal probing. After administrating infiltration anesthesia to perform oral surgery, gingival biotype identification was performed using trans-gingival probing. In the end, immediately after the full-thickness mucoperiosteal flap elevation, a direct measurement of gingival thickness was performed using a modified caliper. The entire testing procedure was performed by the same researcher.

Visual method

A visual method of gingival biotype assessment was performed by observing the appearance of the gingiva in the dental area where oral surgery was indicated and also by observing other teeth of the upper intercanine region as follows: thick biotype – the gingiva around the observed

tooth is thickened and fibrous, the interdental papillae towards the adjacent teeth are short, the contact points are wide, teeth are of square shaped, with pronounced cervical convexity (Figure 1); thin biotype – the gingiva around the observed tooth looks thin and delicate, the interdental papillae are narrow and long, the contact points to adjacent teeth are narrow and more incisally displaced, while the teeth are elongated and triangular in shape (Figure 2).

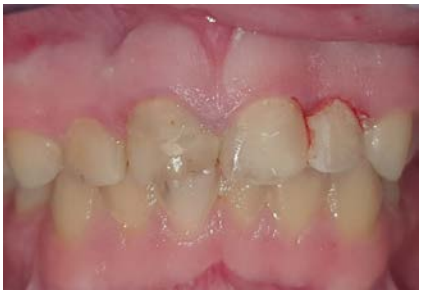


Fig. 1 – Thick gingival biotype.

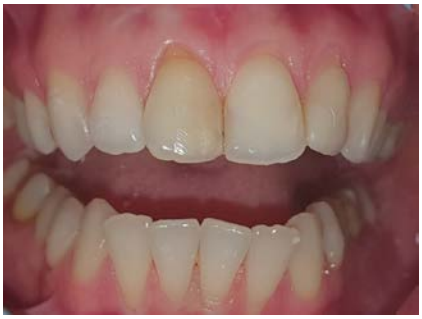


Fig. 2 – Thin gingival biotype. Note the gingival recessions on teeth 11, 21, and 22 – a common clinical finding associated with the thin gingival biotype.

Periodontal probing

Periodontal assessment of gingival biotype was performed using a periodontal probe (WHO Probe 550b, LM Dental). Clinical evaluation was done by sulcus probing in the central part of the vestibular side of the tooth, on which oral surgery was indicated (Figure 3). The gingival biotype was classified according to the visibility of the periodontal probe through the gingival tissue as follows: thick biotype – the periodontal probe is not visible through the gingival tissue; thin biotype – the periodontal probe is visible through the gingival tissue [11].

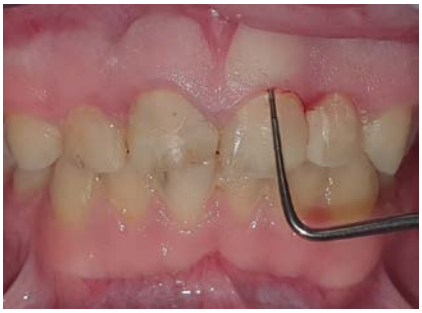


Fig. 3 – Periodontal examination of gingiva thickness.

Trans-gingival probing

Gingival biotype assessment, using trans-gingival (mucosal) probing, was done by measuring its thickness with a root canal instrument number 25 with a rubber stopper (K-file Maillefer, Dentsply). After applying infiltration anesthesia in order to perform the planned oral-surgical intervention, a root canal instrument was used to pierce the soft tissue of the gingiva on the vestibular side of the tooth indicated for surgery at a distance of 3 mm from the marginal gingival edge, set perpendicular relative to the alveolar ridge till the bone contact. The rubber stopper of the root canal instrument was then placed on the surface of the alveolar ridge mucosa (Figure 4). After that, the distance from the tip of the needle to the rubber stopper was measured with a millimeter ruler, based on which the gingival biotype was identified as follows: thick biotype – the distance between the tip of the root canal instrument and the stopper was > 1mm; thin biotype – the distance between the tip of the root canal instrument and the stopper was < 1mm [22].

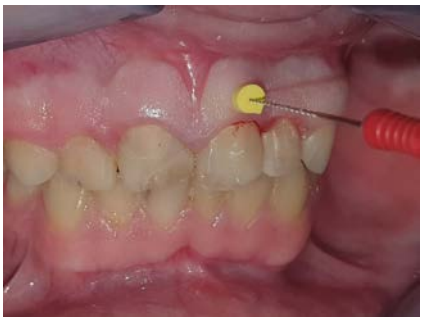


Fig. 4 – Trans-gingival probing.

Direct measurement

The modified caliper with a millimeter ruler (Wax caliper, Odontomed), with tips blunted to minimize the pressure and trauma to the soft tissue, was used for the direct measurement of the gingival thickness (Figure 5). After the full-thickness flap elevation, the gingival thickness on the vestibular side of the tooth was measured at a distance of 3 mm from the edge of the marginal gingiva, based on which the gingival biotype was classified, namely: thick biotype – gingival thickness was > 1 mm; thin biotype – gingival thickness was < 1mm [7].

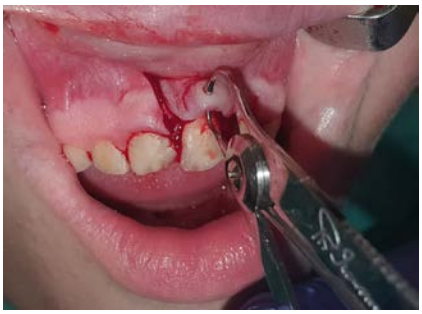


Fig. 5 – Direct measurement.

After all the measurements for each patient, the obtained results were statistically processed. Measures of sensitivity, specificity, and overall accuracy were applied to assess the diagnostic accuracy of the visual method, periodontal biotype identification, and trans-gingival probing in relation to the direct measurement method, used as a gold standard, the most objective method to discriminate the gingival thickness biotype (thin *versus* thick) [7].

Results

To assess the diagnostic accuracy of visual, periodontal, and trans-gingival probing methods for discrimination of gingival thickness biotype (thin to thick), measures of sensitivity, specificity, and overall accuracy, in relation to direct measurement, were applied.

Although invasive, the direct method of measurement is considered the reference method in most studies. The success of all other methods is measured according to the direct method. The results obtained in this study showed that the average gingival thickness, measured by the direct method, was 0.982 mm, with an almost uniform distribution of gingival thickness values larger or smaller than this average (51.5% larger and 48.5% smaller than the mean value). For this reason, we can agree that a borderline value between the gingival thickness for thin and thick gingival biotypes could be considered 1 mm.

By examining the gingival biotype in 33 patients with the visual method, a thin biotype was diagnosed in 8 (24.2%) cases, while a thick biotype was diagnosed in 25 (75.8%) cases (Table 1). When the periodontal examination was used, a thin biotype was found in less and a thick one in more cases, while, when the trans-gingival method was used, a thin biotype was found in most and a thick one in the least number of respondents (Table1). Direct measurements of the gingival thickness, however, resulted in a thin biotype in 51.5% of respondents and a thick one in 48.5% (Table 1).

When examining the diagnostic accuracy of different methods for identifying a thin biotype, the compatibility of results between visual and direct methods was determined in only 7 out of 17 cases. The statistical analysis showed that the value of sensitivity of this method was 41.2% for thin biotype identification, relative to the direct measurement used as a gold standard. On the other hand, the accuracy of this method in identifying the thick biotype was noticed in 15 out of 16 cases identified using the direct method, which indicates a specificity value of 93.8%. Based on the presented results, the calculated overall accuracy value was 66.7% (Tables 2 and 3).

Table 1

Frequency of different gingival biotypes determined by visual method, periodontal probing, trans-gingival method and direct methods

Method	Frequency	Percent	Valid percent	Cumulative percent
Visual				
thin biotype	8	24.2	24.2	24.2
thick biotype	25	75.8	75.8	100.0
Total	33	100.0	100.0	
Periodontal probing				
thin biotype	12	36.4	36.4	36.4
thick biotype	21	63.6	63.6	100.0
Total	33	100.0	100.0	
Trans-gingival				
thin biotype	18	54.5	54.5	54.5
thick biotype	15	45.5	45.5	100.0
Total	33	100.0	100.0	
Direct				
thin biotype	17	51.5	51.5	51.5
thick biotype	16	48.5	48.5	100.0
Total	33	100.0	100.0	

Table 2

Compatibility of results: visual method, periodontal probing, and trans-gingival probing in relation to the direct method

Method	Direct nominal		Total
	thin biotype	thick biotype	
Visual			
thin biotype	7	1	8
thick biotype	10	15	25
Total	17	16	33
Periodontal probing			
thin biotype	11	1	12
thick biotype	6	15	21
Total	17	16	33
Trans-gingival probing			
thin biotype	17	1	18
thick biotype	0	15	15
Total	17	16	33

Table 3

Diagnostic accuracy measures of the tested methods in relation to the direct method

Diagnostic accuracy measures	Visual method	Periodontal probing	Trans-gingival probing
Sensitivity (Se), %	41.2 (18.4–67.1)	64.7 (38.3–85.8)	100.0 (72.7–100.0)
Specificity (Sp), %	93.8 (69.8–99.8)	93.8 (69.8–99.8)	93.8 (69.8–99.8)
Overall accuracy, %	66.7 (48.2–82.0)	78.8 (61.1–91.0)	97.0 (84.2–99.9)

Results are given as mean (95% confidence interval).

With the use of the periodontal probing method, a thin gingival biotype was diagnosed in 11 out of 17 cases determined by direct measurement. When examining the thick biotype, compatibility with the direct measurements was in 15 out of 16 cases, indicating its sensitivity of 64.7%, while the specificity was 93.8%. The overall accuracy of the periodontal probing method was 78.8% (Tables 2 and 3).

The sensitivity of the trans-gingival method in the thin gingival biotype identification, in relation to the direct measurement, was 100.0%, while the specificity was 93.8%. Its overall accuracy was 97.0% (Tables 2 and 3).

Discussion

Gingival biotype is an important clinical parameter that can affect not only the success but also the planning and prognosis of the programmed restorative, periodontal, or implant procedure. The thin gingival biotype around natural teeth increases the risk of gingival recession after surgical, restorative, or even mechanical trauma [6, 22, 23]. A similar phenomenon has also been noticed in the peri-implant mucosa [24]. In addition, this gingival biotype is often associated with the presence of a thin lamellar bone around the teeth, together with the presence of fenestration and dehiscence, which can be a significant limiting factor in terms of possible immediate implant procedures. From a dental implantology point of view, it is important to emphasize that the frequency of gingival recession, around the implant, after the replacement of one lost tooth increases with the reduction of gingival thickness [25]. In addition, Hwang and Wang [26] concluded in their histological study that a thin gingival biotype at the implantation site is more likely to have angulated bone defects, in contrast to a thick biotype where greater stability of the cortical bone is noticed [27]. Finally, the success of numerous periodontal procedures for the coverage of gingival recessions is significantly lower in patients with a thin gingival genotype [27, 28]. Bearing in mind all the mentioned data concerning the significance of the gingival biotype, numerous methods have been developed to evaluate the thickness of the gingival tissue.

The visual method of gingival biotype identification represents the simplest and one of the most commonly used methods. However, its biggest deficiency is the lack of standardization among accurate clinical parameters, so the method itself is often based on the subjective evaluation and experience of the dentist alone. This is the main reason why the precision of this method is insufficient compared to the others available to clinicians [9]. According to the results of this study, when using the visual method in gingival biotype detection, a thin gingival biotype was noticed in only 24.2% of cases, which is markedly different compared to the direct method taken as a reference, where the percentage was 51.5%. This discrepancy is smaller in other examined methods. Concerning the identification of the thick biotype, the diagnostic accuracy of this method showed its sensitivity of 41.2%, while its overall accuracy was 66.7% and specificity 93.8%. In addition, unlike the previous parameters, it does not differ from other examined methods, which indicates that the possibility of erroneous identification of a thin biotype by this method was far greater than that of a thick one.

According to different authors, a much more suitable method for determining gingival biotype is periodontal probing [6, 7]. The procedure is quite simple, with precise clinical parameters, which reduces the possibility of subjective assessments in contrast to the visual method. On the other hand, it is less invasive compared to the trans-gingival and direct methods. The trans-gingival method requires the application of anesthesia in an examined area, while the direct method can be used only during the surgical intervention and cannot be used to determine the gingival biotype in order to plan and predict the success of the future treatment. The results of this study show that the concordance of the measurements of the periodontal and the direct method in determining the thin biotype is higher than when using the visual method. Statistical analysis showed that its sensitivity value was higher compared to the visual method, although still lower compared to the trans-gingival method. Similarly, the value of the overall accuracy was 78.8%, and it is higher compared to the visual method, which gives an advantage to this method for determining the gingival biotype. On the other hand, it is lower compared to the much more invasive trans-gingival method. For this reason, the method of periodontal probing can be recommended as a method of choice in everyday routine practice.

The sensitivity of the trans-gingival method, as well as the overall accuracy, is the highest of all examined methods – 100% and 97%, respectively, and, therefore, almost coincides with the direct method. During the study, a slightly larger deviation in the values of gingival thickness was observed compared to the direct method in the thick biotype (> 1 mm), which is explained by the incomplete insertion of a needle into the thickened gingival tissue. However, these discrepancies do not affect the overall results of this study. Therefore, although invasive and in need of local anesthesia of the examined area, which is considered a shortcoming of this method, compared to the method of periodontal probing, it is still more precise, almost at the level of the direct method. In addition, it can be used for preoperative evaluations.

This almost coincides with the findings of Kan et al. [7], who found the average gingival thickness of 1.06 mm.

Conclusion

The periodontal probing can be recommended for gingival biotype determination as a routine method because its sensitivity and overall accuracy (in relation to direct measurement) are higher compared to the visual method, and it is less invasive compared to the trans-gingival method, although not as accurate.

REFERENCES

1. *Kan JYK, Rungcharassaeng K, Morimoto T, Lozada JL.* Facial gingival tissue stability after connective tissue graft with single immediate tooth replacement in the esthetic zone: Consecutive case report. J Oral Maxillofac Surg 2009; 67(11 Suppl): 40–8.
2. *Kan JYK, Rungcharassaeng K.* Site development for anterior single implant esthetics: The dentulous site. Compend Contin Educ Dent 2001; 22(3):221–6, 228, 230–1; quiz 232.
3. *De Rouck T, Eghbali R, Collys K, De Bruyn H, Cosyn J.* The gingival biotype revisited: Transparency of the periodontal probe through the gingival margin as a method to discriminate thin from thick gingiva. J Clin Periodontol 2009; 36(5): 428–33.
4. *Ochsenbein C, Ross S.* A reevaluation of osseous surgery. Dent Clin North Am 1969; 13(1): 87–102.
5. *Seibert JL, Lindhe J.* Esthetics and periodontal therapy. In: *Lindhe J*, editor. Textbook of Clinical Periodontology. 2nd ed. Denmark, Copenhagen: Munksgaard; 1989. p. 477–514.
6. *Müller HP, Eger T.* Gingival phenotypes in young male adults. J Clin Periodontol 1997; 24 (1): 65–71.
7. *Kan JY, Morimoto T, Rungcharassaeng K, Roe P, Smith DH.* Gingival biotype assessment in the esthetic zone: visual versus direct measurement. Int J Periodontics Restorative Dent 2010; 30(3): 237–43.
8. *Joshi N, Agarwal MC, Madan E, Gupta S, Law A.* Gingival biotype and gingival biofilm: determining factors for periodontal disease progression and treatment outcome. Int J Sci Study 2016; 4(3): 220–5.
9. *Ahmadi RS, Tavassoli R, Sayar F, Ghaffari K, Sarlati F.* Gingival thickness assessment: Visual versus direct measurement. J Islam Dent Assoc Iran (JIDAI) 2016; 28(4): 149–54.
10. *Olsson M, Lindhe J, Marinello CP.* On the relationship between crown form and clinical features of the gingival in adolescents. J Clin Periodontol 1993; 20(8): 570–7.
11. *Kan JYK, Rungcharassaeng K, Umezu K, Kois JC.* Dimensions of peri-implant mucosa: An evaluation of maxillary anterior single implants in humans. J Periodontol 2003; 74(4): 557–62.
12. *Alves PHM, Alves TCLP, Pegoraro TA, Costa YM, Bonfante EA, de Almeida ALPF.* Measurement properties of gingival biotype evaluation methods. Clin Implant Dent Relat Res. 2018; 20(3): 280–4.
13. *Zuhr O, Bäumer D, Hürzeler M.* The addition of soft tissue replacement grafts in plastic periodontal and implant surgery: critical elements in design and execution. J Clin Periodontol 2014; 41 Suppl 15: S123–42.
14. *Linkevicius T, Puisys A, Svediene O, Linkevicius R, Linkeviciene L.* Radiological comparison of laser-microtextured and platformswitchedimplants in thin mucosal biotype. Clin Oral Implants Res 2015; 26(5): 599–605.
15. *Goaslind GD, Robertson PB, Mahan CJ, Morrison WW, Olson JV.* Thickness of facial gingiva. J Periodontol 1977; 48(12): 768–71.
16. *Esfahrood ZR, Kadkhodazadeh M, Talebi Ardakani MR.* Gingival biotype: a review. Gen Dent 2013; 61(4): 14–7.
17. *Kydd WL, Daly CH, Wheeler JB 3rd.* The thickness measurement of masticatory mucosa in vivo. Int Dent J 1971; 21(4):430–41.
18. *Slak B, Daabous A, Bednarz W, Strumban E, Maev RG.* Assessment of gingival thickness using an ultrasonic dental system prototype: A comparison to traditional methods. Ann Anat 2015; 199: 98–103.
19. *Fu JH, Yeh CY, Chan HL, Tatarakis N, Leong DJ, Wang HL.* Tissue biotype and its relation to the underlying bone morphology. J Periodontol 2010; 81(4): 569–74.
20. *Lindhe J, Lang N, Karring Th.* Clinical Periodontology and Implant Dentistry. 5th ed. Hoboken: Wiley-Blackwell; 2008; pp 69–71.
21. *Kolte R, Kolte A, Mahajan A.* Assessment of gingival thickness with regards to age, gender and arch location. J Indian Soc Periodontol 2014; 18(4): 478–81.
22. *Cosyn J, Sabzevar MM, De Bruyn H.* Predictors of inter-proximal and midfacial recession following single implant treatment in the anterior maxilla: a multivariate analysis. J Clin Periodontol 2012; 39(4): 895–903.
23. *Muller HP, Eger T, Sorb A.* Gingival dimensions after root coverage with free connective tissue grafts. J Clin Periodontol 1998; 25(5): 424–30.
24. *Bengazi F, Wennström JL, Lekholm U.* Recession of the soft tissue margin at oral implants. A 2-year longitudinal prospective study. Clin Oral Implants Res 1996; 7(4): 303–10.
25. *Evans CD, Chen ST.* Esthetic outcomes of immediate implant placements. Clin Oral Implants Res 2008; 19(1): 73–80.
26. *Hwang D, Wang HL.* Flap thickness as a predictor of root coverage: a systematic review. J Periodontol 2006; 77(10): 1625–34.
27. *Baldi C, Pini-Prato G, Pagliaro U, Nieri M, Saletta D, Muzzi L,* et al Coronally advanced flap procedure for root coverage. Is flap thickness a relevant predictor to achieve root coverage? A 19-case series. J Periodontol 1999; 70(9): 1077–84.
28. *Kahn S, Almeida RA, Dias AT, Rodrigues WJ, Barceleiro MO, Taba MJr.* Clinical Considerations on the Root Coverage of Gingival Recessions in Thin or Thick Biotype. Int J Periodontics Restorative Dent 2016; 36(3): 409–15.

Received on March 18, 2021
Revised on May 13, 2021
Accepted on May 21, 2021
Online First May 2021

ORIGINAL ARTICLE

UDC: 616.34-073.9-092.9
DOI: https://doi.org/10.2298/VSP211121052S

Early steps of an alternative test meal for gastric emptying scintigraphy

Početni stadijumi razvoja alternativnog test-obroka za scintigrafiju pražnjenja želuca

Hüseyin Şan*, Mehmet Bahadır Çalışkan†

***Ankara City Hospital, Department of Nuclear Medicine, Ankara, Turkey; †Gülhane Training and Research Hospital, Department of Pediatric Surgery, Ankara, Turkey**

Abstract

Background/Aim. Gastric emptying (GE) scintigraphy provides a physiologic and noninvasive measurement of GE. Although GE scintigraphy has been standardized, preparing a meal is still complex and not practical in daily routine. The aim of the study was to prepare a simple, practical, and easily standardizable semisolid meal and investigate its role in estimating the GE function in of rabbits. **Methods.** In the first part of the study (basal condition), the mixture of the macroaggregated albumin (MAA) labeled with 37 MBq (1 mCi) of technetium-99m (^{99m}Tc) and 40 g of barium sulfate (1g/mL) was applied to animals *via* a nasogastric catheter. A series of images (frame/min, 60 min) in the anterior and posterior projections were dynamically acquired, and the motion was corrected after the radiopharmaceutical application. A few days later, the same rabbits were scanned under the same protocol after a 1 mg atropine injection to simulate gastroparesis condition. Eleven rabbits were included according to inclusion and exclusion criteria, and a total of twenty-two imaging data sets were analyzed for quantification. **Results.** In the basal study, total counts of the mixture decreased from 87,800.83 ± 12,622.76 to 42,733.14 ± 6,591.53 at 30 min and to 13,684.19 ± 1,774.90 at 60 min, and these decreases were statistically significant (p = 0.003). Emptying percentages were 51.39 ± 0.78% at 30 min and 84.32 ± 1.56 at 60 min and were statistically significant (p = 0.003). After intravascular atropine sulfate injection, total counts of the mixture decreased from 84,508.78 ± 11,871.48 to 64,995.18 ± 9,298 at 30 min and to 53,507.17 ± 7,258.98 at 60 min, and these decreases were statistically significant (p = 0.003). Emptying percentages were 23.10 ± 1.11% at 30 min and 36.63 ± 1.42 at 60 min and were statistically significant (p = 0.003). The difference between basal and post-atropine sulfate gastric emptying percentage at 30th (p = 0.003) and 60th (p = 0.003) min was statistically significant. **Conclusion.** The meal, used in this study, is non-nutrient, fatty-free, and semisolid and is easy to prepare and administer. Due to its semisolid nature, it offers a chance to evaluate the quantification of regional and total GE as well as the separate roles of the fundus and antrum.

Key words:
gastric emptying; gastroparesis; meals; rabbits; radionuclide imaging.

Apstrakt

Uvod/Cilj. Scintigrafija pražnjenja želuca (GE) obezbeđuje fiziološko i neinvazivno merenje GE. Iako je scintigrafija GE standardizovana, priprema obroka je i dalje složena i nije praktična u svakodnevnoj praksi. Cilj studije bio je da se pripremi jednostavan, praktičan i lako standardizovan polučvrst obrok i ispita njegova uloga u proceni funkcije GE kod zečeva. **Metode.** U prvom delu studije, mešavina mikroagregata albumina (MAA), obeleženog sa 37 MBq (1 mCi) tehnecijuma-99m (^{99m}Tc) i 40 g barijum sulfata (1 g/mL) davana je životinjama putem nazogastričnog katetera. Dinamska studija (*frame*/min, 60 min) je rađena neposredno nakon davanja obeleženog obroka u anteriornoj i posteriornoj projekciji i korigovana na pokretanje. Nekoliko dana kasnije, isti zečevi su skenirani pod istim protokolom nakon injekcije atropina od 1 mg da bi se simuliralo stanje gastropareze. Ukupno 11 zečeva je uključeno u skladu sa kriterijumima za uključivanje i isključenje, a ukupno 22 studije su analizirane za kvantifikaciju. **Rezultati.** U studiji pod bazalnim uslovima ukupan broj prikupljenih impulsa smanjio se sa 87 800,83 ± 12 622,76 na 42 733,14 ± 6591,53 nakon 30 min i na 13 684,19 ± 1 774,90 nakon 60 min i ova smanjenja bila su statistički značajna (p = 0,03). Procenti pražnjenja iznosili su 51,39 ± 0,78% nakon 30 min i 84,32 ± 1,56 nakon 60 min i bili su statistički značajni (p = 0,003). Nakon injekcije atropin sulfata, ukupan broj prikupljenih impulsa smanjio se sa 84 508,78 ± 11 871,48 na 64 995,18 ± 92 98 nakon 30 min i na 53 507,17 ± 7 258,98 nakon 60 min i ova smanjenja bila su

Correspondence to: Hüseyin Şan, Üniversiteler Mahallesi, Ankara City Hospital, Department of Nuclear Medicine, Bilkent, Çankaya, Ankara, Turkey. E-mail: dr.huseyinsan@yahoo.com

statistički značajna (p = 0,003). Procenti pražnjenja iznosili su 23,10 ± 1,11% nakon 30 min i 36,63 ± 1,42% nakon 60 min i bili su statistički značajni (p = 0,003). Razlika između GE u prvom delu studije i posle primene atropin sulfata u 30. min i 60. min bila je statistički značajna (p = 0,003 u oba vremena). **Zaključak.** Obrok, primenjen u ovoj studiji, nije hranljiv, polučvrst je, bez masnoća i lak za pripremu. Zahvaljujući njegovoj polučvrstoj konzistenciji, pruža priliku da se kvantifikuje regionalno i ukupno GE, kao i procene uloge fundusa i antruma.

Ključne reči:
želudac, pražnjenje; gastropareza; obroci; zečevi; radioizotopsko snimanje.

Introduction

Gastroparesis is a term to describe delayed gastric emptying (GE), and patients often present with complaints of dyspepsia, which can be defined as any discomfort in the upper abdomen [1]. Although a less invasive technique such as wireless capsules has been used lately, placement of a tube or catheter-based probe within the gastrointestinal tract to measure pressure, electrical signal, or pH are general motility studies [2]. In contrast to probe methods, GE scintigraphy is performed with a radiolabeled meal so that the counts measured by the gamma camera are directly proportional to the volume of the meal in the stomach [3, 4]. Therefore, GE scintigraphy provides a physiologic and noninvasive measurement of GE without the need for geometric assumptions about the shape of the stomach. The method is quantifiable because the counts measured by scintigraphy correlate directly with the volume of the meal remaining in the stomach. Besides, compared with radiographic methods, scintigraphy involves low radiation exposure and uses commonly ingested foods rather than radiopaque markers [5].

Since the standard breakfast of ordinary food labeled with chromium-51 was used for GE scintigraphy in 1966, the importance of the meal used to obtain accurate results has been recognized over time. The content of the meal used is one of the most important variables needing standardization because GE depends on meal composition [3]. Chicken liver, pancakes, cheese, milk, oatmeal, honey buns, corn flakes and milk, peanut butter sandwiches, egg salad sandwiches, egg burritos, and McDonald's Egg McMuffins were used as test meals. In addition, different approaches for radionuclide labeling and adding time (before and after cooking) were observed in the literature [6].

In the "Consensus Recommendations for Gastric Emptying Scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine" by Abell et al. [3], it was proposed to use the most universally acceptable meal described by Tougas et al. [4]. One hundred twenty-three normal subjects from 11 medical institutions in the United States, Canada, and Europe were studied by Tougas et al. [4] in their multi-institutional protocol. The meal has a caloric value of 255 kcal (nutritional composition: 72% carbohydrates, 24% protein, 2% fat, and 2% fiber) that consists of a scrambled egg substitute (120 g EggBeater, 60 kcal, equivalent to the volume of two large eggs), two slices of bread (120 kcal), strawberry jam (30 g, 75 kcal) and water (120 mL). In addition, imaging was performed in the anterior and posterior projections at four time points (0, 1, 2, and 4 h), causing patients to stay in nuclear medicine clinics. Although above mentioned low-fat test meal was an important step in GE scintigraphy, preparation of this standardized meal is still complex and not easy from a practical point of view in daily routine. In addition, alternative meals must be considered for patients with egg allergies or intolerance to eggs, patients with gluten-sensitive enteropathy, and patients who report symptom exacerbations after eating lipid-rich foods [3]. Researchers have recommended adding butter (10 g) to the low-fat meal. Additionally, others have proposed Liquid Ensure (Abbott Laboratories, Abbott Park, IL) nutrient supplement or an oatmeal meal [3, 7]. However, it is essential to generate specific normal databases for these alternative meals before clinical utilization. Solid foods and fatty nutrients empty more slowly compared to liquids and foods containing proteins or carbohydrates. As a result, caloric ingredients and the form of the food can affect GE [8–10]. Therefore, both solid and liquid GE studies play an important role in assessing patients with upper gastrointestinal symptoms. Since measurement of simple total GE is often insufficient to explain the complaints of a patient with upper gastrointestinal symptoms, the evaluation of separate roles of the fundus and the antrum seems to be necessary [11]. Consequently, there is a need for an inexpensive, easily prepared meal and a relatively shorter study duration to evaluate both solid and liquid phases of GE in patients with upper gastrointestinal symptoms. In our study, we used a non-nutrient, semisolid meal consisting of technetium-99m (^{99m}Tc) macroaggregated albumin (^{99m}Tc-MAA) mixed with barium sulfate, which may be an alternative to the most universally accepted low-fat, solid meal. Although there are studies using barium to evaluate GE in animals radiographically, to the best of our knowledge, this is the first study using barium mixed with ^{99m}Tc-MAA to evaluate GE in animals scintigraphically. The aim of the study was to establish a practical and easily standardizable semisolid meal and investigate its role in estimating the GE functions of rabbits.

Methods

Animals, meal, and administration

The study was performed between July and December 2016. A total of 14 male, three to four months old, New Zealand white rabbits weighing between 2,000 to 2,500 g (mean: 2,215 g) were included in this study. Being healthy, weighing 2,000–3,000 gr, and three to six months old were the inclusion criteria. Rabbits used in prior experiments or showing signs of illness or rabbits unsuitable for image acquisition and data quantification due to extreme motion were the exclusion criteria. Eleven

rabbits were included according to inclusion and exclusion criteria, and a total of 22 imaging data sets were analyzed for quantification. We preferred to use ^{99m}Tc-MAA instead of ^{99m}Tc-sulfur colloid due to our clinical logistics. In the first part of the study (basal study, without atropine sulfate injection), the MAA (Makro-Albumon®, Medi-Radiopharma, Budapest, Hungary) labeled with 37 mBq (1 mCi) of ^{99m}Tc (Molybdenum-99/Technetium-99m generator, Kamrusepa/Samyoung, Turkey) was mixed with 40 g/40 mL barium sulfate (1 g/mL, Radyobarit solution, Recordati Drug Company, Italy) by using a vortex mixer and the mixture was applied to animals *via* a nasogastric catheter. Rabbits were stabilized, but in order not to affect physiological gastric functions, the rabbits were not anesthetized during the dynamic acquisition of images. A few days later, the same rabbits were scanned under the same protocol immediately after 1 mg atropine sulfate (1 mg/1 mL, Biofarma, Turkey) injection into a marginal ear vein to simulate gastroparesis condition. This dosage was determined by a pilot study, in which the minimum standard dose of atropine necessary to produce a significant delay in GE was established. The Local Ethics Committee for animal studies of the Gülhane Military Medical Academy approved our study protocol (2014/28-14/173).

Acquisition and instrumentation

The subjects were fixed on the wooden plate in a prone position on the imaging table of a two-detector gamma camera (GE, Millennium VG, USA) equipped with a low-energy all-purpose (LEAP) collimator, using a 15% window centered over the 140 keV photopeak in a 128 × 128 matrix. A series of images (frame/min, 60 min) in the anterior and posterior projections were dynamically acquired and motion corrected after the radiopharmaceutical application.

Image evaluation

Depth-corrected total gastric counts [geometric mean: $\sqrt{anterior\ counts\ x\ posterior\ counts}$] from 1st, 30th, and 60th frames of the dynamic images were calculated. Decay correction was also performed. The emptying percent for 30th min and 60th min was calculated *via* the following formula:

[(1st frame total gastric counts) – (30th or 60th frame total gastric counts) / 1st frame total gastric counts] X 100.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD). The Wilcoxon signed-rank test was used to evaluate the significance of the difference between the calculations. Statistical analysis was performed using the IBM SPSS Statistics for Macintosh, Version 26.0. (Armonk, NY: IBM Corp.). Differences were considered significant at $p < 0.05$.

Results

When dynamic images were evaluated qualitatively, the mixture of ^{99m}Tc-MAA and barium sulfate was emptied from the stomach regularly. Total counts decreased from 87,800.83 ± 12,622.76 (a) to 42,733.14 ± 6,591.53 (b) at 30 min and to 13,684.19 ± 1,774.90 (c) at 60 min and these decreases were statistically significant (a to b: $p = 0.003$, a to c: $p = 0.003$, and b to c: $p = 0.003$). GE percentages were 51.39 ± 0.78% at 30 min and 84.32 ± 1.56% at 60 min (Figure 1) and were statistically significant (30 to 60 min: $p = 0.003$). Percentages of gastric retention were 48.60 ± 0.23% at 30 min and 15.67 ± 0.47% at 60 min (Figure 2) and were statistically significant (30 min to 60 min: $p = 0.003$).

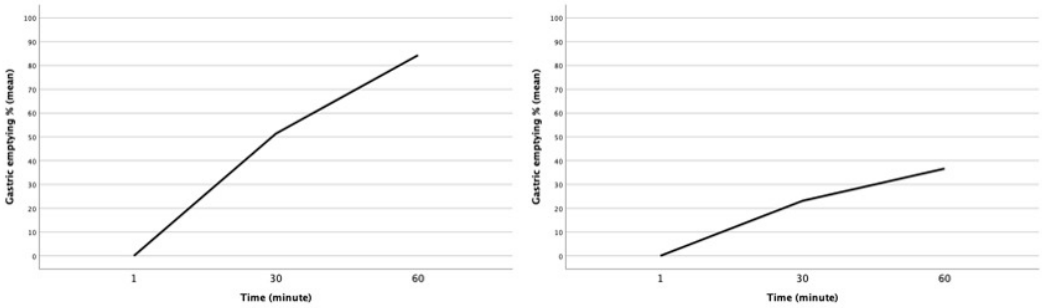


Fig. 1− The line chart shows the percentage of the mean gastric emptying changing over time in the basal study – without atropine sulfate injection (left) and after atropine sulfate injection (right).

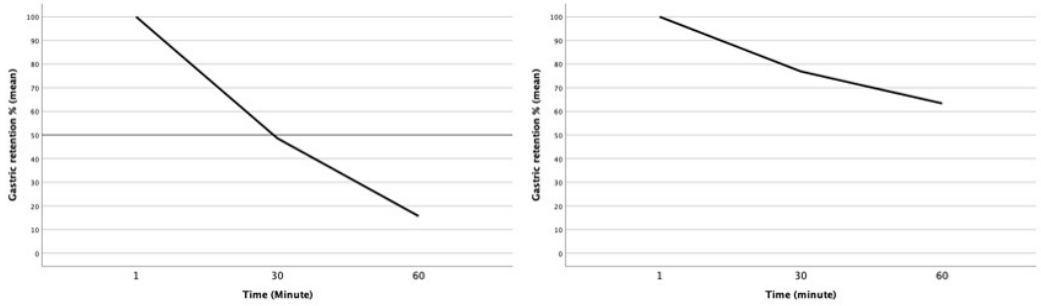


Fig. 2 − The line chart shows the percentage of the mean gastric retention changing over time in the basal study – without atropine sulfate injection (left) and after atropine sulfate injection (right).

After intravascular atropine sulfate injection, the mixture of ^{99m}Tc-MAA and barium sulfate was emptied from the stomach regularly but more slowly. Total counts decreased from 84,508.78 ± 11,871.48 (d) to 64,995.18 ± 9,298.39 (e) at 30 min and to 53,507.17 ± 7,258.98 (f) at 60 min and these decreases were statistically significant (d to e: $p = 0.003$, d to f: $p = 0.003$, and e to f: $p = 0.003$). Emptying percents were 23.10 ± 1.11% at 30 min and 36.63 ± 1.42% at 60 min (Figure 1) and were statistically significant (30 to 60 min: $p = 0.003$). The difference between basal and post-atropine sulfate GE percentage at 30th ($p = 0.003$) and 60th min ($p = 0.003$) was statistically significant. Gastric retention percentages were 76.89 ± 0.33% at 30 min and 63.36 ± 0.42% at 60 min (Figure 2) and were statistically significant (30 min to 60 min: $p = 0.003$).

Irregular regions of interest close to the stomach were manually defined to analyze the total gastric counts on the dynamic image set from anterior and posterior projections (Figure 3).

semisolid mixture was delivered *via* a nasogastric catheter and rapidly dispersed in the stomach.

In GE studies of humans, solid foods transfer from the posteriorly located fundus into the more anteriorly located antrum, and measured counts increase during the anterior projection imaging. To avoid this, dual projection imaging from the anterior and posterior is acquired, and depth-related attenuation correction is performed by calculating the geometric mean. Therefore, the geometric means of decay corrected counts from anterior and posterior projections were used in our study.

Peristaltic contractions transform large solids into smaller 1–2 mm particles with the participation of gastric digestive fluids in the process called 'trituration'. Thus, they discharge monoexponentially at the same rate as the liquids and more rapidly than solids, without lag phase [5, 12]. The liquids require no 'trituration', and liquid-phase GE remained normal until gastroparesis was at an advanced stage. The liquids were less sensitive than solid foods for detecting

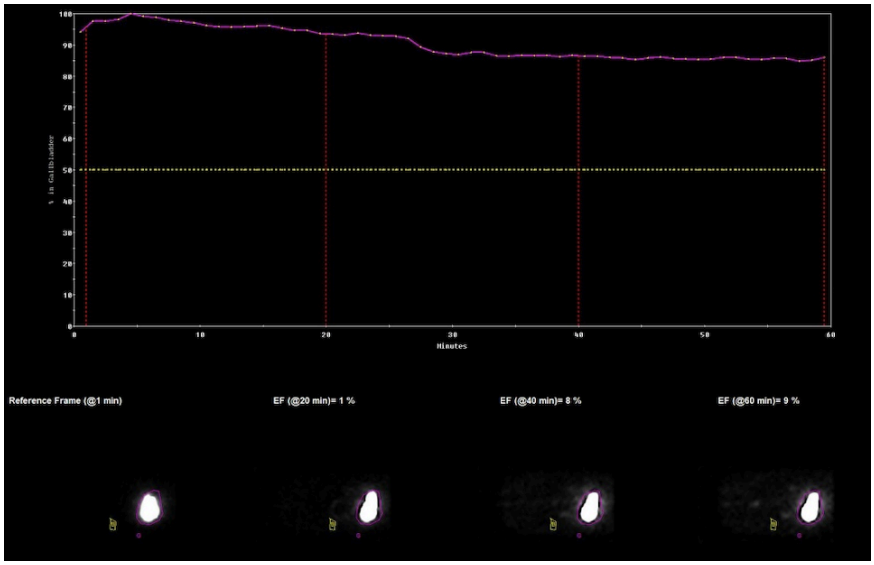


Fig. 3 − An example image dataset of the gastric emptying study in gastroparesis condition after atropine sulfate injection.

Discussion

We prepared a semisolid meal and administered it to rabbits *via* nasogastric catheter to simulate normal gastric function (without atropine sulfate injection) and gastroparesis condition (after atropine sulfate injection) separately and calculated GE rates. Our approach for GE scintigraphy is completely different from the most universally acceptable meal and the protocol, which has the largest normative database [3, 4].

Unlike liquids, solid foods are principally localized in the fundus, referred to as the ‘accommodation response’, and continual slow fundal contractions transfer them to the antrum [5]. Initial and selective localization of solid foods in the fundus can be detected in the first images of a GE study. However, in our study, it was not possible to evaluate the ‘accommodation response’ of the stomach since our early gastroparesis [13], and in order to detect gastroparesis, solid-phase GE scintigraphy is still in use [3]. Conversely, postprandial fullness and early satiety are associated with delayed GE of the liquids [8]. Thus, the liquid-phase GE studies complement solid-phase GE studies because there is a relationship between delayed GE of both and symptoms of postprandial fullness, nausea, and vomiting [5]. In combined dual-isotope solid- and liquid-phase GE, emptying of liquids may be abnormal when the emptying of solids is normal. In one study, 26% of patients were reported to have normal solid-phase GE but delayed liquid-phase GE [14]. Therefore, combined dual-isotope solid- and liquid-phase GE studies are necessary to determine the patients with gastroparesis. For this reason, a semisolid meal with the additional advantage of a low radiation dose may be more convenient and practical for evaluating these patients. Hence, we have mixed ^{99m}Tc-MAA with barium sulfate to prepare an easily

standardizable non-nutrient semisolid meal. Besides, it has been suggested that a non-nutrient, liquid GE study may detect fundal gastric dysmotility [15].

There were no adopted standards for performing GE scintigraphy until a consensus recommendation of a solid-meal GE test, which can provide clinicians with standardized results, was published in 2007 [3]. Normal values were settled not only for the meal but also for the method of acquiring and processing the images in this consensus report; the patients were instructed to stop using medications that affect GE and fasting. Furthermore, since GE studies are complex, the consensus group has identified the limitations of their suggestions, one of them being a need for necessary information on other substitute meals [5]. Our semisolid meal may have a chance as a candidate for a substitute meal in this arena.

The consensus group suggested using a low-fat, solid meal based on normative data from a large multicenter study [4]. The meal comprises 120 g (4 oz) of Eggbeaters (ConAgra Foods) or a generic liquid egg-white equivalent, mixed with 18.5–37.0 MBq (0.5–1.0 mCi) of ^{99m}Tc-sulfur colloid, two slices of white bread, 30 g of strawberry jam, and 120 mL of water. The total energy of the meal is 255 kcal (72% carbohydrates, 24% protein, 2% fat, and 2% fiber). During cooking, ^{99m}Tc-sulfur colloid binds to the egg white. This recipe is not easy to prepare and not practical in the daily routine of a nuclear medicine department. Therefore, we have suggested using the ^{99m}Tc-MAA with a barium sulfate mixture. Since both of them are imaging agents, they are ready to prepare, and the mixture can be administered to the patients easily *via* the oral route. Besides, this semisolid meal may be a useful alternative for patients with egg allergies or intolerance to eggs and patients with gluten-sensitive enteropathy.

The early phase (0–2 h) of a solid GE study mirrors principally fundal function, and the later phase (2–4 h) projects mainly trituration and transfer of the food into the duodenum. Although studies vary in duration from as short as 60 min to as long as 4 h, studies have shown that lengthening the scan to 4 h increases the detection rate of delayed gastric discharge [5, 16]. In the 'Consensus Recommendations for Gastric Emptying Scintigraphy' published by Abell et al. [3], the suggested time points for acquisition of GE scintigraphy images are 0, 60, 120, and 240 min for solid-phase GE scintigraphy. In one study by Guo et al. [16], it was suggested that the 3 h period might be as sensitive as a 4 h study in detecting delayed GE. Further, in another study by Hou et al. [17], it was shown that the 3-h time point is nearly comparable to the 4 h value in detecting patients with delayed GE. Consequently, it may be possible to shorten the duration of the GE study. In contrast to previous studies, we have dynamically imaged the subjects for 60 min to observe the activity passage. Since our meal is semisolid, it has acted as partially liquid, and, therefore, almost half of the activity emptied from the stomach in 30 min. According to our results, our semisolid meal seems to have the potential to give earlier results than the suggested solid meal.

There are incongruities in the quantitative data reported. It has been reported that half-times ($T_{1/2}$ values) of emptying may be potentially less accurate than percentages of retention measured at constant moments in individuals with very prolonged emptying. Furthermore, extrapolation is needed to compute the $T_{1/2}$ value if it was not reached during the study [3]. Studies have shown that percentage of gastric retention has greater sensitivity [10], and it is the most reproducible [11]. Hence, we calculated the percentage of GE and gastric retention at two different time points in our study population.

Abnormal intragastric distribution patterns have also been associated with dyspeptic symptoms due to the separate roles of the fundus and antrum. After the ingestion of solid food, most of it is localized in the upper half of the stomach. This fundal accommodation is a physiologic response to an increase in gastric volume without increasing intragastric pressure. Impaired fundal accommodation can cause dyspeptic symptoms [18]. The invasive barostat testing is the best direct measurement of fundal accommodation [19]; a less invasive water load test can also be used to evaluate the relationship between impaired accommodation and dyspeptic symptoms. Fundal accommodation is best observed in the early images of routine planar GE scintigraphy; the joint report of two different societies suggested evaluating the images for the presence of an abnormal accommodation response [3]. The intragastric distribution of the test meal between the fundus and antrum can be easily evaluated by scintigraphy imaging. However, since our semisolid meal was administered *via* a nasogastric catheter, we could not evaluate the fundal accommodation. If it is administered *via* the oral route, the semisolid properties of our agent may provide information non-invasively regarding the fundal and antral intragastric distribution of activity and quantification of regional GE in humans.

GE depends on the composition of the meal. Solid foods empty more slowly than fluids, and easily digestible soft solids empty more rapidly than solid foods [8]. Further, the emptying of fatty nutrients is slow compared to proteins or carbohydrates. After the inlet of fat nutrients into the duodenum, receptors in the duodenum cause a duodenogastric interaction that induces cholecystokinin release and, as a result, slow entry of the meal into the duodenum occurs [9, 10]. The caloric ingredient and volume of the test meal will also affect GE [3]. Besides, since water drinking has been shown to hinder antral motility after a caloric meal [14], further studies of the physiologic and clinical significance of using a non-alimentary, liquid meal are needed. For this reason, our semisolid mixture is non-nutrient and fat- and calorie-free.

Consensus on performing a solid-phase GE scintigraphy test for clinical practice, based on readily available technology and normative data, which can provide clinicians with standardized results, has been available since 2007 [3, 5, 20].

Study limitations

In this preliminary animal study, the study population was not large enough to get definite conclusions. The

mechanisms conducting gastrointestinal motility in herbivores can be completely different from those in humans, and, therefore, GE scintigraphy results in animals may not reflect the actual physiology in humans. Further, the validity of ^{99m}Tc-MAA mixed barium sulfate as a test meal may not be definitive and need improvement in a large-scale animal study with additional control groups. It would be very appropriate to compare the GE of ^{99m}Tc-MAA mixed barium sulfate with the universally accepted caloric solid test meal. However, ingestion of the universally acceptable caloric test meal by subjects and adjusting the volume of the caloric solid test meal suitable for subjects are the challenges of this process. Another limitation of this study is the significant need to test the labeling stability of ^{99m}Tc-MAA mixed barium sulfate in an acid medium.

Conclusion

The meal used in this study is non-nutrient, fatty-free, and semisolid and is easy to prepare and administer. It seems to have a chance to evaluate the quantification of regional and total GE as well as the separate roles of the fundus and antrum due to its semisolid nature. There are many reasons for the current diversity of imaging and analysis of GE scintigraphy protocols, such as an individual center's meal and analysis preferences, different camera and computer systems, scheduling constraints, and processing software. However, the caloric content and volume of the test meal and consuming water will also affect GE and are important factors to consider. Therefore, further studies of the physiologic and clinical significance of using a non-alimentary, liquid meal are needed. In this preliminary animal study, we aimed to develop the early steps of an alternative approach to the consensus for solid-phase GE scintigraphy, which needs to be improved in larger-scale animal studies.

Conflict of interest

The authors declare no conflict of interest associated with this publication. Additionally, there has been no financial support.

Acknowledgement

In this article, we gratefully acknowledge the help of Alper Özgür Karaçalıoğlu M.D., Mustafa Özdeş Emer M.D., Semra İnce M.D., Nuri Arslan M.D., Murat Kantarcıoğlu, M.D. and Sami Eksert M.D., and Enes Atmaca D.V.M.

REFERENCES

1. *Cuomo R, Sarnelli G, Grasso R, Bruzzese D, Pumpo R, Salomone M*, et al. Functional dyspepsia symptoms, gastric emptying and satiety provocative test: analysis of relationships. Scand J Gastroenterol 2001; 36(10): 1030–6.
2. *Maqbool S, Parkman HP, Friedenberg FK*. Wireless capsule motility: comparison of the SmartPill GI monitoring system with scintigraphy for measuring whole gut transit. Dig Dis Sci 2009; 54(10): 2167–74.
3. *Abell TL, Camilleri M, Donohoe K, Hasler WL, Lin HC, Maurer AH*, et al. Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. Am J Gastroenterol 2008; 103(3): 753–63.
4. *Tougas G, Eaker EY, Abell TL, Abrahamsson H, Boivin M, Chen J*, et al. Assessment of gastric emptying using a low-fat meal: establishment of international control values. Am J Gastroenterol 2000; 95(6): 1456–62.
5. *Maurer AH*. Gastrointestinal Motility, Part 1: Esophageal Transit and Gastric Emptying. J Nucl Med 2015; 56(8): 1229–38.
6. *Farrell MB*. Gastric Emptying Scintigraphy. J Nucl Med Technol 2019; 47(2): 111–9.
7. *Lin HC, Van Citters GW, Zhao XT, Waxman A*. Fat intolerance depends on rapid gastric emptying. Dig Dis Sci 1999; 44(2): 330–5.
8. *Maurer AH, Parkman HP*. Update on gastrointestinal scintigraphy. Semin Nucl Med 2006; 36(2): 110–8.
9. *Kusano M, Minashi K, Maeda M, Shimoyama Y, Kuribayashi S, Higuchi T,* et al. Postprandial water intake inhibits gastric antral motility with increase of cholecystokinin in humans. Scand J Gastroenterol 2005; 40(10): 1176–81.
10. *Schulze K*. Imaging and modelling of digestion in the stomach and the duodenum. Neurogastroenterol Motil 2006; 18(3): 172–83.
11. *Maurer AH*. Gastrointestinal Motility, Part 1: Esophageal Transit and Gastric Emptying. J Nucl Med Technol 2016; 44(1): 1–11.
12. *Siegel JA, Urbain JL, Adler LP, Charkes ND, Maurer AH, Krevsky B*, et al. Biphasic nature of gastric emptying. Gut 1988; 29(1): 85–9.
13. *Couturier O, Bodet-Milin C, Querellou S, Carlier T, Turzo A, Bizais Y*. Gastric scintigraphy with a liquid-solid radiolabelled meal: performances of solid and liquid parameters. Nucl Med Commun 2004; 25(11): 1143–50.
14. *Sachdeva P, Malhotra N, Pathikonda M, Khayyam U, Fisher RS, Maurer AH*, et al. Gastric emptying of solids and liquids for evaluation for gastroparesis. Dig Dis Sci 2011; 56(4): 1138–46.
15. *Ziessman HA, Chander A, Clarke JO, Ramos A, Wahl RL*. The added diagnostic value of liquid gastric emptying compared with solid emptying alone. J Nucl Med 2009; 50(5): 726–31.
16. *Guo JP, Maurer AH, Fisher RS, Parkman HP*. Extending gastric emptying scintigraphy from two to four hours detects more patients with gastroparesis. Dig Dis Sci 2001; 46(1): 24–9.
17. *Hou Q, Lin Z, Dusing R, Gajewski BJ, McCallum RW, Mayo MS*. Optimizing the diagnostic power with gastric emptying scintigraphy at multiple time points. BMC Med Res Methodol 2011; 11(1): 84.
18. *Van den Elzen BD, Boeckxstaens GE*. Review article: a critical view on impaired accommodation as therapeutic target for functional dyspepsia. Aliment Pharmacol Ther 2006; 23(11):1499–510.
19. *Rao SS, Kumar A, Harris B, Brown B, Schulze KS*. Investigation of fundo-antral reflex in human beings. World J Gastroenterol 2005; 11(42): 6676–80.
20. *Maurer A*. Consensus report on gastric emptying: what's needed to prevent tarnishing a gold standard? J Nucl Med 2008; 49(3): 339.

Received on November 21, 2021
Revised on April 20, 2022
Accepted on April 29, 2022
Online First May 2022

UDC: 615.216.2:: 616.314-053.2
DOI: https://doi.org/10.2298/VSP210201022M

A simple calculation of the maximum dose of the local anesthetic in pediatric dentistry with nomogram

Jednostavno izračunavanje maksimalne doze lokalnog anestetika u dečijoj stomatologiji uz pomoć nomograma

Raša Mladenović

University of Kragujevac, Faculty of Medical Sciences, Department of Dentistry, Krgujevac, Serbia

Key words:
anesthetics, local; dose-response relationship, drug; nomograms; pediatric dentistry.

Ključne reči:
anestetici, lokalni; lekovi, odnos doza-reakcija; nomogrami; stomatologija, dečija.

Introduction

The World Health Organization (WHO) defines pain as an unpleasant sensory or emotional experience associated with actual or potential tissue damage. Pain can be controlled by various methods, and the most widely used is to block the transmission of painful impulses by afferent nerve fibers [1]. The application of local anesthetics in the immediate vicinity of nerve fibers temporarily prevents the transmission of painful impulses, as long as its concentration at the site of action is sufficient [2]. Local anesthetics are the most commonly used drugs in dental practice and are used to eliminate pain during a variety of dental interventions [3]. To enhance the effect, a vasoconstrictor is added to local anesthetics, thus prolonging the duration of local anesthesia [4].

The most commonly used local anesthetic in pediatric dentistry is lidocaine, and in recent times, articaine has found increasing use. Lidocaine is the first amide-linked local anesthetic and has been the "gold standard" among local anesthetics for more than 50 years. According to the recommendations of the American Academy of Pediatric Dentistry (AAPD), it is used in infiltration and conduction anesthesia in a concentration of 2%, with or without vasoconstrictors. The maximum single dose of lidocaine for children is 4.4 mg/kg body weight, and the total dose should not exceed 300 mg [5]. Articaine is the fastest metabolized anesthetic of all anesthetics used in dentistry, making it less toxic and can be administered in higher doses. Articaine diffuses better into soft and bone tissues, which ensures good anesthesia. According to the recommendations of the AAPD, it is used in infiltration and conduction anesthesia in a concentration of 4%, with or without vasoconstrictors. The maximum single dose of articaine for children is 7.0 mg/kg body weight, and the total administered dose should not exceed 500 mg [5].

Toxicity of local anesthetics

Toxicity of a local anesthetic means the property of causing side effects in the body. Regardless of the method of applying the anesthetic, the anesthetic after application gradually passes from the place of deposition into the circulation and is distributed through the blood throughout the body before it is excreted. The concentration of anesthetic in plasma depends on the balance between the degree of resorption from the site of application and the rate of its detoxification. Toxic concentrations of anesthetics in the blood can occur if this balance is disturbed. This most often occurs as a consequence of an overdose of local anesthetic, extremely rapid application and resorption, unusually slow detoxification, or slow excretion [2]. If properly applied, in the appropriate dose, and in the right place, local anesthetics do not cause side effects [6].

The manifestations of the local anesthetic systemic toxicity (LAST) may appear 30 sec to 60 min after injection, but they typically appear within 1 to 5 min. The manifestations vary widely but are usually consistent with central nervous system (CNS) excitement (e.g., oral numbness, metallic taste, dizziness or lightheadedness, drowsiness or disorientation, visual or auditory disturbances) [7]. There are five

Correspondence to: Raša Mladenović, University of Kragujevac, Faculty of Medical Sciences, Department of Dentistry, 34 000 Kragujevac, Serbia. E-mail: rasa.mladenovic@med.pr.ac.rs

categories of the LAST manifestation: CNS, cardiovascular, hematologic, allergic, and local tissue responses [8].

Clinicians should identify the risks associated with the use of anesthetics and understand the maximum recommended doses of local anesthetics [9]. The amount of local anesthetic that can be used depends on the type of anesthetic, the patient's health, and the age of the child. Treatment planning and accurate documentation of local anesthetics require careful calculation and dose recording. Errors in calculating the dose of local anesthetics are common due to the widespread use of these agents and the fact that the concentrations of these agents are often presented in non-standard units. Different methods of calculating the dose of a drug have different strengths and weaknesses, and no single method can guarantee error-free calculation. Formulas for calculating the maximum dose of local anesthetics have been described in the literature, but they represent a rather complicated way that takes a lot of time for the dentist [10, 11].

Nomogram

A nomogram is a simple graphical tool on which, without calculation, one can read the result of calculation operations with given numbers (an appropriate nomogram is constructed for each type of problem), i.e., graphical representation of a mathematical formula. Nomograms are widely used in engineering, medicine, statistics, and accounting [12]. Although they have been largely replaced by electronic computers and calculators, nomograms retain numerous advantages over electronic devices. They are easy to use, extremely cheap, and do not require a source of electricity [13].

The nomogram for calculating the maximum dose of local anesthetic enables quick cross-checking of the calculation based on the patient's age or body weight. They are of special importance in the application of local anesthesia in children in order to reduce the chance of toxicity and prolonged duration of anesthesia, which can lead to self-injury of the tongue or soft tissues [13–15].

By drawing a line across the axis, the calculation is performed and a permanent record for medical documentation is provided (Figure 1). The nomogram can be enlarged to ensure greater readability, but it is necessary to keep the original proportions (ratio of width and height) so as not to produce erroneous calculations. Nomograms in Serbian are free for download and easy to use in everyday clinical practice [16].

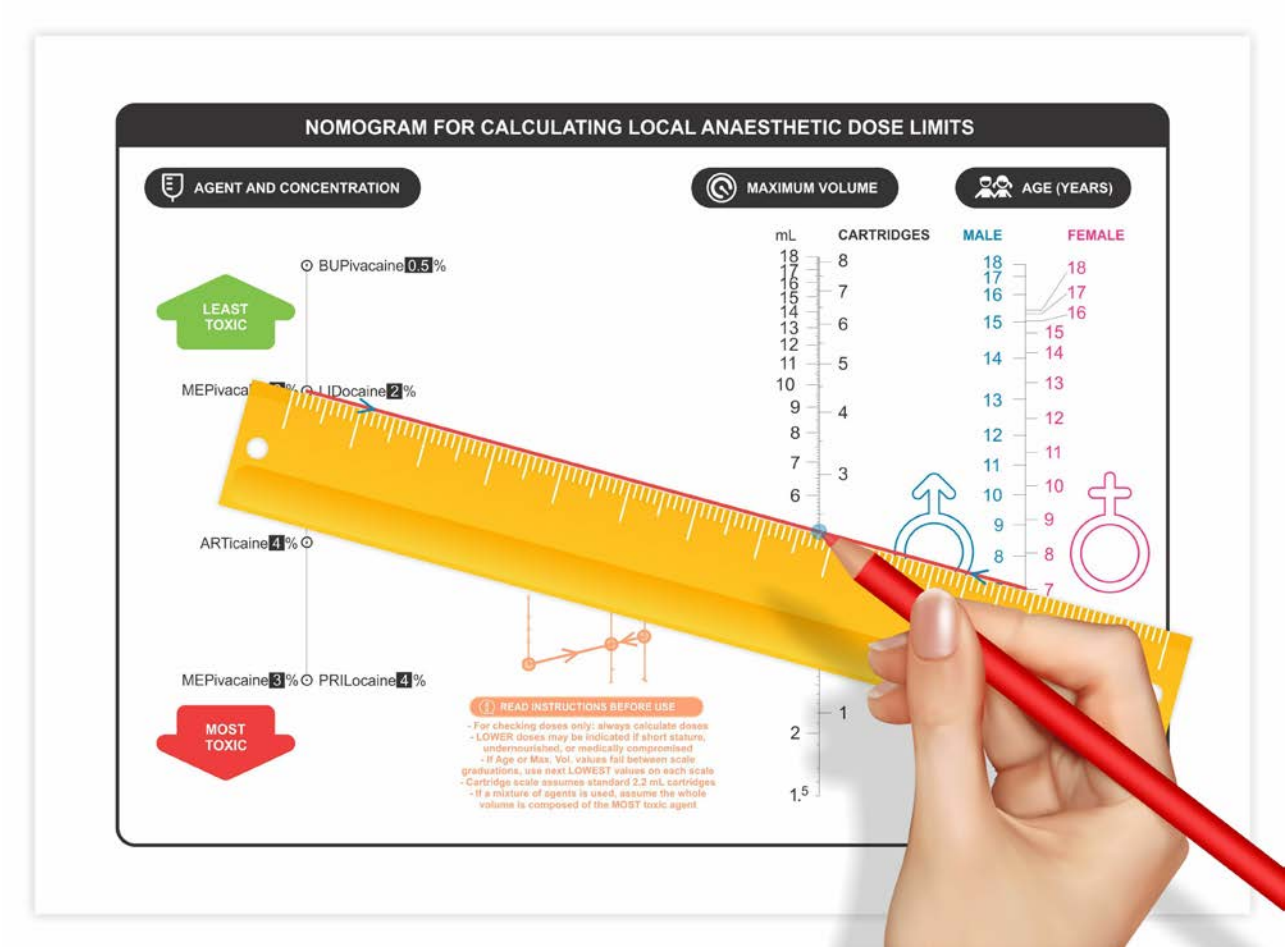


Fig. 1 – How to use the nomogram: by drawing a line from the selected anesthetic on the one hand and the patient's age (or bodyweight) on the other, the value of the maximum dose of the selected anesthetic solution is obtained. Adapted by Raša Mladenović with permission and curtesy of the authors Williams DJ and Walker DJ [14].

Conclusion

This article presents a simple and practical tool that will make it easier for dentists to work with children to calculate the maximum dose of local anesthetic, thus potentially reducing the frequency of toxicity of local anesthetics and improving patient safety.

Acknowledgement

The author would like to thank the designers of the original nomograms (Dr. David Williams and Dr. Jason Walker) for their permission and support in the preparation of the Serbian version.

R E F E R E N C E S

1. *Bennett CR.* Monheim's local anesthesia and pain control in dental practice. 7th ed. St. Louis, Missouri: C.V. Mosby Co; 1984.
2. *Todorović Lj, Petrović V, Avramović K, Stajčić Z.* Anesthesia in dentistry. Belgrade: Zavod za udžbenike i nastavna sredstva; 1990.
3. *Mladenovic R, Pereira L, Djordjevic FS, Vlahović Z, Mladenović K, Cvetanović A*, et al. The use of mobile-aided learning in education of local anesthesia for the inferior alveolar nerve block.Vojnosanit Pregl 2020; 77(8): 839–43.
4. *Ličin I.* Farmacology for dentists. Zagreb: Medicinska naklada; 2011. (Croatian)
5. *American Academy of Pediatric Dentistry.* Use of local anesthesia for pediatric dental patients. Pediatr Dent 2015; 37(Special issue): 199–205.
6. *Slavković D, Kostić Z, Milošević N, Rančić N, Dragojević-Simić V, Stamenković D,* et al. Surgical wound infiltration by local anesthetic: Wound infiltration. Serb J Anest Inten Ther 2019; 41(7): 143–55. (Serbian, English)
7. *Wadlund DL.* Local Anesthetic Systemic Toxicity. AORN J 2017; 106(5): 367–77.
8. *Golembiewski J, Dasta J.* Evolving role of local anesthetics in managing postsurgical analgesia. Clin Ther 2015; 37(6): 1354–71.
9. *American Academy of Pediatric Dentistry.* Use of local anesthesia for pediatric dental patients. The Reference Manual of Pediatric Dentistry. Chicago, Ill.: American Academy of Pediatric Dentistry; 2020. p. 318–23.
10. *Malamed SF.* Handbook of local anesthesia. 6th ed. St Louis: Mosby; 2013.
11. *Rocha JF, Fan S, Dias Ribeiro E.* A new method to calculate the maximum dose of local anesthetics. Arch Health Invest 2020; 9(3): 210.
12. *Evesham H.* The History and development of nomography. Boston, MA, USA: Docent Press; 2011.
13. *Williams D, Splaver T, Walker J.* A Nomogram for Calculation of Maximum Recommended Dose by Volume of Local Anesthetic in Pediatric Dentistry. Pediatr Dent 2017; 39(2): 150–4.
14. *Williams DJ, Walker JD.* A nomogram for calculating the maximum dose of local anaesthetic. Anaesthesia 2014; 69(8): 847–53.
15. *Walker JD, Summers A, Williams DJ..* A nomogram to calculate the maximum dose of local anaesthetic in a paediatric dental setting. Br Dent J 2015; 218(8): 469–71.
16. Nomogram for calculating the maximum dose of local anesthetic [Internet]. www.russiart.info. 2021 [cited 29 January 2021]. Available from: https://russiart.info/nomogram/

Received on February 1, 2021
Revised on March 2, 2021
Accepted on March 3, 2021
Online First March 2021

CASE REPORTS

UDC: 616.36-08
DOI: https://doi.org/10.2298/VSP201116027A

The use of a single pass albumin dialysis for the management of liver failure

Upotreba jednoprotočne albuminske dijalize u lečenju insuficijencije jetre

Tijana Azaševac*†, Violeta Knežević*†, Dejan Ćelić*†, Bojana Ljubičić‡, Tanja Lakić†§, Igor Mitić*†

University Clinical Center of Vojvodina, *Clinic for Nephrology and Clinical Immunology, ‡Department of Emergency Internal Medicine, Emergency Center, §Department of Pathology and Histology, Novi Sad, Serbia; †University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia

Abstract

Introduction. A single pass albumin dialysis (SPAD) is a form of extracorporeal liver support system for removing albumin-bound toxins and water-soluble substances that accumulate in liver failure (LF). **Case report.** We presented three patients hospitalized for LF and treated using the SPAD at the University Clinical Center of Vojvodina, Serbia, from 2018 to 2019. Two of the patients presented with acute LF and one with acute-on-chronic LF. A total of 6 SPAD sessions were performed on each patient, resulting in decreased serum bilirubin and bile acid levels and hepatic encephalopathy grade. On discharge from the hospital, the liver function was improved in all the patients. **Conclusion.** SPAD removes the hepatotoxic substances without improvement of synthetic liver function. It represents a supportive treatment for LF patients who do not respond to the standard of care, offering a longer time for bridging to organ transplantation or spontaneous recovery of the liver function.

Key words:
albumins; liver failure, acute; regeneration; renal dialysis; treatment outcome.

Apstrakt

Uvod. Jednoprotočna albuminska dijaliza (JPAD) je vrsta ekstrakorporalne dijalize kojom se iz krvi obolelih od insuficijencije jetre (IJ) uklanjaju toksini vezani za albumine i hidrosolubilne supstance. **Prikaz bolesnika.** Prikazali smo tri bolesnika sa IJ lečena JPAD metodom u Univerzitetskom kliničkom centru Vojvodine, Srbija od 2018. do 2019. godine. Dva bolesnika su imala akutnu IJ, a jedan akutizaciju hronične lezije jetre. Kod svakog bolesnika sprovedeno je 6 JPAD procedura, koje su dovele do smanjenja nivoa bilirubina i žučnih kiselina u serumu, kao i stepena hepatične encefalopatije. Na otpustu iz bolnice, kod svih bolesnika došlo je do oporavka funkcije jetre. **Zaključak.** JPAD vrši uklanjanje hepatotoksičnih supstanci, bez poboljšanja sintetske funkcije jetre. Koristi se kao suportivan tretman bolesnika sa IJ koji ne reaguju na standardni način lečenja i obezbeđuje stabilizaciju funkcije jetre do njenog spontanog oporavka ili transplantacije.

Ključne reči:
albumini; jetra, insuficijencija, akutna; regeneracija; dijaliza; lečenje, ishod.

Introduction

There is a growing incidence of liver diseases worldwide, accounting for approximately two million deaths per year. Liver failure (LF) is characterized by the lack of metabolic and regulatory functions, resulting in life-threatening complications, such as bleeding, impaired renal function, hepatic encephalopathy (HE) or brain edema, cardiovascular disorders, and immune dysfunction, which eventually may lead to multiple organ failure and death [1, 2]. It is important to identify patients who are not likely to progress after receiving standard medical therapy (SMT) and, accordingly, prepare them for the possibility of liver transplantation. In order to function as a bridge therapy until the recovery of liver function or organ transplantation, extracorporeal liver support systems are used. Extracorporeal albumin dialysis (ECAD) is a mechanical, completely artificial support system that presents detoxification systems of many potential liver toxins which use albumin as a transport protein, such as hydrophobic bile acids, bilirubin, and serum nitric oxide,

Correspondence to: Tijana Azaševac, University of Novi Sad, Faculty of Medicine, University Clinical Center of Vojvodina, Clinic for Nephrology and Clinical Immunology, 1–7 Hajduk Veljkova street, 21 000 Novi Sad, Serbia. E-mail: tijana.azasevac@mf.uns.ac.rs

even though it has not been shown to have an effect on synthetic liver function [3]. Several ECAD systems are in use, but the best-known and the most commonly used are the Molecular Adsorbent Recirculating System (MARS™), the Fractionated Plasma Separation and Adsorption System (Prometheus®), and the Single Pass Albumin Dialysis (SPAD).

Case report

After being admitted to the hospital, patients who were treated with SMT received parenteral fluids (0.9% of sodium chloride solution and 10% of glucose solution) for volume resuscitation and maintenance of normoglycemic state, in addition to proton pump inhibitor [pantoprazole 40 mg per 12 hours (hrs)] for stress ulcer prophylaxis, fresh frozen plasma (10 mL per kilogram of body weight) supplemented with 10 mg of vitamin K prior to the placement of central venous lines, and l-ornithine-l-aspartate for treating HE.

SPAD was performed with a machine for continuous renal replacement therapy (Multifiltrate, Fresenius Medical Care, Bad Homburg, Germany) using high-flux polysulfone membranes (Ultraflux® EMIC2 and AV1000S, Fresenius Medical Care). The standard dialysate solution (multiBIC, Fresenius Medical Care) was enriched with 20% human albumin (CSL Behring GMBH, Marburg, Germany) to a final concentration of 4% albumin in the first case [dialysate flow of 700 mL/h for a seven-h treatment] and 3% albumin (dialysate flow of 1,000 mL/h for a five-h treatment) in the last two cases. Prior to initiation of SPAD, all the patients had a double-lumen hemodialysis catheter inserted into the right internal jugular vein. Systemic anticoagulation was performed by infusion rates of unfractionated heparin. Blood sampling was performed within 30 min before the start and after the termination of the treatment.

Case 1

A 30-year-old male patient started taking anabolic steroids (stanazolol and oxymetholone) one month before the onset of the disease in order to increase his muscle mass. Two weeks prior to admission, the patient had a diffuse maculopapular rash with itching that did not resolve after taking antihistamines and became icteric. On physical examination, the patient was afebrile, oriented, his arterial blood pressure (ABP) was 140/80 mmHg, heart rate (HR) 80 beats per min, with respiratory rate (RR) of 16 breaths per min, Glasgow Coma Scale (GCS) score of 15, and Acute Physiology and Chronic Health Evaluation (APACHE) II score 2. Abdominal ultrasonography and computed tomography (CT) showed hepatomegaly (17 cm) with signs of hepatic steatosis. Gastroduodenoscopy revealed normal findings except for chronic gastric changes. Magnetic resonance cholangiopancreatography showed irregular contour of the bile ducts in the left and right lobes, and the first portion of the common hepatic duct of 7 mm in diameter, without signs of dilatation which could be a sign of edema. The ethylic, viral, metabolic, immunological, and neoplastic etiologies for the liver disease were excluded. Despite the applied SMT, LF persisted and six SPAD sessions were performed. Bilirubin levels during SPAD procedures of all patients are shown in Figure 1. After the SPAD treatment, a liver biopsy was performed, showing the intrahepatic cholestasis that could be caused by drug-induced acute toxic liver damage (Figure 2). The patient was discharged one month later with a regression of jaundice and significant bilirubin reduction. The characteristics of the present case are given in Tables 1 and 2.

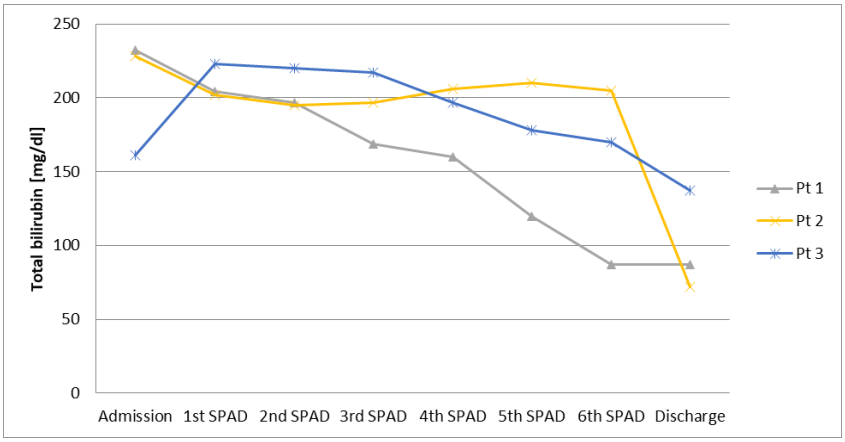


Fig. 1 – Total bilirubin levels during the single pass albumin dialysis (SPAD) sessions. Pt – patient.

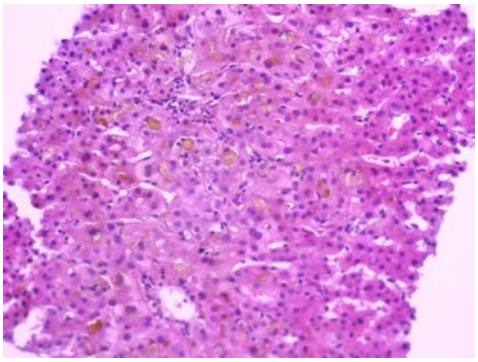


Fig. 2 – Pathohistological finding of liver biopsy of the first patient: the intrahepatic cholestasis (hematoxylin and eosin, ×200).

Table 1

Clinical characteristics of patients and their biochemical parameters on admission and discharge from hospital

Parameter	Pt. 1		Pt. 2		Pt. 3	
Type of liver failure	acute		acute		acute-on-chronic	
Etiology of liver disease	drug intoxication		viral (hepatitis B)		viral (hepatitis A)	
Laboratory finding	adm.	disc.	adm.	disc.	adm.	disc.
AST (IU/L)	27	48	> 7,000	77	7,331	90
ALT (IU/L)	71	167	> 7,000	55	8,808	106
GGT (IU/L)	46	65	62	41	281	282
total bilirubin (mg/dL)	232	87	228	72	161	137
direct bilirubin (mg/dL)	180	71	132	48	122	100
INR	1.5*	0.9	5.3	1.1	2.3	1.0
CRP (mg/dL)	1.5	1	27.6	4	16.2	23.2

***Value measured after two doses of fresh frozen plasma and 10 mg of vitamin K which were administered at admission.**

AST – aspartate transaminase; ALT – alanine aminotransferase; GGT – gamma-glutamyltransferase; INR – international normalized ratio; CRP – C-reactive protein; SPAD – single pass albumin dialysis; adm – admission; disc – discharge; Pt – patient.

Table 2

Biochemical parameters before the first and after the last single pass albumin dialysis (SPAD) procedures

Parameter	Pt. 1		Pt. 2		Pt. 3	
	before SPAD	after SPAD	before SPAD	after SPAD	before SPAD	after SPAD
Hgb (g/L)	158	147	122	90	126	132
Plt ($\times 10^{-9}$/L)	296	254	150	43	181	210
AST (IU/L)	71	167	102	58	4463	190
ALT (IU/L)	50	54	711	92	6893	1755
Total bilirubin (mg/dL)	232	87.2	202.6	193	183	178
Direct bilirubin (mg/dL)	180	71	104	106	141	134
Bile acids (μmol/L)	293	207	156	104	262	154
Albumin (mg/dL)	42	35	27	31	33	31
Urea (mmol/L)	5.8	6.7	2.5	4.4	3.5	8.4
Creatinine (μmol/L)	93	56	26	32	29	68

Hgb – hemoglobin; Plt – platelets; AST – aspartate transaminase; ALT – alanine aminotransferase; Pt – patient.

Case 2

A 49-year-old female patient was admitted to the hospital with fever, jaundice, and abdominal pain. Physical examination revealed the patient to be oriented, without fever, with ABP of 109/53 mmHg, HR 82/min, RR 18/min, painful sensation in the abdomen, GCS score 15, and APACHE II score 2. Diagnosis of acute LF caused by the hepatitis B virus (HBV) was made. Additionally, SMT was initiated together with the nucleoside analogue reverse transcriptase inhibitor, lamivudine, with a daily dose of 100 mg. Despite the applied SMT, on the fifth day of hospitalization, HE developed (stage II) with a worsening coagulation disorder and an increase in bilirubin and bile acid levels. The patient was transferred to the intensive care unit (ICU), and SPAD procedures were initiated. Preparation for the liver transplantation was carried out, but on the 10th day of hospitalization, HE progressed to stage IV, GCS score was 8, and the Model for End-stage Liver disease (MELD) score of 37 points was calculated. Mechanical ventilation was initiated, with the continuation of daily SPAD procedures. Given the performed endocranial CT scan, the signs of diffuse cerebral edema without altered density in the supratentorial and infratentorial regions have been shown (Figure 3). After 6 SPAD sessions, the treatment was discontinued due to the clinical improvement, but jaundice and elevated bilirubin values persisted. The patient was extubated on the 15th day of hospitalization. On the 20th day, she was referred to the Clinic for Infectious Diseases and, after 72 days of hospitalization, discharged with improved laboratory test results.

Case 3

A 59-year-old female patient was hospitalized due to nausea, vomiting, frequent diarrhea, and jaundice that occurred seven days before admission to the Clinic for Infectious Diseases. She has been treated for migraine with analgetics (ibuprofen, diclofenac) and Avamigran® (ergotamine, mecloxamine, camilofin, caffeine, propifenazone) for years. Moreover, she has been acquainted with the elevated aminotransferase levels for ten years but has not been treated for that condition. Physical examination revealed a communicative but disoriented patient, without fiber, with ABP of 130/80 mmHg, HR 100/min, RR 20/min, yellowish discol-

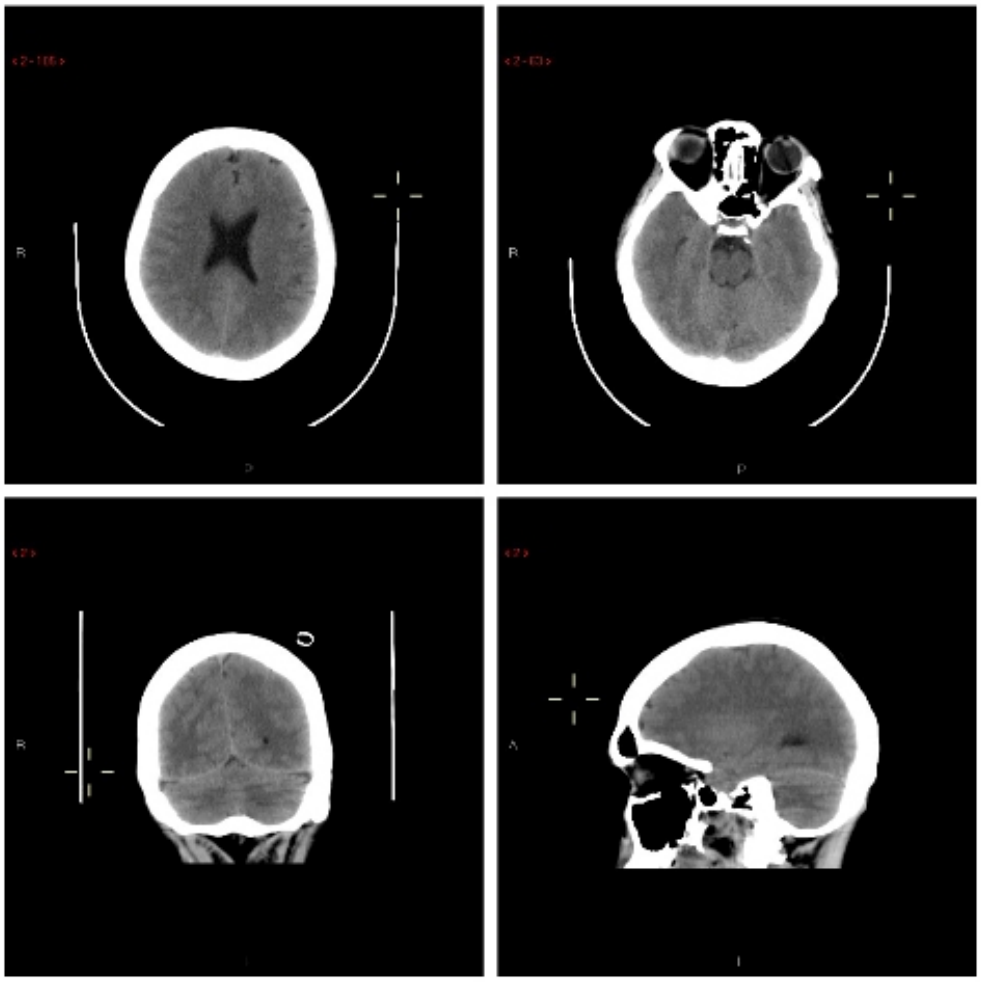


Fig. 3 – Endocranial computed tomography (CT) scan of the second patient shows the signs of a diffuse central edema without altered density in supratentorial and infratentorial regions.

oration of the skin and sclera, painful sensation in the abdomen, GCS score 15 and APACHE II score 4. Diagnosis of an acute hepatitis A virus (HAV) was confirmed by the detection of IgM anti-HAV antibodies and positive epidemiological data (the patient's husband was also diagnosed with acute hepatitis A and had positive IgM anti-HAV antibodies).

The patient was treated with SMT, but on the third day of hospitalization, HE progressed to stage III. Subsequently, she was transferred to ICU, where SPAD sessions were started. After 6 sessions, the HE withdrew, and she was transferred back to the Clinic for Infectious Diseases. Eventually, she was discharged after 37 days with an improved hepatogram and normalization of the coagulation parameters.

Discussion

The use of the ECAD can contribute to the effective removal of albumin-bound toxins, but these procedures cannot substitute the synthetic liver function [2]. Given the fact that the greatest clinical experience in the field of ECAD refers to MARS, SPAD has equal effectiveness in reducing the level of bilirubin as MARS, as well as the same safety profile, while MARS has shown the advantage in reducing the bile acid, creatinine, and urea [4]. Taking into account that the level of bilirubin represents the surrogate marker for protein-bound toxins and correlates positively with the patients' mortality, the greatest significance of the SPAD is in their removal [4–8].

We presented a series of three cases of hospitalized LF patients (1 male and 2 females) who were treated with ECAD by the modality of SPAD between 2018 and 2019. To the best of our knowledge, this type of case series has not been presented in this region before.

Two of our patients had acute LF (ALF), and although one patient was previously diagnosed with liver disease, she consequently developed acute-on-chronic LF (AoCLF). Given the uncertain etiology of the previous liver lesion, the diagnostic criteria for AoCLF remained uncertain without pathohistological findings of liver tissue in that patient. Moreover, prolonged use of migraine medications and elevations of aminotransferase levels in patient history suggested drug-or toxin-induced liver damage, while autoimmune hepatitis could not be ruled out. Less than 1% of acute HAV infections result in ALF, mostly in patients with pre-existing liver disease who are more susceptible to developing an AoCLF in cases of HAV infection [9].

According to the literature data, patients with ALF and AoCLF were frequently eligible for ECAD treatments, comprising three-quarters of implemented ECAD. The viral liver infection was determined in two patients – ALF caused by HBV and AoCLF caused by HAV. The treatment for LF caused by HBV and hepatitis C viruses, mainly by MARS, was described in literature [3], whilst Lee et al. [8] showed the case study of patients with ALF, caused by HAV, which was successfully treated by the SPAD. The use of lamivudine, a potent inhibitor of HBV replication that causes a rapid decline in serum HBV DNA levels, is indicated in patients with a severe form of acute HBV infection, but even then, a small portion of patients with an overwhelming immune response to the virus develop ALF with an expected poor prognosis without liver transplantation and transplant-free survival rates from 26% to 53% [9].

The cause of ALF in one of our cases was the use of anabolic steroids that include a 17-alpha alkyl group that has been linked to the development of jaundice. The literature describes four cases of successful MARS treatment of anabolic steroid-induced liver failure, but, according to our knowledge, this was the first case where the SPAD was used for this indication [10].

The decrease in the bilirubin level has been verified, which correlates to the literature data regarding SPAD [4, 5, 8, 11]. Furthermore, the meta-analysis, which has included ten randomized clinical trials, has shown that the use of the ECAD, as opposed to the isolated application of SMT, has achieved a significant net decrease in a total serum bilirubin level of 8.0 mg/dL [7]. Progressive jaundice and coagulation disorders have been dominant in all the patients, while HE was mild in one patient. A complete withdrawal of HE, including patients with the HE of III and IV grade, was noted after the SPAD treatment, and similar results of the SPAD effect have been presented in literature [4, 12].

Schmuck et al. [11] have shown in an *in vitro* model that optimal detoxification efficiency for albumin-bound substances (bilirubin and bile acids) can be reached with the 3% concentration of albumin in the dialysate and a flow rate of 1,000 mL/h. We used 3% and 4% albumin dialysate solution, as well as 700 mL/h and 1,000 mL/h dialysate flow rate; both albumin concentrations in dialysate solution and dialysate flow rates proved successful in our case series.

Mild thrombocytopenia has been observed in one patient, whereas all other causes of thrombocytopenia were excluded. A meta-analysis conducted by Tsiposis et al. [7] has determined that the application of ECAD has not led to a significant net decrease in the mean platelet count in patients treated by ECAD compared with patients treated with SMT, while another meta-analysis has shown that the use of ECAD was associated with increased risk of thrombocytopenia [12].

All the patients have been discharged from the hospital with liver function improved. The meta-analysis of Alshamsi et al. [12] that included patients with ALF and AoCLF showed that ECAD tended to reduce mortality in these patients.

Conclusion

As one of the ECAD techniques, SPAD has the capacity to remove the hepatotoxic substances without improvement of synthetic liver function. It provides supportive treatment for patients with LF who do not respond to the standard of care and can be used either as a bridge to transplant or for spontaneous recovery of the liver function. However, further prospective studies and meta-analyses are needed to evaluate the efficacy and safety of the SPAD and other ECAD techniques used as "salvage" therapy in LF patients.

R E F E R E N C E S

1. *Asrani SK, Devarbhavi H, Eaton J, Kamath PS*. Burden of liver diseases in the world. J Hepatol 2019; 70(1): 151–71.
2. *Lee KCL, Stadlbauer V, Jalan R*. Extracorporeal liver support devices for listed patients. Liver Transpl 2016; 22(6): 839–48.
3. *Mitzner S*. Albumin dialysis: an update. Curr Opin Nephrol Hypertens 2007; 16(6): 589–95.
4. *Sponholz C, Matthes K, Rupp D, Backaus W, Klammt S, Karailieva D*, et al. Molecular adsorbent recirculating system and single-pass albumin dialysis in liver failure – a prospective, randomized crossover study. Crit Care 2016; 20: 2.
5. *Benyoub K, Muller M, Bonnet A, Simon R, Gazon M, Duperret S*, et al. Amounts of bile acids and bilirubin removed during singlepass albumin dialysis in patients with liver failure. Ther Apheresis Dial 2011; 15(5): 504–10.
6. *Piechota M, Piechota A, Misztal M, Bernas S, Pietraszek-Grzywaczewska I*. An evaluation of the usefulness of extracorporeal liver support techniques in patients with severe liver dysfunction. Arch Med Sci 2019; 15(1): 99–112.
7. *Tsipotis E, Shuja A, Jaber BL*. Albumin Dialysis for Liver Failure: A Systematic Review. Adv Chronic Kidney Dis 2015; 22(5): 38–90.
8. *Lee SW, Joo WC, Kwon SH, Lee JW, Song JH, Kim MJ*. The Use of Single-pass Albumin Dialysis to Correct Severe Hyperbilirubinemia in Acute Hepatitis A: A Case Report. Korean J Nephrol 2010; 29(2): 260–4.
9. *Manka P, Verheyen J, Gerken G, Canbay A*. Liver Failure due to Acute Viral Hepatitis (A-E). Visc Med 2016; 32(2): 80–5.
10. *Diaz FC, Saez-Gonzalez E, Benlloch S, Alvarez-Sotomayor D, Conde I, Polo B*, et al. Albumin dialysis with MARS for the treatment of anabolic steroid-induced cholestasis. Ann Hepatol 2016; 15(6): 939–43.
11. *Schmuck RB, Nawrot GH, Fikatas P, Reutzel-Selke A, Pratschke J, Sauer IM*. Single pass albumin dialysis-a dose-finding study to define optimal albumin concentration and dialysate flow. Artif Organs 2017; 41(2): 153–61.
12. *Alshamsi F, Alshammari K, Belley-Cote E, Dionne J, Albrahim T, Albudoor B*, et al. Extracorporeal liver support in patients with liver failure: a systematic review and meta-analysis of randomized controlled trials. Intensive Care Med 2020; 46(1): 1–16.

Received on November 16, 2020
Revised on March 7, 2021
Accepted on March 11, 2021
Online First March, 2021

CASE REPORT

UDC: 618.14-006-085.849-06:616-006.31
DOI: https://doi.org/10.2298/VSP201207028J

The role of c-MYC expression in the diagnostic and clinical confirmation of radiation-induced angiosarcoma

Uloga ekspresije c-MYC u dijagnozi i kliničkoj potvrdi angiosarkoma izazvanog zračenjem

Luka Jovanović*†, Predrag Filipović*†, Jelena Dedović Stojaković*†, Zorka Milovanović‡, Milica Labudović Borović†, Jasminka Tanasijević§, Predrag Petrašinović*†, Tamara Marinković*†, Vesna Plešinac Karapandžić*†

Institute for Oncology and Radiology of Serbia, *Department of Radiation Oncology, ‡Department of Pathology, Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia; §General Hospital Studenica, Kraljevo, Serbia

Abstract

Introduction. Angiosarcomas (ASs) arising from vascular tissue, account for 3.3% of all sarcomas and have a poor prognosis. Radiation-induced AS is a rare late complication of radiotherapy (RT) treatment and is characterized by a gene expression profile such as amplification of the MYC oncogene, by which we can distinguish primary from secondary induced tumor. **Case report.** For a 77-year-old female patient with early-stage endometrial adenocarcinoma, a radical hysterectomy with bilateral salpingo-oophorectomy was initially done. According to pathological risk factors, the postoperative external beam conformal RT (CRT) of the pelvis was administered with concomitant brachytherapy. Six years after the treatment, on the anterior abdominal wall, in the region of the postoperative irradiation field and surgical scar, an infiltrative AS of the skin and subcutaneous adipose tissue was histologically confirmed. The patient received six cycles of mono-adriamycin chemotherapy with verified partial regression. Additional immunohistochemical analysis (IHC) of c-MYC, Ki67, and CD34 expression showed a high proliferative index (Ki67 around 60%) and c-MYC positivity indicating the molecular pattern of radiation-induced AS. Furthermore, the high proliferative index could explain the positive response to chemotherapy. **Conclusion.** The novel postoperative RT techniques provide better survival and local control in risk-endometrial cancer groups with a decrease in irradiation complications. These patients with longer survival are at a higher risk of developing radiation-induced tumors as late side-effects of RT. When assessing the probability of radiation-induced AS, IHC analysis of c-MYC expression could distinguish secondary from other AS if Cahan's criteria are fulfilled.

Key words:
adenocarcinoma; genetics; immunohistochemistry; neoplasms, radiation-induced; radiotherapy, adjuvant; risk assessment; sarcoma; survival.

Apstrakt

Uvod. Angiosarkomi (AS) koji nastaju iz vaskularnog tkiva, čine 3,3% svih sarkoma i imaju lošu prognozu. Zračenjem indukovani AS su retka kasna komplikacija lečenja radioterapijom (RT), a karakterišu se posebnim profilom genske ekspresije, poput amplifikacije c-MYC onkogena, pomoću kojeg možemo razlikovati primarni od sekundarno indukovanog tumora. **Prikaz bolesnika.** Bolesnici staroj 77 godina, sa adenokarcinomom endometrijuma u ranom stadijumu, inicijalno je urađena radikalna histerektomija sa bilateralnom adneksektomijom. Prema patohistološkim faktorima rizika, indikovana je postoperativna konformalna RT (CRT) karlice, uz brahiterapiju. Šest godina nakon tretmana na prednjem trbušnom zidu, u regiji postoperativnog ožiljka i zračnog polja, histološki je potvrđen infiltrativni AS kože i potkožnog masnog tkiva. Bolesnica je primila šest ciklusa hemioterapije mono-adriamicinom, sa verifikovanom delimičnom regresijom. Dodatna imunohistohemijska analiza (IHH) ekspresije c-MYC, Ki67 i CD34 pokazala je visok proliferativni indeks (Ki67 oko 60%) i pozitivnost c-MYC što je ukazivalo na molekulski obrazac AS izazvanog zračenjem. Visoki proliferativni indeks mogao je objasniti dobar odgovor na hemioterapiju. **Zaključak.** Nove tehnike RT omogućavaju bolje preživljavanje i lokalnu kontrolu bolesnica sa karcinomom endometrijuma visokog rizika. Međutim, produženo preživljavanje tih bolesnica stavlja ih u povišen rizik od razvoja tumora izazvanih radijacijom kao i drugih kasnih efekata RT.

Correspondence to: Luka Jovanović, Institute for Oncology and Radiology of Serbia, Department of Radiation Oncology, Pasterova 14, 11 000 Belgrade, Serbia. E-mail: lukabgd01137@gmail.com

Ukoliko su ispunjeni Cahan-ovi kriterijumi, dodatne IHH analize ekspresije c-MYC mogu pomoći u razlikovanju sekundarnih od ostalih AS, kada se procenjuje mogućnost pojave radijaciom indukovanog AS.

Ključne reči:
adenokarcinom; genetika; imunohistohemija; neoplazme, radijacijom uzrokovane; radioterapija, adjuvantna; rizik, procena; sarkomi; preživljavanje.

Introduction

Angiosarcoma (AS) is a rarely occurring malignancy that arises from vascular tissue and has a poor prognosis. The incidence of all soft tissue sarcomas in Europe ranges from 3.3 per 100,000 in Eastern Europe to 4.7 per 100,000 in Northern Europe, and it is reported that 3.3% of all sarcomas are ASs [1]. By site, ASs are divided as follows: soft tissue AS, bone AS, and cutaneous AS. Furthermore, cutaneous ASs are divided as scalp and face AS, AS in the contest of lymphedema (Stewart-Treves syndrome), epithelioid AS, and radiation-induced AS [2].

AS is characterized by diverse but recurrent chromosomal abnormalities and mutations of genes involved in angiogenesis and endothelial cell receptors [3]. The patterns of mutation are so distinct that they could distinguish secondary, mostly radiation-induced ASs from primary tumors [4].

The first set of criteria used for the diagnosis of radiation-induced malignancy (RIM) was established by Cahan et al. [5] in 1948. Today, modified Cahan's [5] criteria are used to encompass the following: RIM must arise within the boundaries of the irradiation field; duration of the latent period between proposed induced malignancy and previous irradiation must be greater than 4 years; primary malignancy and induced malignancy must be biopsied and of different histology; the tissue that arises from the induced malignancy must be metabolically and genetically normal before irradiation.

From a molecular point of view, around 100 genes are deregulated during secondary AS development, including upregulation of MYC, KIT, and RET genes, as well as concomitant upregulation of MYC and FLT4 and downregulation of CDKN2C gene. Similar genetic patterns are present in other radiation-induced tumors, suggesting the distinct tumorigenic mechanism of radiation [6].

In our study, we analyzed the clinical problem of distinguishing the primary from secondary-radiation induced AS by immunohistochemical (IHC) analysis of c-MYC and other markers expression in the case of a patient with endometrial cancer treated with adjuvant radiotherapy.

Case report

The patient was 77 years old with no prior or family history of malignancy. Initial staging workups revealed endometrial adenocarcinoma of stage IC according to the Union International Cancer Control (UICC) and International Federation of Gynecology and Obstetrics (FIGO). According to the protocol, the patient underwent radical hysterectomy with bilateral salpingo-oophorectomy in November 2011 at the Military Medical Academy (MMA) in Belgrade. Histopathology (HP) findings confirmed endometrial adenocarcinoma FIGO IC (pT1c), infiltrating the uterine wall to a depth of more than 1/2 myometrium.

According to the multidisciplinary board decision, adjuvant conformal external beam RT (CRT) of the pelvis was administered. A total dose of 45 Gy in 25 fractions was given (5 days *per* week) at the MMA Radiotherapy Department, while three applications of intracavitary brachytherapy were performed at the Institute for Oncology and Radiology of Serbia in the period from January to March 2013. Treatment-related early toxicity during RT treatment included diarrheas and tenesmus, which were successfully medically treated.

In March 2019, a patient underwent surgical excision of clinically suspicious multiple cutaneous tumors in the region of the surgery scar and irradiated area of the anterior abdominal wall in the General Hospital in Kraljevo. Initial HP findings were characterized as mesenchymal malignancy. Further IHC analysis revealed infiltrative AS of skin and subcutaneous fat tissue with R1 posterior resection margin.

Further diagnostic workup, which included magnetic resonance imaging (MRI) of the abdomen, detected a soft tissue tumor of the anterior abdominal wall with no further spreading. The patient underwent six cycles of systemic chemotherapy, including mono-adriamycin (75 mg/m^2), with a good partial response (Figure 1).

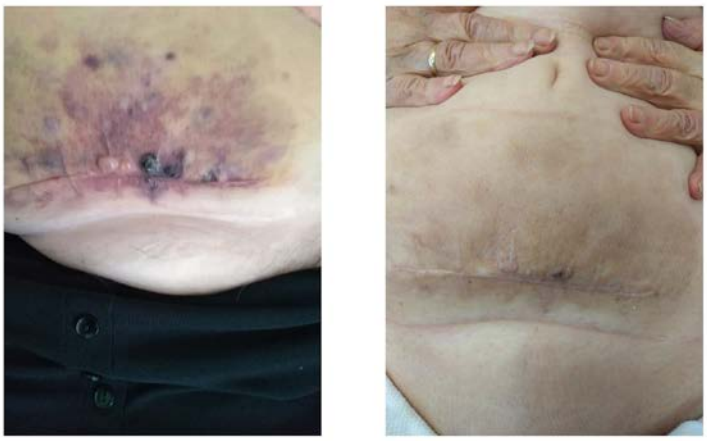


Fig. 1 – Clinical presentation of angiosarcoma in our patient: initially, before chemotherapy with mono-ADM (left) and after chemotherapy (right).

Specimen of skin measuring 105 mm × 40 mm × 30 mm was sent. On the cross-section, we revealed fields of hemorrhage in subcutis.

Sections of 4 μm thickness were sampled. Standard histological analysis was performed using standard hematoxylin and eosin staining. Sections revealed a tumor in the deep portion of the dermis and fat tissue. The tumor consisted of malignant fusiform cells arranged in pseudovascular channels. Neoplastic cells had hyperchromatic *nuclei* and numerous mitotic figures.

The same blocks of tissue, previously prepared for classical pathohistology, were used for IHC analysis.

We performed IHC staining for CD34 (Ventana, RTU, clone QBEnd/10), Ki67 (Ventana, RTU, clone 30-9, RTU), and c-MYC (clone 9E10.3).

Staining for CD34 and Ki67 was performed in immunostainer BenchMark GX, Ventana. C-MYC was stained manually. For c-MYC identification, the demasking procedure was used as the first step in the IHC procedure. For antigen unmasking, a 10 mM citrate buffer (pH6) was used for 21 min in a microwave oven at the maximum power of 800 W. The sections were then washed with TBS [TRIS (hydroxymethyl) aminomethane buffer saline] and incubated with the primary c-MYC monoclonal antibody (Thermo Fisher Scientific Invitrogen, MA5-12080, clone 9E10.3) diluted at 1 : 50 ratio. The sections were treated using the commercial Thermo Scientific UltraVision Quanto Detection System HRP DAB (TL-060-QHD). Immunoreactions were subsequently developed using DAB (diaminobenzidine) as a chromogen. The sections were counterstained with Mayer's hematoxylin.

The quality and the specificity of the developed immunoreactions were controlled by negative controls performed by omitting the primary antibody and applying TBS instead.

CD34 immunoreactivity showed the vascular origin of the tumor. *Nuclei* of malignant cells had a high proliferative index (Ki67 around 60%). C-MYC protein expression was shown in numerous positive tumor cells (Figure 2).

Discussion

Secondary AS occurs as radiation-induced but also in patients with chronic lymphoedema due to prior lymphadenectomy (after breast surgery) or in patients that have chronically altered lymph drainage for other reasons (i.e., Stewart-Treves syndrome) [7, 8].

Radiation-induced secondary malignancies are rare but important late side effects of RT and have an impact on optimal treatment decision-making, especially with expected survival longer than 5 years.

The adverse effects (AEs) of radiation vary depending on the technique and dose applied, and they are generally divided into early and late AEs. Early toxic effects of radiation on healthy tissue are due to acute inflammation (radiation co-

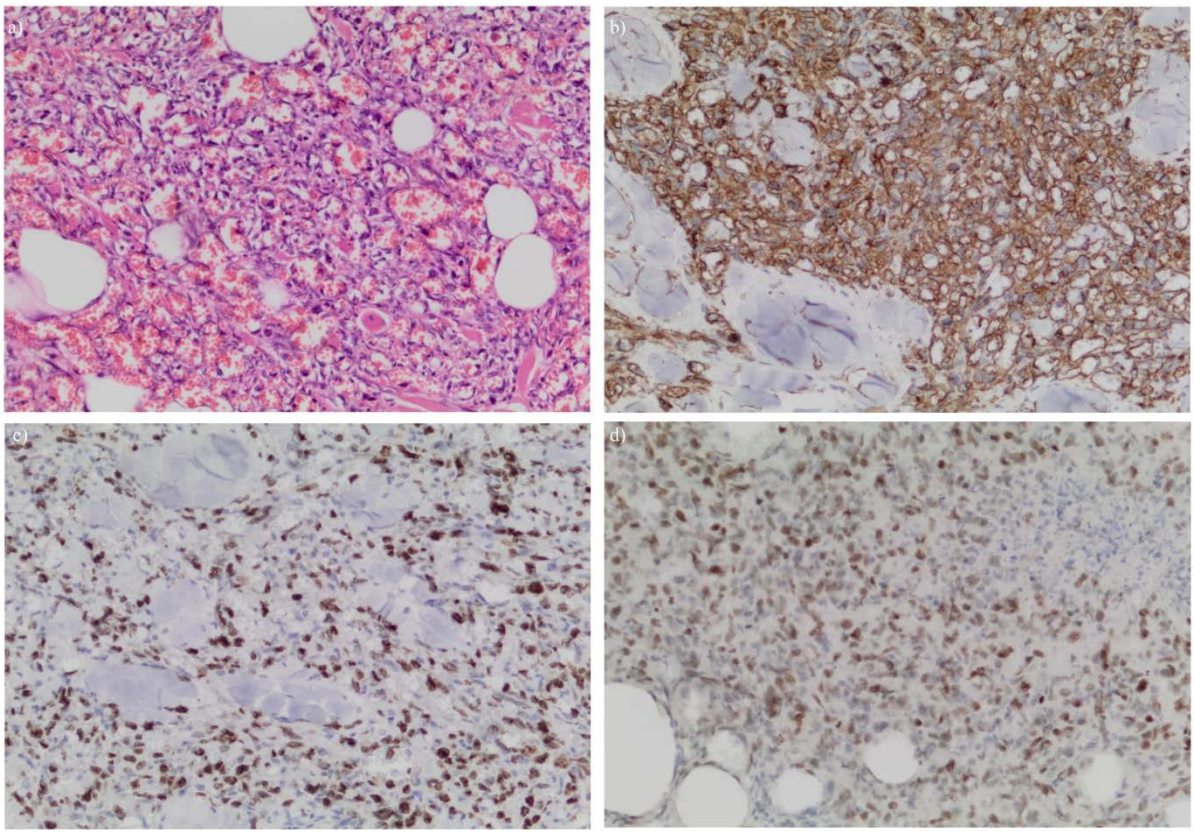


Fig. 2 – Pathohistology (PH) and immunohistochemical (IHC) analysis:
a) neoplastic cells arranged in poorly formed vascular spaces [hematoxylin and eosin (HE), ×400];
b) CD34 immunostaining-positive tumor cells (×400); c) Ki67 immunostaining – high proliferative index (×400); d) c-MYC immunostaining-numerous positive tumor cells (×400).

litis, cystitis, radio-dermatitis, etc.). On the other hand, late AEs are caused by microvascular damage, chronic inflammation, and radiation-induced genetic instability. Whereas early AEs are reversible and have a good prognosis when treated medically, late effects are permanent and generally are less responsive to medications or lifestyle changes.

The development of contemporary RT techniques such as CRT (4 field box-technique) and intensity-modulated RT (IMRT) have improved target dose coverage and reduced early and late treatment toxicities [9]. Some studies, however, found no reduction of gastrointestinal and genitourinary toxicities of radiation when CRT is used and even suggested a positive correlation between the development of early and late toxicities in organs that receive a relatively high dose of radiation during the CRT treatment [10–12].

Nevertheless, contemporary radiation techniques (CRT and IMRT) made escalation of treatment dose possible, which increased the number of long-term cancer survivors at increased risk of developing late AEs of RT, including RIM [13].

Endometrial carcinoma is one of the most common gynecologic malignancies worldwide, with standard treatment protocol in early operable stages, which includes radical hysterectomy followed by adjuvant RT if postoperative histology assesses risk factors for local recurrence (high-grade carcinomas or deep myometrial and cervical stroma invasion) [14, 15]. Adjuvant RT provides a significant improvement in local control and disease-free survival after 5 years of 90% for intermediate-risk patients and around 80% for high-risk patients (high-grade tumors or myometrial invasion) [16]. In our patient, the adjuvant RT was performed due to a microscopic invasion of more than half of the myometrium to achieve better local control (according to hospital protocol at the period).

Modern CRT allows more precise irradiation of a targeted volume, with a sparing effect on normal tissue. However, with these techniques, a larger volume of normal tissue is irradiated with a lower dose. RIM arises mainly in the irradiated tissue or the nearby tissues due to collateral radiation exposure [17, 18]. A large cohort study by Chaturvedi et al. [19] has shown that after radiation treatment of gynecological malignancies (external beam RT and brachytherapy), there is a secondary malignancy incidence increase of 12% compared with the cohort that did not receive radiation treatment. RIM was detected with a median follow-up of 12.2 years. The most common RIM observed were anal, colorectal, and gynecological malignancies [19]. Concerning endometrial carcinoma treatment, (PORTEC)-1 trial showed that 22% of the patients that received RT developed secondary neoplasm after 15 years, while 16% of non-irradiated patients developed secondary neoplasms [20].

In several clinical series, comparing c-MYC gene amplification and expression between secondary AS of the skin and primary AS showed that c-MYC expression is statistically significantly more prevalent in secondary AS [21, 22]. Moreover, a c-MYC expression is present in secondary AS associated with chronic lymphedema (Stewart-Treves sy) [23]. Nevertheless, in a minority of primary skin ASs, the c-MYC expression could be found too, as well as in primary AS of other sites [24].

The study by Styring et al. [6] underlined the role of MYC, KIT, and RET genes upregulation in the pathogenesis of radiation-induced AS and its diagnostic application as the basis for the therapeutic use of kinase inhibitors in these sarcomas. This study also found that over 100 genes are significantly deregulated between primary and secondary ASs, for example, the upregulation of FLT4, a tyrosine kinase receptor for vascular endothelial growth factor (VEGF), and other vascular-specific receptor tyrosine kinases such as TIE1, KDR, and FLT1. This somatic mutation pattern could be of diagnostic importance since it is common in radiation-induced sarcomas and other RIM.

In the end, it should be noted that the overall prognosis of radiation-induced AS is poor [25]. Although surgical treatment is the therapy of choice in other sarcomas, it seems that re-occurrence of the AS, including radiation-induced ones, is high, even when radical excision is made [25]. This could be explained by the multifocality of the tumor, so a truly negative resection margin is hard to be achieved [26]. Hematogenic spread in the lungs, pleura, and bone, as well as spread to regional lymph nodes, is possible [27, 28]. At the time, doxorubicin-based chemotherapy remained the standard treatment for metastatic or unresectable AS, but other chemotherapeutic agents such as taxanes showed activity against AS [29]. Overall response rates to chemotherapy are variable from 20% to 60% [30]. In our patient, a high proliferative index (Ki67 around 60%) and c-MYC positivity could explain a positive response to chemotherapy.

Target therapy could be a potential new approach in the treatment of radiation-induced AS, such as tyrosine kinase inhibitors sorafenib, brivanib, and sirolimus, as well as KIT inhibitor imatinib, anti-VEGF antibody bevacizumab, and thalidomide but the experience is still limited, and further studies are necessary [26].

Conclusion

The development of novel RT techniques provided longer survival and better local control of patients with high-risk endometrial cancer. Longer survival of these patients put them at a higher risk of developing RIM and other late side-effects of RT. When assessing the probability of radiation-induced AS, IHH finding of c-MYC expression could help distinguish secondary from other ASs if Cahan‘s criteria are fulfilled. Additional IHH analysis of other molecular markers such as KIT or RET kinases and VEGF expression could be of diagnostic and therapeutic importance in the era of target therapy in oncology.

R E F E R E N C E S

1. *Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD*, et al. Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. Eur J Cancer 2013; 49(3): 684–95.
2. *Morgan MB, Swann M, Somach S, Eng W, Smoller B.* Cutaneous angiosarcoma: a case series with prognostic correlation. J Am Acad Dermatol 2004; 50(6): 867–74.
3. *Itakura E, Yamamoto H, Oda Y, Tsuneyoshi M.* Detection and characterization of vascular endothelial growth factors and their receptors in a series of angiosarcomas. J Surg Oncol 2008; 97(1): 74–81.
4. *Huang SC, Zhang L, Sung YS, Chen CL, Kao YC, Agaram NP,* et al. Recurrent CIC Gene Abnormalities in Angiosarcomas: A Molecular Study of 120 Cases With Concurrent Investigation of PLCG1, KDR, MYC,and FLT4 Gene Alterations. Am J Surg Pathol 2016; 40(5): 645–55.
5. *Cahan WG, Woodard HQ, Higinbotham NL, Stewart FW, Coley BL.* Sarcoma arising in irradiated bone: report of eleven cases. Cancer 1948; 1(1): 3–29.
6. *Styring E, Seinen J, Dominguez-Valentin M, Domanski HA, Jönsson M, von Steyern FV*, et al. Key roles for MYC, KIT and RET signaling in secondary angiosarcomas. Br J Cancer 2014; 111(2): 407–12.
7. *Sheppard DG, Libshitz HI.* Post-radiation sarcomas: a review of the clinical and imaging features in 63 cases. Clin Radiol 2001; 56(1): 22–9.
8. *Sharma A, Schwartz RA.* Stewart-Treves syndrome: pathogenesis and management. J Am Acad Dermatol 2012; 67(6): 1342–8.
9. *Bucci MK, Bevan A, Roach M.* Advances in radiation therapy: conventional to 3D, to IMRT, to 4D, and beyond. CA Cancer J Clin 2005; 55(2): 117–34
10. *Stankovic V, Džamic Z, Pekmezovic T, Tepavcevic DK, Dozic M, Saric M*, et al. Acute and Late Genitourinary Toxicity after 72 Gy of Conventionally Fractionated Conformal Radiotherapy for Localised Prostate Cancer: Impact of Individual and Clinical Parameters. Clin Oncol (R Coll Radiol) 2016; 28(9): 577–86.
11. *Stankovic V, Nikitovic M, Pekmezovic T, Pekmezovic D, Kisic Tepavcevic D, Stefanovic Djuric A,* et al. Toxicity of the lower gastrointestinal tract and its predictive factors after 72Gy conventionally fractionated 3D conformal radiotherapy of localized prostate cancer. J BUON 2016; 21(5): 1224–32.
12. *Koper PC, Stroom JC, van Putten WL, Korevaar GA, Heijmen BJ, Wijnmaalen A,* et al. Acute morbidity reduction using 3DCRT for prostate carcinoma: a randomized study. Int J Radiat Oncol Biol Phys 1999; 43(4): 727–34.
13. *Dracham CB, Shankar A, Madan R.* Radiation induced secondary malignancies: review article. Radiat Oncol J 2018; 36(2): 85–94.
14. *Soslow RA, Tornos C, Park KJ, Malpica A, Matias-Guiu X, Oliva E,* et al. Endometrial Carcinoma Diagnosis: Use of FIGO Grading and Genomic Subcategories in Clinical Practice: Recommendations of the International Society of Gynecological Pathologists. Int J Gynecol Pathol 2019; 38 (1 Suppl 1): S64–S74.
15. *Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J*, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer. Ann Oncol 2016; 27(1): 16–41.
16. *Creutzberg CL, Nout RA.* The role of radiotherapy in endometrial cancer: current evidence and trends. Curr Oncol Rep 2011; 13(6): 472–8.
17. *Liauw SL, Sylvester JE, Morris CG, Blasko JC, Grimm PD.* Second malignancies after prostate brachytherapy: incidence of bladder and colorectal cancers in patients with 15 years of potential follow-up. Int J Radiat Oncol Biol Phys 2006; 66(3): 669–73.
18. *Moon K, Stukenborg GJ, Hein S, Theodorescu D.* Cancer incidence after localized therapy for prostate. Cancer 2006; 107(5): 991–8.
19. *Chaturvedi AK, Engels EA, Gilbert ES, Chen BE, Storm H, Lynch CF,* et al. Second cancers among 104,760 survivors of cervical cancer: Evaluation of long-term risk. J Nat Cancer Inst 2007; 99(21): 1634–43.
20. *Creutzberg CL, Nout RA, Lybeert ML, Wárlám-Rodenhuis CC, Jobsen JJ, Mens JW* et al. Fifteen-year radiotherapy outcomes of the randomized PORTEC-1 trial for endometrial carcinoma. Int J Radiat Oncol Biol Phys 2011; 81(4): e631–8.
21. *Laé M, Lebel A, Hamel-Viard F, Asselain B, Trassard M, Sastre X*, et al. Can c-myc amplification reliably discriminate postradiation from primary angiosarcoma of the breast? Cancer Radiother 2015; 19(3): 168–74.
22. *Fraga-Guedes C, André S, Mastropasqua MG, Botteri E, Toesca A, Rocha RM*, et al. Angiosarcoma and atypical vascular lesions of the breast: diagnostic and prognostic role of MYC gene amplification and protein expression. Breast Cancer Res Treat 2015; 151(1): 131–40.
23. *Wang LL, Cui LF, Gao Y, Jiang ZC.* Clinicopathologic features of Stewart-Treves syndrome. Int J Clin Exp Pathol 2019; 12(3): 680–8.
24. *Ginter PS, Mosquera JM, MacDonald TY, D'Alfonso TM, Rubin MA, Shin SJ.* Diagnostic utility of MYC amplification and anti-MYC immunohistochemistry in atypical vascular lesions, primary or radiation-induced mammary angiosarcomas, and primary angiosarcomas of other sites. Hum Pathol 2014; 45(4): 709–16.
25. *Seinen JM, Styring E, Verstappen V, Vult von Steyern F, Rydholm A, Suurmeijer AJ* et al. Radiation-associated angiosarcoma after breast cancer: high recurrence rate and poor survival despite surgical treatment with R0 resection. Ann Surg Oncol 2012; 19(8): 2700–6.
26. *Bonito FJP, de Almeida Cerejeira D, Dahlstedt-Ferreira C, Oliveira Coelho H, Rosas R.* Radiation-induced angiosarcoma of the breast: A review. Breast J 2020; 26(3): 458–63.
27. *Virgilio E, Lombardi M, Stefano DD, Marchetti P, Cavallini M, Chieco PA,* et al. Angiosarcoma of the Breast: A Rare and Dismal Complication of Breast Surgery Associated with Radiation. Am Surg 2017; 83(3): e71–3.
28. *Shah S, Rosa M.* Radiation-Associated Angiosarcoma of the Breast: Clinical and Pathologic Features. Arch Pathol Lab Med 2016; 140(5): 477–81.
29. *Fujisawa Y, Yoshino K, Kadono T, Miyagawa T, Nakamura Y, Fujimoto M.* Chemoradiotherapy with taxane is superior to conventional surgery and radiotherapy in the management of cutaneous angiosarcoma: a multicentre, retrospective study. Br J Dermatol 2014; 171(6): 1493–500.
30. *Li GZ, Fairweather M, Wang J, Orgill DP, Bertagnolli MM, Raut CP.* Cutaneous radiation-associated breast angiosarcoma: radicality of surgery impacts survival. Ann Surg 2017; 265(4): 814–20.

Received on December 7, 2020
Accepted on March 12, 2021
Online First March 2021

CASE REPORT

UDC: 616.351-006.326
DOI: https://doi.org/10.2298/VSP200627025E

Rectum lipoma incarcerated in the anus as a cause of abundant rectorrhagia

Lipom rektuma inkarceriran u anusu kao uzrok obilne rektoragije

Katarina Erić*, Marko Miladinov*, Zoran Krivokapić*†‡

***University Clinical Center of Serbia, Clinic for Digestive Surgery, First Surgical Clinic, Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia; ‡Serbian Academy of Sciences and Arts, Belgrade, Serbia**

Abstract

Introduction. Lipomas are slow-growing, benign tumors of mesenchymal origin. In most cases, they are incidental findings during endoscopic examinations. Lipomas in the gastrointestinal tract are rare entities with the lowest rate of occurrence in the rectum. They are mostly asymptomatic but can cause pain, intussusception, bleeding, volvulus, prolapse, and weight loss if larger than 4 cm. The aim of the presented case report was to demonstrate that abundant rectorrhagia can be caused by a rare entity such as rectal lipoma. **Case report.** We presented the case of a fifty-year-old male patient with a soft-structure prolapse through the anus accompanied by extensive bleeding. After repositioning, the structure was endoscopically removed. Histopathological analysis confirmed the presence of a lipoma. **Conclusion.** Rectorrhagia can be a clinical manifestation of this rare condition – incarcerated rectal lipoma – and should be considered in practice as a differential diagnosis in cases where the existence of more common conditions like hemorrhoidal disease and malignant tumors is excluded.

Key words:
diagnosis, differential; gastrointestinal hemorrhage; hemostasis, endoscopic; histological techniques; lipoma; rectal neoplasms.

Apstrakt

Uvod. Lipomi su spororastući, benigni tumori mezenhimalnog porekla. U većini slučajeva se njihovo prisustvo slučajno otkriva tokom endoskopskih ispitivanja. Lipomi u gastrointestinalnom traktu su retki i najređe su prisutni u rektumu. Uglavnom su asimptomatski, ali ukoliko su veći od 4 cm mogu uzrokovati bolove, intususepciju, krvarenje, volvulus, prolaps i gubitak telesne mase. Cilj prikaza bio je da se pokaže da uzrok obilne rektoragije može biti i redak entitet kao što je lipom rektuma. **Prikaz bolesnika.** Prikazali smo slučaj 50-godišnjeg bolesnika sa prolapsom mekotkivne strukture kroz anus, praćenim obilnim krvarenjem. Nakon repozicije, struktura je endoskopski uklonjena. Histopatološkom analizom potvrđeno je prisustvo lipoma. **Zaključak.** Rektoragija može biti klinička manifestacija ovog retkog stanja – inkarceriranog lipoma rektuma – i trebalo bi da se razmatra u praksi kao diferencijalna dijagnoza u slučajevima kada je isključeno postojanje češćih uzroka rektoragije, kao što su hemoroidalna bolest i maligni tumor.

Ključne reči:
dijagnoza, diferencijalna; krvarenje, gastrointestinalno; hemostaza, endoskopska; histološke tehnike; lipom; rektum, neoplazme.

Introduction

Lipoma is a benign tumor of mesenchymal origin, produced by the proliferation of mature fat cells [1]. Lipoma in the colon was first described in 1757 by Bauer [2]. Lipomas are most frequently located in the right colon [1]. Rectal lipomas account for 5% of all lipomas in the gastrointestinal tract [3]. They are the third most prevalent tumors among all benign tumors in the rectum, after hyperplastic polyps and adenomas [4]. In most cases, lipomas are incidental findings made during diagnostic procedures and surgical interventions. In rare cases, they can lead to clinical manifestations such as pain, intussusception, bleeding, volvulus, prolapse, and weight loss [3], and extremely rarely to extensive bleeding. The aim of the presented case report is to demonstrate that abundant rectorrhagia can be caused by a rare entity such as rectal lipoma.

Correspondence to: Marko Miladinov, University Clinical Center of Serbia, Clinic for Digestive Surgery, First Surgical Clinic, Dr. Koste Todorovića 6, 11 000 Belgrade, Serbia. E-mail: marko.miladinov90@gmail.com

Case report

We presented the case of a fifty-year-old patient who came to the Outpatient Clinic of the Digestive Surgery Clinic within the University Clinical Center of Serbia because of abundant hemorrhage from the perianal region. The patient was bleeding so profusely that blood was present on his trousers, underwear, and both thighs. Inspection of the perianal region revealed a prolapse of polypoid neoplastic formation, blood-saturated, with a soft consistency and eroded bleeding surface. The structure was examined and carefully manually repositioned into the anus. The first assumption was that the prolapsed formation was an incarcerated hemorrhoid since the patient had suffered from the hemorrhoidal disease for several years. Although he often suffered bleeding from hemorrhoids, the patient was treated conservatively and was never examined anoscopically. Differentially, both benign and malignant rectal polyps and rectal mucosal prolapse were considered. After the repositioning and the anoproctoscopy, a flexible rectoscopy was performed on the unprepared intestine, and the described polypoid tumor on the peduncle on the right lateral wall of the distal rectum was verified, some 3.5 cm from the dentate line of the anus (Figure 1). Incarceration was probably caused by tumor peduncle torsion and reflex anal sphincter spasm. The tumor was electroresected entirely after infiltrating the peduncle with a solution of adrenaline and sent for histopathological examination. Instant hemostasis was achieved by clipping the tumor base residue. The patient tolerated the intervention well and was released shortly after the procedure. The lesion, 28 × 25 × 24 mm in size (Figure 2), was submitted for histopathological examination (Figures 3 and 4), which confirmed the finding of lipoma.

Discussion

Lipomas are rare, most commonly solitary tumors in the gastrointestinal tract, with an overall incidence ranging between 0.2% and 4.4% [5]. They occur equally in both genders, most frequently in the sixth decade of life [6]. The most common location of lipomas in the gastrointestinal tract is the right colon [6], and very rarely in the rectum – in about 3.9% of all reported cases [7]. Lipomas are predominantly presented as sessile

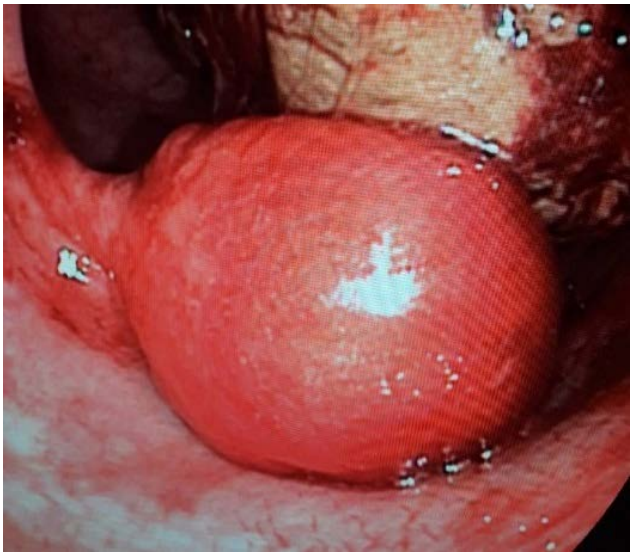


Fig. 1 – Colonoscopic presentation of a tumor in the distal rectum.

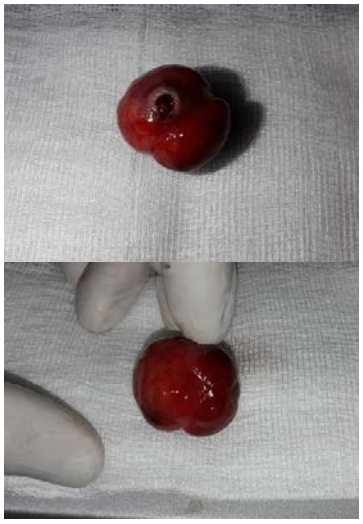


Fig. 2 – Macroscopic presentation of a sessile polypoid tumor, with a base strongly saturated with blood.

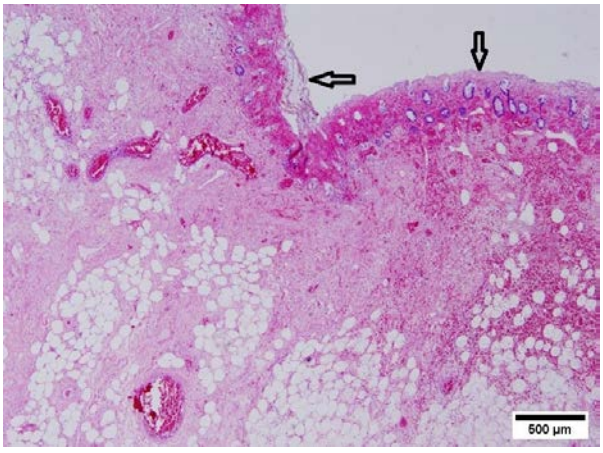


Fig. 3 – Histological presentation of submucosal rectum lipoma with eroded mucosa accompanied by bleeding focuses (arrows) (hematoxylin and eosin, ×40).

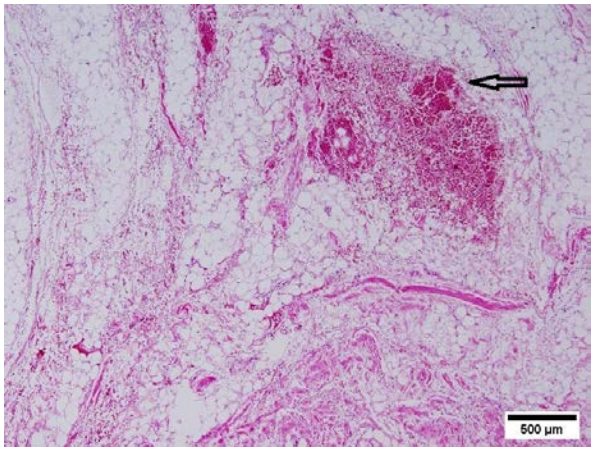


Fig. 4 – Histological presentation of submucosal rectum lipoma with intralipomal bleeding (arrow) (hematoxylin and eosin, ×40).

or pedunculated pseudopolypoid tumors. Macroscopically, sections reveal a yellow, clearly defined structure. In 90% of cases, they are submucosal, and the remaining 10% are subserous and intramuscular [8]. Lipomas are generally asymptomatic, whilst those larger than 4 cm always manifest clinically [6]. The clinical manifestations of colon and rectum lipomas are abdominal colic, rectal bleeding, peristalsis disorder, colonic-colon intussusception, weight loss, sigmoid colon volvulus, and spontaneous lipoma expulsion [6]. In the case presented here, rectal lipoma was manifested by extensive rectal bleeding, which was cited by Crocceti et al. [6] as a complication in 46% of patients. Bleeding can be acute and chronic and can cause anemia when it is occult. The likelihood of a lipoma causing rectal bleeding is thought to correlate directly to tumor size. In lipomas larger than 4 cm, rectal bleeding has been reported in 10% of cases [9]. In the present case, the patient had profuse secondary bleeding even though the maximum tumor size was less than 3 cm. Bleeding was caused by incarceration in the anus, necrosis within the tumor, and mucosal erosion. Lipoma prolapse through the anus is rare [10] and has been described in several papers. In all cases, including ours, rectal prolapse or prolapsed hemorrhoids were first contemplated [11, 12]. Spontaneous lipoma expulsion is a very rare clinical manifestation. It occurs in large pedunculated lipomas. These entities produce a twist of the peduncle and its necrosis leading to tumor autoamputation and its spontaneous expulsion through the anus [9]. Intussusception, in addition to rectal bleeding, is the rectal lipoma's most important urgent complication [13]. In adult patients, the incidence of intussusception is about 1% [4]. Preoperative diagnosis of lipoma can be made based on the radiographic findings – computed tomography and magnetic resonance imaging – and during colonoscopy. Three signs help diagnose lipomas during colonoscopy: "tenting sign" (grasping the overlying mucous), "cushion sign", and "naked fat sign" (fat tissue extrusion after colon mucosal biopsy) [6]. During these diagnostic procedures, it is sometimes difficult to distinguish lipoma from polypoid cancers. In 2016, Chakrabarti and Goenka [3] described the case of confocal laser endomicroscopy first used for diagnostic purposes. It is a novel method used to diagnose gastrointestinal lesions, predominantly in polypoid colorectal lesions, and to provide tissue diagnosis at both cellular and subcellular levels. Regardless of the state-of-the-art technologies used in diagnostic medicine, histopathology is still the gold standard in diagnosis [8]. The surgical approach depends solely on the size of the tumor, its location, accompanying complications, and suspected malignancy [9]. Lipomas smaller than 2 cm in size should be removed endoscopically [14]. For large lipomas, surgical resection in the form of colotomy or segmental colon resection is recommended [6]. Laparoscopic colon and rectum resection is the gold standard in removing giant lipomas because of shorter hospitalization, faster recovery, and lower risk of complications [15]. Rectal lipomas that prolapse through the anus are surgically removed by transanal resection or local excision [10, 11]. The presented patient underwent local endoscopic excision of the lipoma that prolapsed through the anus.

Conclusion

Rectal lipoma is a rare entity. Bleeding from the rectum can be a clinical manifestation of incarcerated rectal lipoma, and it should be considered in practice as a differential diagnosis concerning the presence of hemorrhoidal disease and malignant tumors.

REFERENCES

1. *Krishnan P, Adlekha S, Chadha T, Babu A.* Rectal lipoma associated with genital prolapse. Ann Med Health Sci Res 2013; 3(Suppl 1): S18–20.
2. *Bagherzade G, Etemad O.* Recto-sigmoid lipoma: a case report and review of the literature. J Coloproctol (Rio J) 2016; 37(1): 50–4.
3. *Chakrabarti A, Goenka MK.* Submucosal lipoma of the rectum presenting with rectal prolapse and appearing as an adenomatous polyp on confocal laser endomicroscopy: a case report and review of literature. Gastroenterol Hepatol Open Access 2016; 5(1): 00126.
4. *Wei R, Xu W, Xiao Y, Yeng F, Mao S.* Laparoscopic segmental resection of the rectum for upper rectal intussusception caused by a giant rectal lipoma. Medicine 2018; 97(39): e1227.
5. *Dultz LA, Ullery BW, Sun HH, Huston TL, Eachempati SR, Barie PS,* et al. Ileocecal valve lipoma with refractory hemorrhage. JSLS. 2009; 13: 80–83. (English)
6. *Crocetti D, Sapienza P, Sterpetti AV, Paliotta A, De Gori A, Pedulla G et al.* Surgery for symptomatic colon lipoma: A systematic review of the literature. Anticancer Res. 2014; 34(11): 6271–6.
7. *Manickavasagam K, Chandramohan S, Lakshmi J, Vairavan K, Madhusudhanan J.* Intussuscepting rectal lipoma: A case report and review of literature. Int J Gastroenterol 2012; 11(1): doi: 10.5580/2bd3.
8. *Yaman I, Derici H, Demirpolat G.* Giant colon lipoma. Ulus Cerrahi Derg. 2015; 31(2): 76–81.
9. *Kouritas VK, Baloyiannis I, Koukoulis G, Mamaloudis I, Zacharoulis D, Efthimiou M.* Spontaneous expulsion from rectum: a rare presentation of intestinal lipomas. World J Emerg Surg 2011; 6: 19.
10. *Martellucci J, Civitelli S, Tanzini G.* Transanal resection of rectal lipoma mimicking rectal prolapse: description of a case and review of the literature. ISRN Surg 2011; 2011: 170285.
11. *Kose E, Cipe G, Demirgan S, Oguz S.* Giant colonic lipoma with prolapse throuh the rectum treated by external local excision: A case report. Oncol Lett 2014; 8(3): 1377–9.
12. *Ghanem OM, Slater J, Singh P, Heitmiller RF, DiRocco JD.* Pedunculated colonic lipoma prolapsing through the anus. World J Clin Cases 2015; 3(5): 457–61.
13. *Shobeirian F, Mehrnahad M, Soleimantabar H.* Rectal lipoma as a lead point for colo-colonic intussusception. Raiol Case Rep 2018; 13(2): 431–3.
14. *Fernandes J, Libanio D, Giestas S, Araújo T, Martinez-Ares D, Certo M,* et al. Giant symptomatic rectal lipoma resected by endoscopic submucosal dissection. Endoscopy. 2018; 50: E63–E64.
15. *Arora R, Kumar A, Bansal V.* Giant rectal lipoma. Abdom Imaging 2011; 36(5): 545–7.

Received on June 27, 2020
Revised on March 21, 2021
Accepted on March 22, 2021
Online First March 2021

CASE REPORT

UDC: 616-053.2:616.37-056.7
DOI: https://doi.org/10.2298/VSP200512042S

Partial annular pancreas in a 12-year-old girl

Parcijalni anularni pankreas kod devojčice od 12 godina

Djordje Savić*†, Dragan Prokić*†, Blagoje Grujić*†, Miroslav Vukadin*, Vesna Milojković Marinović*†, Petar Rašić*, Maja Miličković*†

***Mother and Child Health Care Institute of Serbia "Dr. Vukan Čupić", Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia**

Abstract

Introduction. Annular pancreas is a rare congenital anomaly in which a band of the pancreatic tissue, in continuity with the pancreatic head, completely or incompletely surrounds the descending part of the duodenum. An abnormal pancreatic development can cause complete annular pancreas, partial annular pancreas, and pancreas divisum. Complete annular pancreas is diagnosed in newborns, while the diagnosis of the partial annular pancreas is more frequently established in adults. The most reliable diagnostic methods are computed tomography and magnetic resonance cholangiopancreatography. The anomaly is treated surgically, using bypass procedures. **Case report.** A 12-year-old girl presented malnourished, with occasional feeding problems, vomiting, heartburn, and pain from infancy. The upper gastrointestinal series showed an extremely dilated stomach, the first and the second part of the duodenum. An endoscopic exam revealed the dilated stomach, pylorus, and the first and the second part of the duodenum with retained contrast, while the entrance of the endoscope into the third part of the duodenum was not possible. Computed tomography showed pancreatic tissue encircling the second part of the duodenum and the characteristic "crocodile jaw" sign. Roux-en-Y duodenojejunostomy was performed as a bypass procedure. **Conclusion.** The complete annular pancreas is a well-known and easily diagnosed anomaly in newborns. The partial annular pancreas is often poorly recognized, especially in patients who do not present with marked duodenal obstruction. Unrevealed, it causes chronic problems in food intake, with possible serious complications. Although a very rare condition in the pediatric population, partial annular pancreas should be taken into consideration in unclear cases of chronic poor oral food intake and vomiting.

Key words:
annular pancreas; child; diagnosis; endoscopy, digestive system; tomography, x-ray computed.

Apstrakt

Uvod. Anularni pankreas je retka kongenitalna anomalija kod koje deo tkiva pankreasa, u kontinuitetu sa glavom pankreasa, potpuno ili nepotpuno okružuje nishodni deo dvanaestopalačnog creva. Kao rezultat nepravilnog razvoja pankreasa mogu nastati kompletni anularni pankreas, parcijalni anularni pankreas i pankreas *divisum*. Kompletni anularni pankreas se dijagnostikuje kod novorođenčadi, dok se dijagnoza parcijalnog anularnog pankreasa češće postavlja kod odraslih osoba. Najpouzdanije dijagnostičke metode za otkrivanje ovog oboljenja su kompjuterizovana tomografija i magnetna holangiopankreatografija. Ova anomalija se leči hirurški, korišćenjem jedne od *bypass* procedura. **Prikaz bolesnika.** Prikazana je neuhranjena devojčica od 12 godina, koja je imala povremene probleme sa hranjenjem, povraćanjem, gorušicom i grčevitim bolovima od odojačkog uzrasta. Radiografskim pregledom otkriven je izuzetno proširen želudac, prvi i drugi deo dvanaestopalačnog creva. Endoskopskim pregledom viđen je proširen želudac, pilorus, prvi i drugi deo dvanaestopalačnog creva, sa zadržanim kontrastom, dok ulaz endoskopa u treći deo dvanaestopalačnog creva nije bio moguć. Kompjuterizovanom tomografijom otkriveno je tkivo pankreasa koje je okruživalo nishodni deo dvanaestopalačnog creva i karakterističan znak „čeljusti krokodila". *Roux-en-Y* duodenojejunostomija je učinjena kao *bypass* procedura. **Zaključak.** Kompletni anularni pankreas je dobro poznata anomalija, koja se lako dijagnostikuje kod novorođenčadi. Parcijalni anularni pankreas je često slabo prepoznatljiv, naročito kod bolesnika koji nemaju izraženu opstrukciju dvanaestopalačnog creva. Neotkriven, on izaziva hronične probleme u unosu hrane, sa mogućim ozbiljnim komplikacijama. Iako je veoma redak u pedijatrijskoj populaciji, parcijalni anularni pankreas treba uzimati u obzir kod nejasnih slučajeva hronično lošeg oralnog unosa hrane i povraćanja.

Ključne reči:
pankreas, prstenasti; deca; dijagnoza; endoskopija, gastrointestinalna; tomografija, kompjuterizovana, rendgenska.

Correspondence to: Djordje Savić, Mother and Child Health Care Institute of Serbia "Dr. Vukan Čupić", Majke Jevrosime 10/17, 11 000 Belgrade, Serbia. E-mail: savic.60.djordje@gmail.com

Introduction

Annular pancreas (AP) is a rare congenital anomaly in which a band of pancreatic tissue, in continuity with the pancreatic head, completely or incompletely surrounds the second part of the duodenum (D2). The malformation was first described by Tiedemann in 1818 and named as annular pancreas by Ecker in 1862 [1]. The first operation on the annular pancreas was performed by Vidal in 1905. The anomaly is a result of abnormal pancreatic development. The pancreas develops from a dorsal bud and two ventral buds that appear in the 5th gestational week. By the 7th week, the duodenal rotation causes the clockwise rotation of the fused ventral buds, passing behind the duodenum from right to left to fuse with the dorsal bud [1]. The ventral bud forms the posterior part of the pancreatic head and uncinate process, while the dorsal bud forms the anterior part of the pancreatic head, body, and tail [2]. There are several theories explaining the development of AP. There is an opinion that the ventral pancreatic bud fuses with the duodenal wall and rotates incorrectly around the duodenum [2]. Some authors stated that the reason for the development of AP is the abnormal movement of the ventral pancreatic bud [2]. On the other hand, some suggested that the reason for the condition is a primary duodenal abnormality, and the pancreas only fills the space around a narrowed duodenum. The hypertrophy of both dorsal and ventral pancreatic buds may be the cause of this condition [3]. However, most authors consider the ventral pancreatic bud as the cause of AP [1, 2, 4]. As a result of abnormal pancreatic development, complete annular pancreas (CAP), partial annular pancreas (PAP), and pancreas divisum (PD) can arise.

The prevalence of AP is about 5–15 cases per 100,000 adults according to autopsy studies [5], and 400 cases per 100,000 adults according to endoscopic retrograde cholangiopancreatography (ERCP) studies [6]. A review of 103 cases with AP suggested the same prevalence in children and adults [7]. PAP has a lower incidence than CAP. Moreover, according to recorded data, PAP is detected less frequently in children than adults. In the adult population, PAP is most frequently detected between 20 and 50 years of age, presenting with upper abdominal pain (70%), nausea, and vomiting (47%) as a result of incomplete duodenal obstruction [8]. Peptic ulcer, acute, chronic, or recurrent pancreatitis, and jaundice are complications of this anomaly [9–11]. Symptoms of duodenal obstruction predominate in children, whereas PAP most commonly presents as pancreatitis in adults [12]. The diagnosis is made using ultrasonography, upper gastrointestinal (GI) series, endoscopic procedures, computed tomography (CT), and magnetic resonance cholangiopancreatography (MRCP). The treatment of PAP is surgical and mainly directed to the relief of duodenal obstruction. The surgical options include duodenoduodenostomy, duodenojejunostomy, gastrojejunostomy, and Roux-en-Y duodenojejunostomy [8]. The long-term postoperative results are excellent in the absence of severe associated anomalies such as congenital heart diseases and anomalies of the alimentary tract.

Case report

A 12-year-old girl presented with occasional feeding problems, such as sporadic vomiting mostly occurring after dinner, which started from her infancy and worsened in the last 6 months. She was complaining of night heartburn and short-term spasmodic abdominal pain. She had a poor appetite, chewing food for a long time. During the last three months, before her admission to the local hospital, she lost 2.5 kg of body weight and menarche had not appeared. Upper GI series, performed in another hospital, had shown food retention in the distal esophagus, dilation, and ptosis of the stomach, while the endoscopic procedure had shown inflammatory changes in the gastric mucosa. The girl had only received antiulcer therapy and nutrition advice. She had also been reviewed by the endocrinologist and psychiatrist.

On admission to our hospital, the girl was very malnourished, aging 12 years and 8 months, with 145 cm of body height and 24.5 kg of body weight. Secondary gender characteristics were poorly developed.

An ultrasound exam revealed the distended stomach and D1 and D2 portions, with a larger amount of denser content, with peristaltic and antiperistaltic waves. At the level of mesenteric forceps, the duodenum was about 4.5 mm in diameter in the transverse plane.

The upper GI series showed extreme dilation and ptosis of the stomach down to the bispinal line. D1 and D2 were markedly dilated, with a transverse diameter of 4.5 cm (Figure 1).

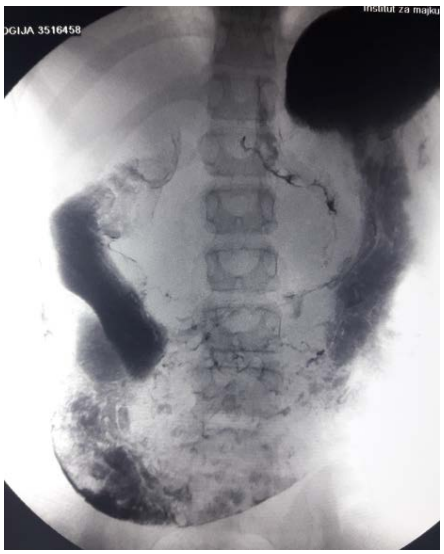


Fig. 1 – Preoperative radiogram showing dilated stomach and first two duodenal portions.

Endoscopic findings revealed hyperemia of the distal part of the esophagus and elongated stomach with retained food and contrast medium for more than 16 hours. Pylorus was dilated, D1 and D2 portions strongly dilated and edematous, while the entrance into D3 was impossible. Upon the suspicion of upper mesenteric artery syndrome, a CT scan was performed and revealed the presence of barium contrast in the stomach, which was given 10 days earlier. The dilation of the

stomach, D1 and D2 portions of the duodenum, and collapsed D3 portion were evident. In the area of the major duodenal papilla, the pancreatic tissue was seen encircling D2 on the posterior and lateral sides ("crocodile jaw" sign). There were no signs of intestinal malrotation, dilation of the choledochus, or pancreatitis. Intraoperatively, a strong dilation of D1 and D2 was revealed, caused by the head of the pancreas, which surrounded 3/4 of the D2 circumference (Figure 2).

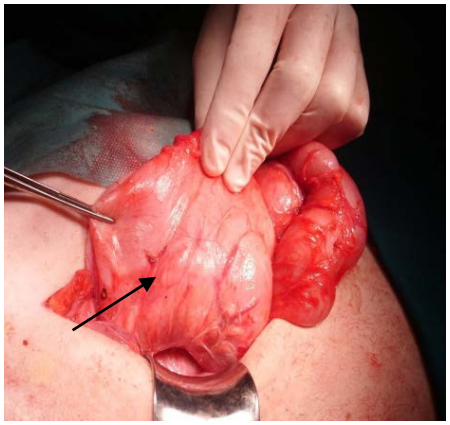


Fig. 2 – Intraoperative finding of pancreatic tissue on lateral duodenal wall (arrow).

The incision of the D2 showed the absence of a duodenal membrane. A Roux-en-Y duodenojejunostomy was performed as a bypass procedure. At the level of 20 cm from the ligament of Treitz, the jejunum was transected, and its distal portion anastomosed termino-laterally (T-L) with the D2. The proximal portion of the transected jejunum is T-L anastomosed with the distal portion of the jejunum, thus the conduit was about 25 cm long (Figure 3).

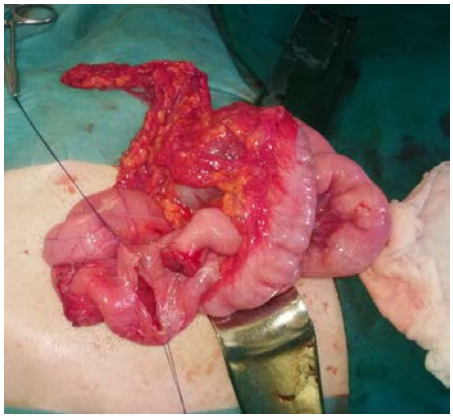


Fig. 3 – Duodenojejunal anastomosis.

The oral intake was started on the 4th postoperative day and was well-tolerated. The only postoperative problem was prolonged serious abdominal drainage due to hypoalbuminemia. During a 6-month follow-up period, the child had normal food intake, gaining 1 kg of body weight *per* month without any medical problem, so control radiographic and endoscopic exams were not indicated. Psychological and gynecological problems were resolved due to better food intake and weight gain.

Discussion

CAP is a well-known entity, presenting in newborns in the first days of life. Nowadays, the diagnosis of CAP is made in the early fetal period, according to an ultrasound exam. The time of onset of symptoms, clinical features, diagnostic methods, and treatment protocols are clearly explained in a huge number of literature data and are well-known because of clear and explicit symptoms of duodenal obstruction. On the contrary, PAP is a less frequent anomaly, and there is not enough literature data, especially considering the pediatric population [7]. The previous studies state that PAP, although a congenital anomaly, is most frequently detected between 20 and 50 years of age [5, 8]. The most prominent entities in the differential diagnosis of PAP are intestinal malrotation, perforated duodenal web, and upper mesenteric artery syndrome. There is a great delay in establishing the diagnosis of PAP. Therefore, patients suffer from duodenal obstruction symptoms, such as anorexia, vomiting, failure to thrive, and weight loss for a long time. A peptic ulcer can complicate the disease due to duodenogastric reflux caused by duodenal obstruction. The decreased outflow of pancreatic juice may give rise to acute or chronic pancreatitis. Jaundice can be present as a result of choledochal stenosis caused by the annular pancreas or as a result of biliary calculosis due to impaired biliary outflow [8]. In our patient, anorexia, vomiting, heartburn, and spasmodic abdominal pain were present sporadically 12 years prior to diagnosis. She was very malnourished, and her body weight was 24.5 kg at admission. A low body height of 145 cm and the absence of menarche were the results of chronic illness and poor food intake and were not caused by primary endocrinological disturbances. Our patient's symptoms were caused by duodenal obstruction, not by complications, which is the fact in accordance with literature data that children, unlike adults, seldom have complications of PAP [7]. Ultrasound exam should be indicative for PAP [10], although in our patient was not significant, suggesting superior mesenteric artery syndrome. Endoscopic ultrasonography has higher accuracy and is preferred [13]. The endoscopic exam can detect only complications of PAP but not the anomaly itself. CT scan and MRCP are reliable diagnostic tools in establishing the diagnosis, showing the characteristic "crocodile jaw" sign [14–16]. Two decades ago, in more than 40% of cases, the correct diagnosis was made only at laparotomy [17]. In our patient, a CT scan revealed the exact cause of the patient's problems and indicated operative treatment. Duodenoplasty, duodenoduodenostomy, duodenojejunostomy, gastrojejunostomy, and Roux-en-Y duodenojejunostomy are surgical options in the treatment of this anomaly [18, 19]. All these procedures can also be performed laparoscopically [20]. Although reported in the literature, the method of pancreatic resection has a higher incidence of complications, such as pancreatitis, pancreatic fistulae, and pancreatic insufficiency, and should be avoided [15]. Literature data do not suggest the preferable surgical procedure, so we

decided to perform Roux-en-Y duodenojejunostomy on our patient. In our opinion, duodenoplasty is not efficient for solving the problem. We consider that duodenoduodenostomy and duodenojejunostomy, the preferred methods in newborns, are inadequate in elder children because of a great dilation and disturbed motoric activity of the proximal duodenum and the stomach. Direct duodenojejunostomy and gastrojejunostomy can also lead to the postoperative reflux of jejunal content in dilated and hypoperistaltic duodenum and stomach [21]. Roux-en-Y duodenojejunostomy enables adequate duodenal emptying and prevents reflux of jejunal content; hence we consider this procedure an appropriate method for the treatment of PAP in elder children.

Conclusion

PAP is a rare congenital anomaly, unlike the well-known entity of CAP. The small incidence of this anomaly causes a great delay in establishing the correct diagnosis. It is very important to consider this anomaly as a potential cause of chronic feeding problems and vomiting. Operative treatment enables total cure and prompt recovery.

REFERENCES

1. *Mittal* S, *Jindal* G, *Mittal* A, *Singal* R, *Singal* S. Partial annular pancreas. Proc (Bayl Univ Med Cent) 2016; 29(4): 402–3.
2. *Singh SK, Agrawal DK, Suganita, Singh GN, Singh AK.* Annular pancreas causing duodenal obstruction: a case report. J Evolution Med Dent Sci 2016; 5(5): 296–300.
3. *Glazer G, Margulis A.* Annular pancreas: etiology and diagnosis using endoscopic retrograde cholangiopancreatography. Radiology 1979; 133(2): 303–6.
4. *Dowsett JF, Rode J, Russell RC.* Annular pancreas: a clinical, endoscopic, and immunohistochemical study. Gut 1989; 30(1): 130–5.
5. *Sandrasegaran K, Patel A, Fogel EL, Zyromski NJ, Pitt HA.* Annular pancreas in adults. AJR Am J Roentgenol 2009; 193(2): 455–60.
6. *Baggott BB, Long WB.* Annular pancreas as a cause of extrahepatic biliary obstruction. Am J Gastroenterol 1991; 86(2): 224–6.
7. *Zyromski NJ, Sandoval JA, Pitt HA, Ladd AP, Fogel EL, Mattar WE, Sandrasegaran K,* et al. Annular pancreas: dramatic differences between children and adults. J Am Coll Surg 2008; 206(5): 1019–25; discussion 1025–7.
8. *Urayama S, Kozarek R, Ball T, Brandabur J, Traverso L, Ryan J*, et al. Presentation and treatment of annular pancreas in an adult population. Am J Gastroenterol 1995; 90(6): 995–9.
9. *Chen YC, Yeh CN, Tseng JH.* Symptomatic adult annular pancreas. J Clin Gastroenterol 2003; 36(5): 446–50.
10. *Kandpal H, Bhatia V, Garg P, Sharma R.* Annular pancreas in an adult patient: diagnosis with endoscopic ultrasonography and magnetic resonance cholangiopancreatography. Singapore Med J 2009; 50(1): e29–31.
11. *Noor R, Mohamad Z, Farhan S, Nor T.* Annular pancreas causing duodenal obstruction in two consecutive sisters. Saudi Surg J 2017; 5(2): 87–8.
12. *Udgirkar S, Chandnani S, Zanwar V, Surude R, Contarctor Q, Rathi P.* Duodenal and Bile Duct Narrowing Due to Partial Annular Pancreas: A Rare Association. J Assoc Physicians India 2018; 66(6): 92–4.
13. *Lin SZ.* Annular pancreas. Etiology, classification and diagnostic imaging. Chin Med J (Engl) 1989; 102(5): 368–72.
14. *Jadvar H, Mindelzun RE.* Annular pancreas in adults: imaging features in seven patients. Abdom Imaging 1999; 24(2): 174–7.
15. *Wani AA, Maqsood S, Lala P, Wani S.* Annular pancreas in adults: a report of two cases and review of literature. JOP 2013; 14(3): 277–9.
16. *Mittal P, Gupta K, Mittal A, Gupta R.* Imaging findings in incomplete annular pancreas in adults with crocodile jaw appearance: report of two cases. Int J Health Allied Sci 2016; 5(4): 278–80.
17. *Inamoto K, Ishikawa Y, Itoh N.* CT demonstration of annular pancreas: case report. Gastrointest Radiol 1983; 8(2): 143–4.
18. *Johnston DW.* Annular pancreas: a new classification and clinical observations. Can J Surg 1978; 21(3): 241–4.
19. *Anish N, Adarash M, Kumar VV, Prabkharan M.* Incomplete annular pancreas presenting with gastric outlet obstruction and pancreatitis-a case report. Int J Sci Res 2019; 8(9): doi: 10.36106/ijsr.
20. *Hill S, Koontz CS, Langness SM, Wulkan ML.* Laparoscopic versus open repair of congenital duodenal obstruction in infants. J Laparoendosc Adv Surg Tech 2011; 21(10): 961–3.
21. *Ignjatović D, Cuk V, Misović S.* War, traffic and iatrogenic injuries of D3 duodenal segment. Vojnosanit Pregl 2005; 62(1): 69–72. (Serbian)

Received on May 12, 2020
Revised on April 17, 2021
Accepted on April 20, 2021
Online First April 2021

HISTORY OF MEDICINE

UDC: 61(091)
DOI: https://doi.org/10.2298/VSP210204031G

Pre-Hippocratic medicine

Medicina pre Hipokrata

Miloš Glišić*, Tatjana Lazarević†, Zoran Kovačević‡, Katarina Janićijević§, Mirjana A. Janićijević Petrović‖, Biljana Ljujić¶

University of Kragujevac, Faculty of Medical Sciences, *Department of Physiology, †Department of Internal Medicine, §Department of Social Medicine, ‖Department of Ophthalmology, ¶Department of Genetics, Kragujevac, Serbia; ‡University Clinical Center of Kragujevac, Department of Internal Medicine, Kragujevac, Serbia

Key words:
egypt, ancient; greece, ancient; history, ancient; medicine, persian; medicine, traditional.

Ključne reči:
egipat, drevni; grčka, drevna; istorija, drevna; medicina, persijska; medicina, tradicionalna.

Introduction

Traditionally and conventionally, Hippocrates is considered the father of medicine. In that sense, medicine is divided into pre-Hippocratic and post-Hippocratic.

Pre-Hippocratic medicine is not well known. There were no precise records in that period. Very few places had economics and legislation that would enable the development of culture and science, and thus medicine. However, this does not mean that the data do not exist [1]. There is even knowledge of some systems of organs, and the most detailed information about the organic system is related to blood circulation. Although sometimes completely wrong, they still give us a significant introduction to the way they used to think and treat people. Often, they would come to a knowledge that coincides with the present one. They knew about the bloodstream [2]. Moreover, the bloodstream was mentioned by the Indians, the Chinese, Ebers papyrus, Alcmaeon, Asclepius, Diogenes, and Empedocles. It is also possible to follow their system of thinking and sometimes come across valuable research material, such as their empirical knowledge of plant use. Many pharmaceutical preparations have been created in this way. They also had a fascinating knowledge of surgery, especially considering the lack of equipment, the knowledge of asepsis, antisepsis, and hemodynamics of blood vessels. In the pre-Hippocratic period, medical specializations, hygiene, public health, and family planning were conceived. Pre-Hippocratic medicine was based on religion, magic, and practical methods. That being said, it was believed that diseases were caused by supernatural forces; both Homer and the Bible mentioned stroke. In addition to those beliefs, the authors of the Bible placed the soul in the heart [3, 4]. Perhaps one of the most interesting procedures of pre-Hippocratic medicine was trepanation. The survival rate of ancient trepanations was surprisingly high. Trepanation was mainly done in Neolithic Europe, South America, Africa, and the Pacific [5]. It is considered the root of material understanding of diseases in Egyptian medicine [6]. This paper deals with the knowledge of pre-Hippocratic medicine in ancient civilizations such as Mesopotamia, Ancient Jews, Persia, India, China, Greece, and Egypt.

Mesopotamian pre-Hippocratic medicine

Mesopotamia is the oldest civilization. It was created by the Ancient Sumerians and was practically the first human society. It is the place where the first letter in the world was written. The letter was preserved on clay tablets in the form of a cuneiform letter. The existence of law and literacy led people to take care of their health systematically, and that was practically the beginning of medicine as we know it. Mesopotamia originated in the Euphrates and Tigris basins in 5000 BC. After Sumer, Mesopotamian civilization was continued by the Babylonians and Assyrians.

The medical data of this society come from the oldest medical record in the world. Namely, the most important part of the Mesopotamian corpus of knowledge was the Nippur tablets (Figure 1). They are the remnants of the library of King Ashurbanipal from the 7th century BC. Today, 20,000 tablets are in the British Museum, 1,000 of which are dedicated to medical information. They contained recipes for herbal and mineral medicines and healing incantations.

Correspondence to: Mirjana A. Janićijević Petrović, University of Kragujevac, Faculty of Medical Sciences, Department of Ophthalmology, Svetozara Markovica 69, 34 000 Kragujevac, Serbia. E-mail: mira.andreja@yahoo.com

Astronomy and mathematics had a significant place in Mesopotamian society. They considered the stars to be manifestations of their Gods. Diseases were thought to be caused by angry Gods and stellar movements. They corrupted the spirit in a man, which was seen as a disease. The cure was practically to cast out the devil. They believed that the heart was the center of intelligence and the blood the most important juice of life.

Fig. 1 – One of the Nippur tablets, 4663 BC [6]. (http/hal.archives-ouvertes.fr/hal-01139613/document-page 20)

In 2000 BC, the first law in the world – the Code of Hammurabi – was passed, and the first medical regulations were written there. The Hammurabi code of laws stipulated that if a slave died due to a doctor's mistake, the doctor had to buy a new slave for the owner, and if a free man died due to a doctor's mistake, both hands of the doctor had to be cut off. This was the first mention of medical responsibility. Such rigorous punishments hindered the most important thing for the development of medical procedures – experimentation. Nevertheless, Mesopotamian medicine left speculative records about maintaining the cleanliness of food and drink. It is also interesting that they isolated the lepers. However, there was no systematic advice for the people in this sense (Figure 1) [1, 5].

Ancient Jewish pre-Hippocratic medicine

Many diseases were described in the ancient Jewish books, but not the cure. That being said, it was considered that diseases were given by God as a part of God's plan and that the treatment was meaningless. Circulation, stroke, and brain leads were described. The heart was considered to be the place where the soul was. Even today, that belief is even symbolically deeply woven into the Christian tradition. There was a talk of leprosy. The two-week isolation was ordered for lepers, as well as wearing special clothes and verbally warning others of their disease. The treatment was left to the priests, and the hygienic and preventive measures were part of the religious practice.

The doctor as a special profession was mentioned only in the 2nd century BC. Interestingly, the cult of the doctor began to be built then, as the verses testify: "Respect a doctor because you need him. Because God created him, too; the doctor acquires wisdom from God; and receives gifts from the king; knowledge raises the head of the doctor; he stands proudly in front of the princes." The Talmud mentioned six doctors. Supervision of treatment was, however, the responsibility of a rabbi. They also issued hygiene regulations [1, 5].

Ancient Persian pre-Hippocratic medicine

Zoroastrianism ruled Old Persia. The essence of that religion, which still exists today, was the eternal dualism of good and evil. The God of good was Ormuzd, and the God of evil was Ahriman. Naturally, Ahriman was the one who caused the diseases. Because of that, there were no doctors in Old Persia. People were treated by priests. Even surgeons, who later appeared, were subordinate to them, being seen as artisans who were healing war wounds. Medical data were found in religious books called Avesta. Hygiene and prevention had a religious connotation. The cemeteries were built on hills, and the lepers were isolated [1, 5]. Healing was carried out with prayers, incantations, and herbs. In traditional Persian medicine, amenorrhea and oligomenorrhea were treated with cinnamon, nettle, mint, sesame, peony, hemp, fenugreek, and fennel [7, 8].

Indian pre-Hippocratic medicine

There are not many records of healing from the Incas, Maya poeple, and Aztecs. They saw disease as a punishment from Gods and demons. They prayed, offered sacrifices, performed trepanation, and used herbs for treatment. It is known that they used *Erythroxylum coca* and tobacco. They gave *Carapichea ipecacuanha* in amoebic dysentery as an emetic and quinine for malaria. The Maya protected themselves from demons by tattooing. Their medicine was somewhat similar to the Chinese one. However, it is still not known whether this was due to the backwardness of Asian teachings in the first people who crossed the Bering Strait or whether the two teachings developed independently of each other [1, 5, 7, 8]. Some of the plants that the Cherokees used and still use today are yarrow (for wound healing), ginseng (anti-inflammatory effect), blue cohosh (antirheumatic), black cohosh (serotoninergic), and Scutellaria (anxiolytic) [9].

Ancient Indian pre-Hippocratic medicine

The first Indian medical records were made between 1500 and 500 BC. Diseases were perceived as a magical act and treated with a mixture of rituals and herbs; surgery was also present. They were found in the holy books called the Vedas. Medical records also appeared in the oldest Vedas – the Rigveda. That book talked about doctors, surgeons, and herbal treatments. Younger Vedas or Atharva Veda talked

about leprosy, rheumatism, venereal and eye diseases, abscesses, tumors, jaundice, pain, and other problems. They paid great attention to hygiene and forbidden meals. Alcohol, mushrooms, onion, and garlic were banned. There were three Indian medical systems: Ayurveda, Siddha, and Unani. Ayurveda was a part of the Atharva Veda and had a special significance for ancient Indian medicine. Ayurveda had eight books on medicine. Around 500 BC, medicine in India completely passed into the hands of priests. They believed that the body consisted of 3 principles – air, bile, and phlegm – which built seven systems: lymph, flesh, bones, fat, blood, marrow, and semen. It was believed that diseases occurred if these elements were not in balance. This system of thinking was quite obviously a precursor to the hypocritical temperaments and Avicenna's myiasis, especially since, in different historical periods, there were fusions or overflows of knowledge between these cultures. The famous doctors Sushruta, Charaka, and Wabhata wrote one book each. Sushruta's book had a description of about 1,000 diseases, and there were a lot of surgeries in it. He also wrote about inhalation and baths. They performed rudimentary auscultation, palpation, and pulse examination and used smell and taste. Although they knew little about anatomy because they did not dissect corpses, they performed hernia surgery, rhinoplasty, laparotomy, amputations, lithotomy, and eye surgeries. They were the first to perform cataract surgery. In the fifth century BC, they had their first hospitals [1, 10].

Chinese pre-Hippocratic medicine

Chinese pre-Hippocratic medicine is believed to have originated 5000 years BC. However, Shen Nung is considered the father of Chinese medicine. He wrote a collection of 350 medicinal herbs with their effects. However, Huang-ti made an even greater impact, making a great medical book between 2698 and 2598 BC. Chang Chung-ching, who was called the Hippocrates of China, worked during the Han dynasty between 206 and 220 BC.

According to the Chinese belief, there was a male principle of yang (health, activity) and a female principle of yin (illness, passivity). For the health to subsist, yang had to prevail. Everything consisted of five elements: fire, earth, water, wood, and metal. Everything was in balance, nothing disappeared, but one arose from the other. The Chinese understood diseases in this philosophical-metaphorical way. Namely, there were five main organs – heart, lungs, liver, kidneys, and spleen – and five secondary – gallbladder, small intestine, stomach, colon, and bladder. They even thought that relations between the organs existed – the liver was the mother, the stomach was the son, and the kidneys were the enemy of the heart. Chinese doctors did not deal with surgery and obstetrics; midwives gave birth. Only fractures were treated with extension. Herbs and pulse examination were used; as many as 200 types of pulse were distinguished. The Chinese doctors would also examine a person for several hours. They gave iron for anemia, gelatin for bleeding, and opium for pain, and they knew about anesthesia. Even today, acupuncture still has an impact on treatment. Their strength was in observation, so they knew about infectious diseases (smallpox, dysentery, cholera, variola, syphilis, taenia, Ascaris). The first attempts at immunization were a valuable contribution of Chinese medicine. They took pus from the smallpox lixivium, dried it and ground it, and gave the patient to snort it, even recording successes [1, 9]. Recent discoveries have shown that, contrary to the attitudes to date, trepanation was widely practiced in China as well. The misconception is thought to have been caused by problems while translating ancient writings. This practice dates back several thousand years, even with a solid survival rate. Moreover, there are records of surgeons, such as Hua Tuo [11], who performed cranial interventions.

Greek pre-Hippocratic medicine

As everywhere in the ancient world, diseases were attributed to supernatural causes that had physical manifestations. That is why the healing was mostly magical. The terms used in Greek medicine in the pre-Hippocratic period to refer to medicines were *iatromantis*, pholarcos, ouliads, and asclepiads. Only later did they move to a rational conception of medicine or techneiatrike [7, 12].

One of the first Greek physicians was Alcmaeon of Croton on the territory of today's Southern Italy in the 6th century BC. Many consider him the father of scientific medicine. There are 18 preserved texts about him, and five are believed to have been written by himself. They are mostly about physiology and psychology. He was also a philosopher, and many of his works on epistemology have been preserved. Herodotus also praised his healing skills [11]. Alcmaeon gave a description of the strokes and brain injuries, Alexandrian authors described nerves, and Galen connected cognition to the brain. Ancient authors even divided the brain into parts according to functions [4, 12]. Herodotus' writing about the Persian king Cambyses II is one of the most significant and earliest records of epilepsy. Specifically, he attributed the king's illness to either an angry God or a holy disease. He also speculated about the option that diseases were somatic. It is the first record to consider epilepsy as a somatic disease. This was crucial for beginning the transition of understanding the etiology of the disease as unnatural to somatic [13]. It is believed that Homer's description of the Trojan War in the Iliad contained the earliest records of neurological injuries. A description of the following was given: brain stem injuries, penetrating head injuries, signs of brain death, damage to the spinal cord, brachial plexus, and peripheral, all with signs of injuries. Note that this data is 5,000 years old [14]. Trepanation was performed in Ancient Greece in the Bronze Age. Paleo surgery is an area that dealt with trepanation in ancient times. In 2014, Papagrigorakis et al. [15] described the trepanation of the right parietal bone of the skull of a 30-year-old man found in the Delphi area. The tests were performed on computed tomography (CT). It was concluded that metal tools had been used and that they dated between 1900 and 1600 BC.

Egyptian pre-Hippocratic medicine

Ancient Egypt is a great civilization that lasted from 3300 to 525 BC. Among the oldest medical records are those that originate from ancient Egypt. The Egyptians significantly contributed to our understanding of the vascular system. However, perhaps the greatest medical significance of the ancient Egyptian data is precisely in the fact that they were the first to set up a concept that we understand today as public health. It should be noted that ancient Egyptian medical sources and the confluence of Greek pre-Hippocratic medicine were claimed by Pliny the Elder and Galen [1, 2, 15].

As in Mesopotamia, scholars in Egypt also developed mathematics and astronomy. The country had a long cultural continuity. They started as nomads but established a strong system of law and rule. This allowed for relative stability in economic and political terms. Such societies could record data, impose rules and standards, and train people for a variety of occupations. All this is a prerequisite for medical development. They were able to impose different laws, and that was of special importance for public health.

Naturally, a mystical view of the world was to be expected. In that sense, they also observed the human body. The ancient Egyptians saw diseases as magical entities with physical manifestations. They believed that spirits, demons, and Gods created diseases by blocking the standard channels of movement of the spirit in the human body. The entity that was physically blocking the channels was allegedly Wekhedu. They thought there were 46 channels, and once they were blocked, pus appeared. They thought the Gods could get angry by bad behavior or in some other way. Like every pantheon, the Egyptian Gods had their own sectors, even in the domain of health. The goddess of medicine was Heka. During pregnancy, women were protected by Bes. Serket protected and healed from the scorpion bites. During the treatment, these channels were magically unblocked. In that sense, patients were treated with a combination of magical and physical methods. In the beginning, there was no doctor as a profession. The treatment was performed mostly by priests of Sekhmet. Sekhmet was an Egyptian warrior goddess and the goddess of healing, curses, and threats. Only in the later period did the forerunners of today's doctors appear. Egyptian doctors were widely known in foreign countries. As magical means, they used prayers, incantations and rituals, amulets and good luck charms, tattoos, statues, and gifts. The first recorded Egyptian physician was Hesy-Ra in 2700 BC during the reign of King (pharaoh) Djoser. He was the commander of doctors and dentists. The first recorded Egyptian physician was Peseshet about 2400 BC, but both of them are thought to have existed at least 500 years earlier. The best doctors worked in the palace. There were medical supervisors and specializations (ophthalmologist, dentist, stomach doctor, proctologist forerunner). Interestingly, the proctologist was called "*neryphuyt*", which literally means anal shepherd [2, 15]. Interestingly, the punishments for bad treatment were so rigorous that the doctor could have been sentenced to death.

From the physical methods, they used rudimentary surgery, herbs, and some tools. There is even a display of Isis on the birthing stool. They learned about the human body through the process of mummification. The economic potential enabled them to procure medicines and plants from the most remote areas for at least some members of society. Moreover, in the absence of immediate existential care, they were able to experiment with treatment and raise the quality of life standards.

There are records stating they took care of the health of the builders of the pyramids and that the builders were given some variant of compensation in case of illness. For the time they lived in, it was surrealistically advanced. Needless to say, the average resident could not even dream of this level of quality of life and health care.

The medical literature of ancient Egypt is one of the richest of that period. The Ebers papyrus (Figure 2) is among the oldest medical records. It is believed to have been written in about 1500 BC. The document may possess material dating back to 3400 BC. The Ebers papyrus contains over 700 magical formulas, incantations, and prescriptions for medicines. Some of the data from the Ebers papyrus still have credibility today. Osteology was quite credible at that time, and they also knew something about how the brain and liver worked. In addition to the Ebers papyrus as the most valuable preserved document written in the hieratic script (13 medical papyri in total), the Edwin Smith papyrus is also worth mentioning. Edwin Smith was a rich American who bought the papyrus in 1862 and donated it to the library of the New York Academy of Sciences. This text is a real textbook of orthopedics and traumatology. However, the most important medical knowledge of ancient Egypt was related to blood circulation. The Ebers papyrus [16] mentions hemiplegia and brain injuries [2, 15].

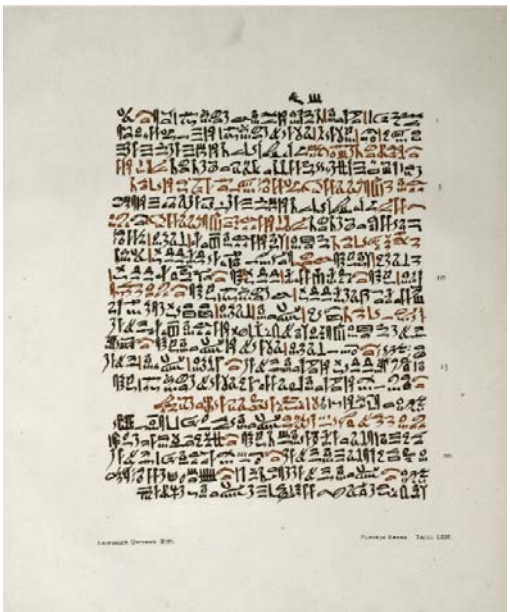


Fig. 2 – Papyrus Ebers, Leipzig University [16]. (http://digi.ub.uni-heildelberg.de/digilit /ebers 1875bd1/-page 21)

Egyptian vision of blood flow

The channel system was believed to look like this: Vessels come from the heart and carry the appropriate fluid for each part of the body. It is believed that the air goes first to the heart, then to the lungs, and then to the stomach. The nostrils have four vessels – two carry mucus and two carry blood. The 'breath of life' enters the right ear, and the 'breath of death' enters the left ear. Four vessels lead to the parietal part of the head, and if something is not right with them, the person goes bald. There are four vessels in the forehead for the eyes, and the problem with them causes eye diseases. Six vessels reach the soles of the feet and six lead down the arms to the fingers. Four vessels bring air and fluid into the lungs and spleen. Two vessels carry urine to the bladder. Four vessels supply the liver with liquid and air and must not be overfilled because it causes diseases. Two vessels enter the testicles and provide semen. Two vessels go into the glutei muscles. Finally, four vessels that carry air and feces go into the anus.

Although they made many mistakes in describing blood flow, it is still fascinating how much they understood it at all [17]. However, they had access to blood vessels visually only through the process of mummification. In no way could they functionally examine the blood vessels. Yet, they understood that the heart was the center of the bloodstream and that it pumped blood into the body through blood vessels. Furthermore, they considered the heart to be the meeting point for vessels that carried not only blood but urine, tears and semen, and feces. That system of vessels was called Metu. It was thought that feces could contaminate parts of the body that it did not need. For that reason, the enema was considered a treatment for many diseases, such as malaria and smallpox. However, they thought that the most important physiological function was breathing [2, 17].

Diseases of ancient Egypt

Ancient Egyptians used opium or thyme for pain. For headaches, they advised to mash together flour, incense, the wood of we, wane plant, mint, a horn of a stag, sycamore seeds, mason's plaster, and water and apply to the head; or use poppy seeds or aloe. They used honey, milk, and frankincense for asthma.

Sandalwood, mint, garlic, and juniper were used for digestion, and mint for bad breath. Emetic was mustard, and antiemetic mint. Epilepsy was treated with camphor. Interestingly, they treated the cold with incantations. They also had a rudimentary knowledge of gynecology. They were acquainted with the signs of pregnancy and herbal contraception. They used a plug of crocodile scale for contraception as a kind of diaphragm. They knew about some gynecological diseases as well. Ancient Egypt had the knowledge of dental treatment. For gingivitis, they used cumin, incense, and onion. The pain was relieved with opium. They even drilled holes in the bones to heal the abscess. It was not noticed that they extracted the teeth; they filled their teeth. They understood some mental disorders such as dementia and depression, believing that they were the act of angry Gods and evil spirits that blocked the channels. They knew about some skin, intestinal, and eye diseases. They treated burns with aloe, and willow leaf wraps cured inflammations [18].

Ancient Egyptians performed some surgical procedures. Specifically, they knew how to repair broken bones and dislocated joints, perform amputations, and operate on abscesses and tumors. This surgical knowledge was also a part of the mummification. For instance, they inserted a hook through the nose to break bones (probably *lamina cribrosa*) and remove the brain. They used hooks, spoons, knives, and even forceps. They tried to fill the bone holes with oyster eggs, probably as a variant of homeopathy. They had prostheses, but only when mummified – they were not a therapeutic tool. They also performed circumcisions. They sutured the wounds; they burned wounds and hemorrhoids (primitive cauterization). They drew blood [1, 2, 18].

However, perhaps the beginning of medical hygiene was the greatest contribution of ancient Egypt to medicine. They understood the concept of infection and dirt. They taught people that rotten food, especially fish, and dirty water were dangerous. They advised bathing and cleaning food. They highly valued cleanliness so much that they connected it to religious practices. They believed that not only cleanliness but also makeup around the eyes contributed to the protection from diseases. The priests wore white linen robes and regularly washed the body, dishes, and clothes. They used mosquito nets because malaria was common. They even had primitive toilets [1, 18].

Conclusion

Pre-Hippocratic medicine is the beginning of the systematization of human treatment. Everything we know today about medicine dates back to the pre-Hippocratic period. Ethics, medical responsibility, phytotherapy, surgery, hygiene, public health, family planning, immunization, and even medical specializations have their roots in pre-Hippocratic medicine.

This period is often associated with the mystical in medicine. If this knowledge were applied today, in most cases, it would certainly belong to pseudoscience. However, things must be viewed in the context of a given time and historical-cultural circumstances. With the few resources and options they had, these people laid the foundations of today's scientific medicine. Not to mention that we still use some of their conclusions to this day. Surgeons often use their procedures, and their work with plants is largely the basis of pharmacology. Pre-Hippocratic medicine needs to be well researched. Some forgotten empirical knowledge of the old doctors may prove to be life-saving.

R E F E R E N C E S

1. *Janjić M, Timotić B.* Development of medical thought and practice with the basics of biomedical scientific informatics. Kragujevac: Faculty of Medicine; 1994. (Serbian)
2. Ancient Egyptian Medicine. Available from: http://www.reshafim.org.il/ad/egypt/timelines/topics/medicine.html.
3. *Loukas M, Tubbs RS, Louis RG Jr, Pinyard J, Vaid S, Curry B.* The cardiovascular system in the pre-Hippocratic era. Int J Cardiol 2007; 120(2): 145–9.
4. *Rose FC.* Cerebral localization in antiquity. J Hist Neurosci 2009; 18(3): 239–47.
5. *González-Darder JM.* Cranial trepanation in primitive cultures. Neurocirugia (Astur) 2017; 28(1): 28–40. (Spanish)
6. *Proust C.* Does a Master Always Write for His Students? Some Evidence from Old Babylonian Scribal Schools. Scientific Sources and Teaching Contexts Throughout History: Problems and Perspectives. New York, US: Springer; 2014. p. 69–94.
7. *Lips Castro W, Urenda Arias C.* Medicine in the pre-Hippocratic civilization of ancient Greece. Gac Med Mex 2014; 150(Supp 3): 369–76. (Spanish)
8. *Moini Jazani A, Hamdi K, Tansaz M, Nazemiyeh H, Sadeghi Bazargani H, Fazljou SMB,* et al. Herbal Medicine for Oligomenorrhea and Amenorrhea: A Systematic Review of Ancient and Conventional Medicine. Biomed Res Int 2018; 2018: 3052768.
9. *Setzer WN.* The Phytochemistry of Cherokee Aromatic Medicinal Plants. Medicines (Basel) 2018; 5(4): 121.
10. *Rao MS.* The history of medicine in India and Burma. Med Hist 1968; 12(1): 52–61. doi: 10.1017/s002572730001276x.
11. *Hobert L, Binello E.* Trepanation in Ancient China. World Neurosurg 2017; 101: 451–6.
12. *Santacroce L, Charitos IA, Topi S, Bottalico L.* The Alcmaeon's School of Croton: Philosophy and Science. Open Access Maced J Med Sci 2019; 7(3): 500–3.
13. *York GK, Steinberg DA.* The sacred disease of Cambyses II. Arch Neurol 2001; 58(10): 1702–4.
14. *Sahlas DJ.* Functional neuroanatomy in the pre-Hippocratic era: observations from the Iliad of Homer. Neurosurgery 2001; 48(6): 1352–7.
15. *Papagrigorakis MJ, Toulas P, Tsilivakos MG, Kousoulis AA, Skorda D, Orfanidis G,* et al. Neurosurgery during the Bronze Age: a skull trepanation in 1900 BC Greece. World Neurosurg 2014; 81(2): 431–5.
16. *Veiga P.* Oncology and Infectious Diseases in Ancient Egypt: The Ebers Papyrus' Treatise on Tumours 857-877 and the Cases Found in Ancient Egyptian Human Material [dissertation]. England, Manchester: University of Manchester, Faculty of Life Sciences; 2008. Available from: https://vtechworks.lib.vt.edu/handle/10919/71527?show=full
17. *Barr J.* Vascular medicine and surgery in ancient Egypt. J Vascul Surg 2014; 60(1): 260–3.
18. *Mark JJ.* Egyptian medical treatments. Horsham, United Kingdom: World History Encyclopedia; 2017.

Received on February 4, 2021
Revised on March 9, 2021
Accepted on March 11, 2021
Online First March 2021

INSTRUCTIONS TO THE AUTHORS

The VSP publishes only papers not published before, nor submitted to any other journals, in the order determined by the Editorial Board. Any attempted plagiarism or self-plagiarism will be punished. When submitting a paper to the VSP electronic editing system **(http://aseestant.ceon.rs/index.php)**, the following should be enclosed: a statement on meeting any technical requirements, a statement signed by all the authors that the paper on the whole and/or partly has not been submitted nor accepted for publication elsewhere, a statement specifying the actual contribution of each author, no conflict of interest statement that make them responsible for meeting any requirements set. What follows subsequently is the acceptance of a paper for further editing procedure. The manuscripts submitted to the VSP pass in-house and external peer review. All authors pay "Article Processing Charge" for coverage all editing and publishing expenses. Domestic authors pay 5,000 RSD, and those from aboard 150 euros. The editing and publishing fee is required for substantive editing, facts and references validations, copy editing, and publishing online and in print by editorial staff of the Journal. No additional fees, other than stated above, are required even if an author who already paid the fee would have more articles accepted for publishing in the year when fee was paid. All authors who pay this fee may, if want, receive printed version of the Journal in year when fee is payed. Please note that the payment of this charge does not guarantee acceptance of the manuscript for publication and does not influence the outcome of the review procedure. The requirement about paying "Article Processing Charge" does not apply to reviewers, members of the Editorial Board and the Publisher's Council of the Journal, young researchers and students, as well as any of the subscribers of the Journal.

The VSP publishes: **editorials, original articles**, **short communications**, **reviews**/**meta-analyses, case reports**, **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, and other. Original articles, short communications, meta-analyses and case reports are published with abstracts in both English and Serbian.

General review papers will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided as reserved for subtitles. Original articles, reviews, meta-analyses and articles from medical history should not exceed 16 pages; current topics 10; case reports 6; short communications 5; letters to the editor and comments 3, and reports on scientific meetings and book reviews 2.

All measurements should be reported in the metric system of the International System of Units (SI), and the standard internationally accepted terms (except for mmHg and °C).

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office** (**Excel**, **Word Graph**)**.** The use of colors and shading in graphs should be avoided.

Papers should be prepared in accordance with the **Vancouver Convention**.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

1. ***Title page***

a) The title should be concise but informative, while subheadings should be avoided;

b) Full names of the authors signed as follows: ***, †, ‡, §, ‖, ¶, **, ††, ...** .

c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

d) Conclusion could be a separate chapter or the last paragraph of the discussion;

e) Data on the corresponding author.

2. ***Abstract and key words***

The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

Methods. The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethnics Committee for the tests in humans and animals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al*. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

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

Tables

Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper left corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

Illustrations

Any forms of graphic enclosures are considered to bi figures and should be submitted as additional databases in the System of Assistent. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure (**Figure 1**, **Figure 2** and so on). If a figure has been published, state the original source.

Legends for illustrations are typed on a separate page, with Arabic numbers corresponding to the illustrations. If used to identify parts of the illustrations, the symbols, arrows, numbers, or letters should be identified and explained clearly in the legend. Explain the method of staining in photomicrographs.

Abbreviations and acronyms

Authors are encouraged to use abbreviations and acronyms in the manuscript in the following manner: abbreviations and acronyms must be defined the first time they are used in the text consistently throughout the whole manuscript, tables, and graphics; abbreviations should be used only for terms that appear more than three times in text; abbreviations should be sparingly used.

An alphabetical list of all abbreviations used in the paper, followed by their full definitions, should be provided on submission.

Detailed Instructions are available at the web site:

www.vma.mod.gov.rs/vsp

UPUTSTVO AUTORIMA

VSP objavljuje radove koji nisu ranije nigde objavljivani, niti predati za objavljivanje redosledom koji određuje uređivački odbor. Svaki pokušaj plagijarizma ili autoplagijarizma kažnjava se. Prilikom prijave rada u sistem elektronskog uređivanja „Vojnosanitetskog pregleda“**(http://aseestant.ceon.rs/index.php)** neophodno je priložiti izjavu da su ispunjeni svi postavljeni tehnički zahtevi uključujući i izjavu koju potpisuju svi autori da rad nije ranije ni u celini, niti delimično objavljen niti prihvaćen za štampanje u drugom časopisu. Izjavu o pojedinačnom doprinosu svakog od autora rada potpisanu od svih autora, treba skenirati i poslati uz rad kao dopunsku datoteku. Takođe, autori su obavezni da dostave i potpisanu izjavu o nepostojanju sukoba interesa čime postaju odgovorni za ispunjavanje svih postavljenih uslova. Ovome sledi odluka o prihvatanju za dalji uređivački postupak. Rukopisi pristigli u Redakciju časopisa podležu internoj i eksternoj recenziji. Svi autori dužni su da plate “Article Processing Charge” za pokriće troškova jezičke, stručne i tehničke obrade rukopisa, kao i njegovog objavljivanja. Domaći autori plaćaju iznos od 5 000 dinara, a inostrani 150 eura. Dodatna plaćanja nisu predviđena čak i u slučaju da autor koji je već prethodno platio traženi iznos, ima više prihvaćenih radova za objavljivanje u godini u kojoj je izvršio uplatu. Svi autori koji su platili “Article Processing Charge” mogu, ukoliko žele, dobijati štampanu verziju časopisa tokom godine u kojoj je izvršena uplata. Plaćanje ovog iznosa ne garantuje prihvatanje rukopisa za objavljivanje i ne utiče na ishod recenzije. Od obaveze plaćanja pokrića navedenih troškova oslobođeni su recenzenti, članovi Uređivačkog odbora i Izdavačkog saveta VSP, studenti i mladi istraživači, kao i pretplatnici časopisa.

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme, metaanalize**, **kazuistika, seminar praktičnog lekara,** članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Pre objave, rad se upućuje autoru određenom za korespodenciju na konačnu saglasnost.

Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi **– Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

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.

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

Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp