YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2021; March Vol. 78 (No. 3): pp. 285–384.

Vojnosanitetski Pregled 2021 March Vol. 78 (No. 3): pp. 285–384.

vol. 78, br. 3, 2021

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
Univerzitet odbrane, MO Republike Srbije

ISSN 0042-8450
eISSN 2406-0720

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). 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

vol. 78, br. 3, 2021

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

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

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). 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

CONTENTS / SADRŽAJ

Monument to the Chernobyl victims in front of the Chernobyl Nuclear Power Plant (Ukraine)
Spomenik žrtvama černobiljskog akcidenta ispred nuklearne elektrane u Černobilju (Ukrajina)

This year marks the 35th anniversary of the Chernobyl accident (April 26, 1986) and the 10th anniversary of the Fukushima accident in Japan (March 11, 2011), two largest nuclear accidents in history whose causes and consequences for human health are still the subject of scientific research and controversy. This is an opportunity to remind ourselves once again of how to use the acquired knowledge and experience in improving the existing preparedness and plans for disaster management.

Ove godine navršava se 35 godina od černobiljskog akcidenta (26. april 1986) i 10 godina od akcidenta u oblasti Fukušima, Japan (11. mart 2011), dva najveća nuklearna akcidenta u istoriji, čiji su uzroci i posledice po ljudsko zdravlje još uvek predmet naučnih istraživanja i polemika. Ovo je prilika da se još jednom podsetimo kako iskoristiti stečeno znanje i iskustvo u poboljšanju postojeće pripremljenosti i planova za upravljanje katastrofama.

ORIGINAL ARTICLES

UDC: 616.31
https://doi.org/10.2298/VSP190225043E

Anti-inflammatory effect of amalgam on periapical lesion cells in culture

Anti-inflamacijski efekat amalgama na ćelije iz periapeksne lezije u kulturi

Mile Eraković*, Miloš Duka*†, Marina Bekić‡, Marijana Milanović†, Sergej Tomić‡, Dragana Vučević†‡, Miodrag Čolić†‡§

Military Medical Academy, *Clinic for Stomatology, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ‡Institute for the Application of Nuclear Energy, Belgrade-Zemun, Serbia; University of East Sarajevo, §Faculty of Medicine, Foča, Republic of Srpska, Bosnia and Herzegovina

Abstract

Background/Aim. Amalgam has been used for years in dentistry, but the controversy on its adverse effects, both on local oral/dental tissues and systemic health, still exists. When used for retrograde filling in apical surgery, amalgam comes in close contact with the periapical tissue, and it is sometimes responsible for the induction of periapical lesion (PL) or its exacerbation. Therefore, the aim of the study was to examine the effect of amalgam on cytotoxicity and production of pro-inflammatory cytokine by cells isolated from PL. **Methods.** Conditioned medium from freshly prepared amalgam (ACM) was performed according to the ISO 10993-12 by incubating the alloy in RPMI medium (0.2 g/mL) for 3 days at 37°C. Cells were isolated from 20 human PLs after apicoectomy by collagenase/DNA-ase digestion and cultured with different dilutions of ACM. Cytotoxicity was determined by MTT assay (n = 7 cultures) and apoptosis/necrosis assays (n = 8 cultures), whereas cytokine production was measured by a Flow Cytomix Microbeads Assay (n = 8 cultures). **Results**. Undiluted (100%) and 75% ACM was cytotoxic due to induction of apoptosis of PL cells. Non-cytotoxic concentrations of ACM (50% and 25%) inhibited the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8), concentration-dependently. **Conclusion.** For the first time, our results showed an unexpected anti-inflammatory property of amalgam on PL cells, which could be beneficial for PL healing after apicoectomy.

Key words:
dental amalgam; periapical tissue; cytokines; cytotoxicity, immunologic; inflammation; apicoectomy.

Apstrakt

Uvod/Cilj. Amalgam se godinama koristi u stomatologiji, ali i dalje postoje kontroverze o njegovim neželjenim efektima na lokalno oralno/dentalno tkivo i sistemsko zdravlje. Kada se koristi za retrogradno punjenje u apikalnoj hirurgiji, amalgam dolazi u blizak kontakt sa periapeksnim tkivom, što je ponekad povezano sa indukcijom periapeksne lezije (PL) ili njenom egzacerbacijom. Zato je cilj ovog rada bio da se ispita efekat amalgama na citotoksičnost i produkciju pro-inflamacijskih citokina od strane ćelija izolovanih iz PL. **Metode.** Od sveže napravljenog amalgama pripremljen je kondicionirani medijum (ACM) inkubiranjem legure na 37°C u RPMI medijumu u toku 3 dana (0.2 g/mL) kako je predloženo standardom ISO 10993-12. Ćelije su izolovane iz 20 humanih PL nakon apikoektomije, digestijom tkiva pomoću kolagenaze/DNA-aze, a zatim su korišćene za kulturu u prisustvu različitih razblaženja ACM. Citotoksičnost je ispitivana pomoću MTT testa (n = 7 kultura) i detekcijom apoptoze/nekroze (n = 8), dok je nivo produkovanih citokina meren simultano pomoću eseja sa mikrokuglicama uz pomoć protočne citometrije (n = 8). **Rezultati.** Nerazblažen ACM (100%) i onaj od 75% pokazali su citotoksični efekat, indukujući apoptozu PL ćelija. Necitotoksične koncentracije ACM (50% i 25%) inhibirale su produkciju pro-inflamacijskih citokina (TNF-α, IL-1β, IL-6 i IL-8) na dozno-zavisan način. **Zaključak.** Naši rezultati po prvi put pokazuju neočekivano antiinflamacijsko svojstvo amalgama na PL ćelije, što može biti korisno za zarastanje lezije nakon apikoektomije.

Ključne reči:
amalgam, stomatološki; periapeksno tkivo; citokini; citotoksičnost, imunološka; zapaljenje; apikoektomija.

Correspondence to: Miodrag Čolić, Institute for the Application of Nuclear Energy, Banatska 31b, 11 080 Belgrade-Zemun, Serbia.
E-mail: mjcolic@eunet.rs

Introduction

Dental amalgam is one of the most versatile restorative materials that has been used in dentistry for about 170 years, particularly as the first choice for restoring posterior teeth. However, it has myriads of uses, including root-end filling in periapical surgery [1–3]. This procedure prevents the invasion of irritants from infected root canals into the periapical tissues. The advantage of using amalgam for retrograde filling for such a long period of time is its self-sealing capacity, easy manipulation, radio-opacity, and insolubility in tissue fluids [2]. The preferred amalgam is a high copper-zinc-free amalgam, composed of silver 40%–70%, tin 12%–30%, and copper 12%–24%. However, it has many disadvantages, such as the production of corrosive by-products [4, 5], cytotoxicity of mercury and other dissolved metal ions, moisture sensitivity, and staining of hard and soft tissues [1, 6, 7]. There is a possibility of releasing non-resorbable scattered particles during amalgam manipulation, which may be difficult to retrieve [2]. Moreover, amalgam does not properly seal the root end three-dimensionally, has poor marginal adaptation, and does not prevent the leakage of microorganisms and their products in the peri-radicular tissue [2]. However, despite these disadvantages and evidence of a decrease in its use, amalgam's cost, durability, and ease of manipulation have persuaded many dentists to continue to use it, and amalgam remains a standard to which other materials are compared [2, 8].

The major concern for using amalgam in dentistry is its cytotoxic effect, which has been documented in many human and animal cells as well as in established cell lines *in vitro* [6, 9–11]. In the past few decades, however, potential systemic and local toxic effects have been described *in vivo* [2, 3, 12, 13]. Patients may suffer from hypersensitivity reactions to mercury or other amalgam components. Other reactions to amalgam with a variety of clinical symptoms, collectively termed "amalgam disease," have been reported, including adverse immunological effects and autoimmune phenomena [12, 14, 15].

Clinical and histopathological studies show that amalgam, implanted subcutaneously or in the bone, is well tolerated [16, 17]. This is in contrast with some studies showing the capability of amalgam particles to cause periapical lesions [18] and to cause a cytotoxic effect on periodontal ligament cells and periodontal fibroblasts [19–21]. However, there is no study investigating the effect of amalgam on human periapical lesion (PL) cells *in vitro*, which was the main goal of our study. This knowledge is important since the alloy communicates with the periapical tissue for a long period of time. Our results showed for the first time an unexpected anti-inflammatory effect of amalgam on PL cells which could be beneficial for PL healing.

Methods

Periapical lesion samples

Human PLs (n = 20) were extracted during apicoectomy at the Department of Oral Surgery, Clinic for Stomatology, Military Medical Academy (MMA), Belgrade, Serbia. The study was approved by the Ethics Committee of the MMA, followed by informed consent from patients. The exclusion criteria included the following: patients with malignant, autoimmune, and other chronic inflammatory diseases, as well as those on immunosuppressive/immunomodulatory therapy. The patients included had not been treated with antibiotics for one month prior to the PLs excision. PLs were diagnosed by clinical and radiographic criteria. No distinction was made between age, sex, tooth type, size, and clinical presentation of PLs. After extraction, PLs were immediately placed in a medium consisting of RPMI-1640 (Sigma, Munich, Germany) and antibiotics/antimycotics and transported to the laboratory.

Isolation of cells from PLs

The cells from PLs were isolated by a procedure that has been previously introduced by our research group [22]. Briefly, periapical tissue was placed in a Petri dish containing 1 mL RPMI-1640 medium and cut into 2–3 mm diameter pieces using a scalpel. The tissue was then digested for 20 min with 0.05% collagenase type IV (Sigma) and 0.02% DNA-ase (Sigma) dissolved in RPMI-1640 medium in a cell incubator at 37 °C. After that, the tissue was pressed through a stainless-steel mesh using a syringe plunger, filtered, and resuspended in RPMI-1640 medium containing 1 mM EDTA. The released cells were pooled, washed twice by centrifugation in the RPMI medium at room temperature (400 g for 10 min), and counted. The viability of cells, determined by Trypan Blue dye, was 93% ± 3%. The cells were used for *in vitro* experiments. Eight periapical lesions were used to study cytokine production and apoptosis/necrosis. Twelve PLs containing either a larger number of cells (higher than 2.0×10^6 cells; n = 4 PLs) or pooled PLs from the same donors (n = 8 PLs from 3 patients) were used for the MTT assay. The total number of individual cultures for this assay was 7.

Preparation of conditioned medium

Amalgam, consisting of the encapsulated alloy (Extracap) and mercury, was purchased from Galenika, Belgrade, Serbia. One-gram (g) powder of the alloy contained silver (500 mg), tin (299 mg), and cooper (201 mg). The alloy mass was 0.360 g, and the mercury mass was 0.400 g. Amalgam specimens were prepared by triturating amalgam alloy powder with pure mercury in an amalgamator, and after the mixture, disc-form specimens, diameter around 10 mm, thickness about 1–2 mm, were prepared. The freshly prepared amalgam discs were used for the preparation of amalgam conditioned medium (ACM) by placing the amalgam disc in a glass tube containing RPMI-1640 medium with an addition of antibiotics/antimycotics. The mass of amalgam to the volume of RPMI medium was 0.2 g/mL according to ISO 10993-5 and ISO 10993-12. The conditioning lasted for 3 days. Control CM was prepared by incubating control inert material, polystyrene, under the same conditions. ACM and control (C)-CM were supplemented with 10% FCS. There was no need for pH adjustment, which remained 7.4. Such prepared CM were further used for PL cell culture experiments.

Cell cultures

The cells isolated from PLs were cultivated in 96-wells, with round-bottomed plates (ICN, Costa Mesa, CA) (1×10^5 cells/well, 200 μL) in the complete culture medium consisted of RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) (Sigma) and standard culture solutions of antibiotics [22]. The cultures were treated with different dilutions of ACM or C-CM. Undiluted CM was considered 100% CM. After 24 h, the cell supernatants were collected, centrifuged, and frozen at -70 °C until the levels of cytokines were determined. The cells were used for apoptosis/necrosis assay.

MTT assay

PL cells were cultivated in 96-well plates (1×10^5/well; triplicates) in either fresh complete RPMI medium, different dilutions of ACM or C-CM. After a 24-hour incubation period, the plates were centrifuged, and the medium was carefully removed. The solution of 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) (100 μL/well, final concentration 100 μg/mL), was added. Wells with an MTT solution without cells served as blank controls. The plates were incubated with MTT for 3 hours in an incubator at 37 °C. Dissolution of formazan was done by incubating the MTT-treated cultures with 0.1N HCl/10% SDS (sodium dodecyl sulphate) (100 μL/well) overnight. The next day, the optical density (OD) of the developed colour was read atw 570/650 nm (ELISA reader, Behring II). The results were expressed as the relative metabolic activity compared to the metabolic activity of control cultures.

The relative metabolic activity was calculated as follows: metabolic activity (%) = (OD of cultures with ACM/OD of cultures with control fresh medium) × 100.

Apoptosis/necrosis assay

Apoptosis/necrosis was detected by Annexin-V–fluorescein isothiocyanate (FITC) and Propidium iodide (PI) staining kit (R&D), following the manufacturer's protocol. Briefly, cultivated PL cells were collected, washed with binding buffer, followed by incubation with Annexin-V–FITC and PI. The labeled cells were analyzed on a flow cytometer (Partec, Cube 6). Annexin-V-FITC$^+$ cells were recognized as primary apoptotic cells (early phase of apoptosis), PI$^+$ cells were primary necrotic cells, whereas double-positive cells were apoptotic/secondary necrotic cells (late phase of apoptosis).

Cytokine assays

The concentrations of interleukin (IL)-1β, IL-6, IL-8, and TNF-α in culture supernatants were detected by a FlowCytomix Microbeads Assay. This is a bead-based ELISA-like assay optimized for flow cytometry, allowing the simultaneous detection of several cytokines in a volume of samples (50 μL). The inflammation kit, containing microbeads coupled with antibodies to pro-inflammatory cytokines, was purchased from Biolegend. The levels of cytokines were determined by constructing standard curves based on the known concentration of these cytokines.

Statistical analysis

The Student's *t*-test was used for comparison of parametric variables between two groups. The Friedman's test (paired one-way ANOVA) was used for comparison between groups for non-parametric variables with Dunn's multiple comparison post-test. The values of $p < 0.05$ were considered to be statistically significant. Software SPSS version 23.0 (IBM, Armonk, New York, USA) was used to analyze the data.

Results

The first aim of this study was to examine the cytotoxicity of ACM on PL cells in culture. By using the MTT test (Figure 1), we showed that only concentrated (100%) and 75% ACM significantly reduced the viability of PL cells ($p < 0.001$ and $p < 0.01$, respectively). The cytotoxicity was due to the induction of apoptosis (Figures 2A and 2B). Figure 2B shows that ACM increased the proportion of late apoptotic/secondary necrotic cells.

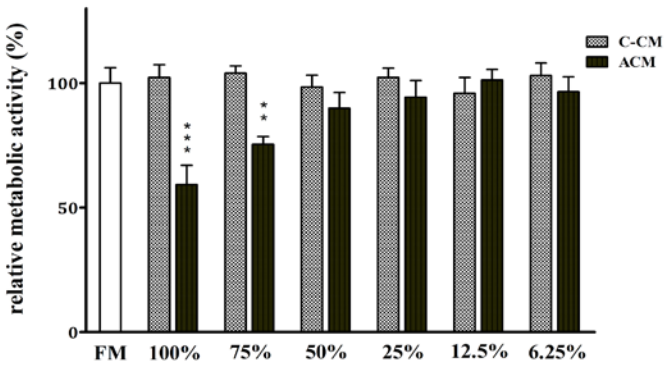


Fig. 1 – Cytotoxicity effect of amalgam on periapical lesion cells (PL) in culture. PL cells, prepared as described in Materials and methods, were cultured with different dilutions of amalgam conditioned medium (ACM) for 24 hours. The viability of PL cells was determined by the MTT test, as described. Values are given as mean ± SD (n = 7 cultures) of relative metabolic activity of cells. **$p < 0.01$; ***$p < 0.001$ compared to control cultures. FM – fresh medium; C-CM – control-conditioning medium.

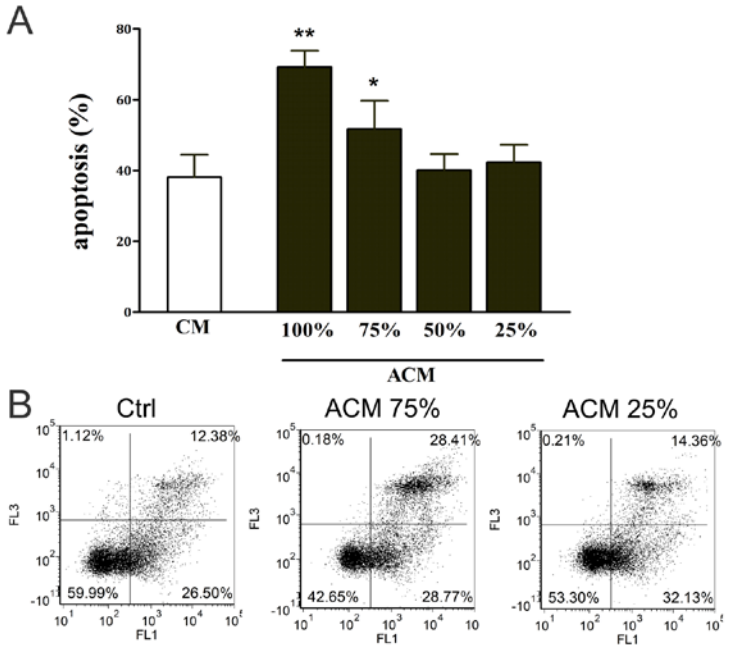


Fig. 2 – Effect of amalgam on apoptosis of periapical lesion (PL) cells in culture. PL cells, prepared as described in Materials and methods, were cultured with different dilutions of amalgam conditioned medium (ACM) for 24 hours. The apoptosis of PL cells was determined by the Annexin V-FITC/ PI assay, as described.
A) Values are given as mean ± SD (n = 8 cultures) of apoptotic cells (*$p < 0.05$; **$p < 0.01$ compared to control cultures).
B) Representative histograms showing that ACM accelerate apoptosis of PL cells, manifested by an increase of late apoptotic/secondary necrotic cells.
CM – control medium.

The second aim was to investigate the effect of ACM on the production of pro-inflammatory cytokines (IL-1β, TNFα, IL-6, and IL-8) by PL cells. We used non-cytotoxic concentrations (50%, 25%, and 12.5%) of ACM. The 50% and 25% concentrations of ACM suppressed the production of all four cytokines dose-dependently (Figure 3), whereas the 12.5% concentration did not show any modulatory effect (data not shown).

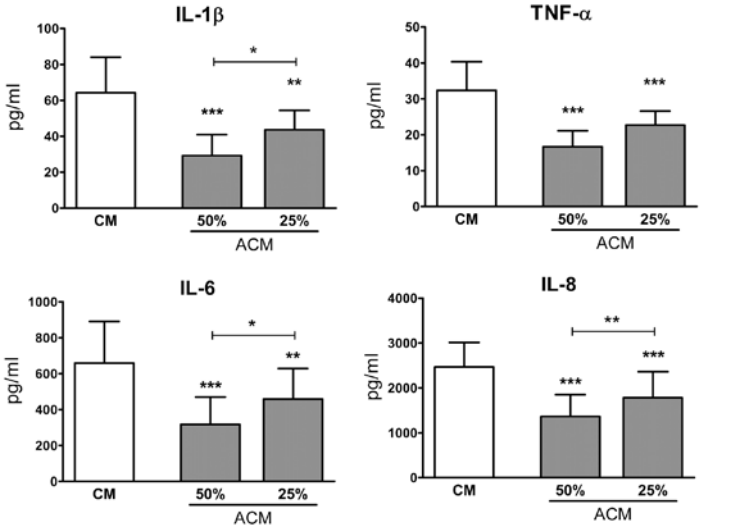


Fig. 3 – Effect of amalgam on the levels of pro-inflammatory cytokines in the culture of periapical lesion (PL) cells. PL cells, prepared as described in Materials and methods, were cultured with different dilutions of amalgam conditioned medium (ACM) for 24 hours. The levels of pro-inflammatory cytokines in culture supernatants were determined by Flow Cytomix Microbeads Assay. Values are given as mean ± SD (n= 8 cultures) levels of cytokines (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to control cultures or compared to 50% ACM, indicated by corresponding bars). CM – control medium.

Discussion

The first aim of this study was to examine the cytotoxicity *in vitro* of a copper-zinc-free amalgam, which is the oldest root-end filling material in apical surgery. Apicotomy is a common procedure for removing periapical lesions (granuloma or cysts) when the conventional endodontic treatment is not efficacious. Amalgam is still used for this purpose because of its self-sealing capacity, radio-opacity, insolubility in tissue fluids, and low price. However, since amalgam does not properly seal the root-end three-dimensionally, has poor marginal adaptation, and does not prevent the leakage of microorganisms in the peri-radicular tissue successfully [1–3, 23], we hypothesized that amalgam, due to its cytotoxic effect, could aggravate periapical inflammation. Therefore, cells isolated from PLs, which are dominantly composed of infiltrating inflammatory cells [22, 24], were the most suitable target to test this hypothesis, and this was our original approach.

Before starting with crucial experiments, it was necessary to determine the cytotoxicity of amalgam by using this culture model. Up to now, many different tests have been used for assessing amalgam cytotoxicity, but MTT, based on the evaluation of cellular metabolic activity, is the most acceptable as a first screening assay [6]. It is known that amalgam causes cytotoxicity either in direct contact with examined cells or indirectly by metallic ions released from the alloy [2, 6, 9]. We decided to study the effect of amalgam indirectly by analyzing the effect of ACM in which its leachable products are present and which are considered dominant cytotoxic factors [9, 25]. The study was conducted exactly as recommended by the ISO 10993-5 standard. We showed that only high concentrations of ACM (concentrated and 75%) were cytotoxic for PL cells due to apoptosis induction, suggesting that amalgam is generally cytotoxic alloy as similarly shown on other target cells. A relatively high proportion of apoptotic cells were also observed in control PL cell cultures, and the most sensitive cells were granulocytes, followed by macrophages, whereas lymphoid cells were more resistant (data not shown). These observations are in line with the already known facts about the high apoptotic rate of extravasated neutrophils as terminally differentiated cells [26].

We did not examine the concentrations of released ions from amalgam because this has been extensively investigated and published [5, 6]. In fact, all metal ions can be released in CM from amalgam, such as mercury, silver, copper, and thin. Out of them, cooper is the most cytotoxic, but it can be hypothesized that other ions act synergistically in inducing cytotoxicity [1, 2, 5, 6, 9]. This hypothesis was based on previous publications which thoroughly investigated the release and cytotoxicity of metal ions from amalgams of different composition. In this context, Kaga et al. [9] have demonstrated that pure copper showed the highest cytotoxicity among the metals tested in zinc-free amalgams. Silver and mercury showed reduced cytotoxicity, while tin was non-cytotoxic. In contrast, zinc-containing amalgams are more cytotoxic due to the easy release of Zn ions. The toxic effects of mercury are believed to exist due to the high reactivity of mercury species toward thiol-groups and other functional groups, notably in proteins [27]. It has been shown that both organic and inorganic mercury induce apoptosis of different cells, including human lymphocytes [27, 28].

The second part of this study was related to the effect of ACM on the production of pro-inflammatory cytokines by PL cells. We tested non-cytotoxic concentrations of ACM because toxic concentrations would not be relevant for a proper conclusion, partly due to the spontaneous release of cytokines from dead cells. We observed an unexpected result where ACM at non-cytotoxic concentrations significantly inhibited the secretion of pro-inflammatory cytokines (IL-1β, TNFα, IL-6, and IL-8). Therefore, our hypothesis was rejected.

The anti-inflammatory effect of ACM is contrary to the data published on the proinflammatory effect of amalgam particles which could induce the PL development if released into the periapical tissue during endodontic surgery [18]. Similarly, amalgam has been found to cause an inflammatory response in the dental pulp, which is transitory and significantly decreased in due time [29]. These differences (pro-inflammatory *versus* anti-inflammatory properties of amalgam) can be explained by the difference in setting experiments. Namely, cytotoxic effects of amalgam on periodontal tissue *in vivo* can provoke an inflammatory reaction due to direct contact, where, in the vicinity of the alloy, relatively high concentrations of cytotoxic metallic ions can be released. This effect dominates over anti-inflammatory effects seen at non-cytotoxic concentrations of leachable amalgam components.

No one has ever published a study related to amalgam nor examined the changes of multiple pro-inflammatory and other cytokines. The most relevant paper is the one published by Schedle et al. [30], who investigated the effects of dental amalgam on cytokine production by human peripheral blood mononuclear cells (PBMC) from healthy donors. To induce cytokine production, they stimulated PBMC in culture with lipopolysaccharide, phytohemagglutinin, or staphylococcal enterotoxin A in the presence of fresh amalgam, aged amalgam, or ACM prepared from fresh amalgam. They showed that freshly prepared amalgam, as well as ACM, reduced the production of interferon-γ (IFN-γ) and IL-10 but increased the levels of TNF-α. Both fresh amalgam and ACM had no effects on the levels of IL-2, IL-6, or granulocyte-macrophage colony-stimulating factor. Amalgam aged for 6 weeks did not modulate the concentrations of any of the above cytokines. To investigate which heavy metal cations released from amalgam caused the observed immunomodulatory effects, Cu^{2+}, Hg^{2+}, and Sn^{2+}, which were detected in ACM, were added as salts to PBMC cultures. Cu^{2+} and Hg^{2+} decreased the IFN-γ and IL-10 levels. However, Hg^{2+} increased TNF-α concentrations, whereas Sn^{2+} had no modulatory effect.

It is evident that our results, showing a decrease in TNF-α production, are opposite. The difference could be due to the following reasons, respectively: different concentrations of ACM (concentrated vs. diluted ACM); different

cells (stimulated PBMC vs. non-stimulated PL cells); different mass/volume ratio for ACM preparation (1.92 g/mL vs. 0.2 g/mL); different incubation time for cell cultures (48 h vs. 24 h). Some other studies investigated the effect of mercury. In this context, Soleo et al. [31] showed an increase in the number of $CD4^+$ cells in peripheral blood of subjects exposed to mercury from dental amalgam together with a decrease of serum IL-8 levels. Podzimek et al. [32] examined cytokine production (IL-1β, IL-4, IL-6, TNF-α, and IFN-γ) by human lymphocytes in cultures treated with mercury and found increased production of TNF-α and IFN-γ. Ilday et al. [33] observed reduced clinical periodontal findings in patients after overhang amalgam restoration removal, but these findings did not correlate with the changes in the levels of IL-6, IL-8, and TNF-α in the gingival crevicular fluid. This is in contrast with another study, published previously, which showed that removal of dental amalgam restorations was associated with decreased concentrations of Th1-type pro-inflammatory cytokines in serum, supporting the hypothesis that amalgam could be responsible for stimulating the Th1-type response *in vivo* [34].

It is known that cytokines play a key role in the pathogenesis of PLs [35]. Pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, and TNF-α, orchestrate the recruitment and activation of innate immune cells, presumably neutrophil granulocytes and monocytes in the early inflammatory phase and T and B cells in the later inflammatory phase, respectively. In this context, cytokines of T cells are the main controllers of the immune/inflammatory reactions. T-helper 1 (Th1) cells and Th-17 cells, by producing interferon-γ (IFN-γ) and IL-17, respectively, are involved in the progression of PLs and bone destruction, whereas T-helper 2 (Th2) cytokines, such as interleukin 4 (IL-4), IL-5, IL-10, and IL-33, are involved in the humoral immune response and attenuation of the tissue damage [22, 35, 36]. Therefore, further experiments investigating the effect of amalgam on this panel of cytokines could make a much better conclusion.

Conclusion

By using inflammatory cells isolated from human PL, we showed, for the first time, a potent anti-inflammatory effect of non-cytotoxic concentrations of ACM. This finding is in contrast with the previous findings, which state that particular amalgam particles released during retrograde filling can cause chronic apical periodontitis. Our results suggest that, in contrast to the high release of toxic ions from amalgam, slow release of leachable components from this amalgam, by down-modulating the production of pro-inflammatory cytokines, may control an excessive inflammation and promote PL healing.

Acknowledgement

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (grant No: OI 175102) and grants from the Ministry of Defence, Republic of Serbia (MFVMA/7/17-19 and MFVMA/9/16-18).

R E F E R E N C E S

1. *Mahler DB.* The high-copper dental amalgam alloys. J Dent Res 1997; 76(1): 537–41.
2. *Bhagat K, Goel M, Bhagat N.* Root End Filling Materials and Recent Advances: A Review. EC Dent Sci 2017; 12: 46–57.
3. *Mjor IA, Jokstad A, Qvist V.* Longevity of posterior restorations. Int Dent J 1990; 40(1): 11–7.
4. *Hohenfeldt PR, Aurelio JA, Gerstein H.* Electrochemical corrosion in the failure of apical amalgam. Report of two cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1985; 60(6): 658–60.
5. *Pleva J.* Corrosion and mercury release from dental amalgam. J Orthomol Med 1989; 4(3): 141–58.
6. *Kaga M, Seale N, Hanawa T, Ferracane J, Waite D, Okabe T.* Cytotoxicity of amalgams, alloys, and their elements and phases. Dent Mater 1991; 7(1): 68–72.
7. *Bodrumlu E.* Biocompatibility of retrograde root filling materials: a review. Aust Endod J 2008; 34(1): 30–5.
8. *Osborne JW, Norman RD, Gale EN.* A 14-year clinical assessment of 12 amalgam alloys. Quintessence Int 1991; 22(11): 857–64.
9. *Kaga M, Seale N, Hanawa T, Ferracane J, Okabe T.* Cytotoxicity of amalgams. J Dent Res 1988; 67(9): 1221–4.
10. *Leirskar J.* On the mechanism of cytotoxicity of silver and copper amalgams in a cell culture system. Eur J Oral Sci 1974; 82(1): 74–81.
11. *Meryon S.* The effect of zinc on the biocompatibility of dental amalgams in vitro. Biomaterials 1984; 5(5): 293–7.
12. *Enwonwu C.* Potential health hazard of use of mercury in dentistry: critical review of the literature. Environ Res 1987; 42(1): 257–74.
13. *Moszczyński P.* Mercury compounds and the immune system: a review. Int J Occup Med Environ Health 1997; 10(3): 247–58.
14. *Enestrom S, Hultman P.* Does amalgam affect the immune system? A controversial issue. Int Arch Allergy Immunol 1995; 106(3): 180–203.
15. *Meurman JH, Porko C, Murtomaa H.* Patients complaining about amalgam-related symptoms suffer more often from illnesses and chronic craniofacial pain than their controls. Eur J Oral Sci 1990; 98(2): 167–72.
16. *Flanders DH, James GA, Burch B, Dockum N.* Comparative histopathologic study of zinc-free amalgram and Cavit in connective tissue of the rat. J Endod 1975; 1(2): 56–9.
17. *Torabinejad M, Hong CU, Pitt Ford TR, Kaiyawasam SP.* Tissue reaction to implanted super-EBA and mineral trioxide aggregate in the mandible of guinea pigs: a preliminary report. J Endod 1995; 21(11): 569–71.
18. *Nair PN.* On the causes of persistent apical periodontitis: a review. Int Endod J 2006; 39(4): 249–81.
19. *Keiser K, Johnson CC, Tipton DA.* Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. J Endod 2000; 26(5): 288–91.
20. *Tai KW, Chang YC.* Cytotoxicity evaluation of perforation repair materials on human periodontal ligament cells in vitro. J Endod 2000; 26(7): 395–7.

21. *Lin CP, Chen YJ, Lee YL, Wang JS, Chang MC, Lan WH*, et al. Effects of root-end filling materials and eugenol on mitochondrial dehydrogenase activity and cytotoxicity to human periodontal ligament fibroblasts. J Biomed Mater Res 2004; 71(2): 429–40.
22. *Colic M, Gazivoda D, Vucevic D, Vasilijic S, Rudolf R, Lukic A*. Proinflammatory and immunoregulatory mechanisms in periapical lesions. Mol Immunol 2009; 47(1): 101–13.
23. *Letzel H, van 't Hof MA, Vrijhoef MM, Marshall GW Jr, Marshall SJ*. A controlled clinical study of amalgam restorations: survival, failures, and causes of failure. Dent Mater 1989; 5(2): 115–21.
24. *Čolić M, Lukić A, Vučević D, Milosavljević P, Majstorović I, Marjanović M*, et al. Correlation between phenotypic characteristics of mononuclear cells isolated from human periapical lesions and their in vitro production of Th1 and Th2 cytokines. Arch Oral Biol 2006; 51(12): 1120–30.
25. *Ferracane JL, Mafiana P, Cooper C, Okabe T*. Time-dependent dissolution of amalgams into saline solution. J Dent Res 1987; 66(8): 1331–5.
26. *Greenlee-Wacker MC*. Clearance of apoptotic neutrophils and resolution of inflammation. Immunol Rev 2016; 273(1): 357–70.
27. *Schwenk M, Klein R, Templeton DM*. Immunological effects of mercury (IUPAC Technical Report). Pure Appl Chem 2009; 81(1): 153–67.
28. *Shenker BJ, Berthold P, Decker S, Mayro J, Rooney C, Vitale L*, et al. Immunotoxic Effects of Mercuric Compounds on Human Lymphocytes and Monocytes. II. Alterations in Cell Viability. Immunopharmacol Immunotoxicol 1992; 14(3): 555–77.
29. *Baek SH, Plenk Jr H, Kim S*. Periapical tissue responses and cementum regeneration with amalgam, SuperEBA, and MTA as root-end filling materials. J Endod 2005; 31(6): 444–9.
30. *Schedle A, Rausch-Fan XH, Samorapoompichit P, Franz A, Leutmezer F, Spittler A,* et al. Effects of dental amalgam and heavy metal cations on cytokine production by peripheral blood mononuclear cells in vitro. J Biomed Mater Res 1998; 42(1): 76–84.
31. *Soleo L, Colosio C, Alinovi R, Guarneri D, Russo A, Lovreglio P,* et al. Immunologic effects of exposure to low levels of inorganic mercury. Med Lav 2002; 93(3): 225–32.
32. *Podzimek S, Tomka M, Nemeth T, Himmlova L, Matucha P, Prochazkova J*. Influence of metals on cytokines production in connection with successful implantation therapy in dentistry. Neuro Endocrinol Lett 2010; 31(5): 657–62.
33. *Ilday NO, Celik N, Dilsiz A, Alp HH, Aydin T, Seven N,* et al. The effects of overhang amalgam restoration on levels of cytokines, gingival crevicular fluid volume and some periodontal parameters. Am J Dent 2016; 29(5): 266–70.
34. *Bjorkman L, Brokstad KA, Moen K, Jonsson R*. Minor changes in serum levels of cytokines after removal of amalgam restorations. Toxicol Lett 2012; 211(2): 120–5.
35. *Braz-Silva PH, Bergamini ML, Mardegan AP, De Rosa CS, Hasseus B, Jonasson P*. Inflammatory profile of chronic apical periodontitis: a literature review. Acta Odontol Scand 2019; 77(3): 173–80.
36. *Naufel AO, Aguiar MCF, Madeira FM, Abreu LG*. Treg and Th17 cells in inflammatory periapical disease: a systematic review. Braz Oral Res 2017; 31: e103.

Received on February 25, 2019.
Accepted on March 19, 2019.
Online First April, 2019.

ORIGINAL ARTICLE

UDC: 616.716.1/.4-089.168
https://doi.org/10.2298/VSP180906058S

Cephalometric evaluation of skeletal relationships after bimaxillary surgical correction of mandibular prognathism

Rendgen kraniometrijska procena skeletnih odnosa nakon bimaksilarne hirurške korekcije mandibularnog prognatizma

Vladimir Sinobad*, Ljiljana Strajnić†, Tamara Sinobad‡

University of Belgrade, Faculty of Dental Medicine, *Clinic for Maxillofacial Surgery, Belgrade, Serbia; †Clinic for Dentistry of Vojvodina, Novi Sad, Serbia; ‡Zepter Dental Polyclinic, Belgrade, Serbia

Abstract

Background/Aim. In recent years, bimaxillary surgery has widely been accepted as an effective surgical procedure for the correction of mandibular prognathism. The aim of this study was to determine how bimaxillary surgical correction can change the skeletal dimensions and relations typical of mandibular prognathism and whether the postoperative results can be compared with biometric values of these dimensions in subjects with normal occlusion. **Methods.** The study included 50 subjects divided into two groups. The analyzed group consisted of 20 patients with mandibular prognathism, mean age 19.8 ± 5.3 years. The control group consisted of 30 subjects with skeletal class I and normal occlusion, mean age 21.5 ± 3.5 years. Cephalometric studies were conducted on 70 lateral cephalograms made on subjects of the analyzed group before and after surgery and in controls. All radiographs were transformed into a digital form. Using the computer program "Dr. Ceph", 30 linear and angular skeletal variables were analyzed and compared on each radiograph. The values of examined variables in the analyzed group were compared before and after surgery and with the values of the same variables in the control group. **Results.** Bimaxillary osteotomies changed most of the variables that characterize the mandibular prognathism. Changes in the sagittal plane were reflected in a significant increase of angles SNA (by 4° on the average), ANB (6°), and a significant reduction in angles SNB (3°), ArGoMe (8°), NGoMe (6.2°), Bjork's sum (7°) and the angle of skeletal convexity NAPg (2°). Changes in vertical relationships were reflected in a significant reduction in overall anterior face height N-Me (by 5 mm on average), the lower anterior face height ANS-Me (4 mm), in a significant increase in the total posterior face height S-Go (2.5–3 mm), lower posterior face height PNS-Go (4 mm), in a significant reduction of the basal angle PP/MP (5°) and angle that mandibular plane closes with the anterior cranial base NS/MP (4°). Comparison of investigated variables in the analyzed group after surgery with the same values in the control group showed that they were significantly closer to biometric standards. **Conclusion.** Bimaxillary surgery significantly alters the skeletal relationships and facial dimensions typical of mandibular prognathism and normalizes the skeletal profile and appearance in operated patients.

Key words:
malocclusion, angle class III; cephalometry; oral surgical procedures; orthognathic surgical procedures; treatment outcome.

Apstrakt

Uvod/Cilj. Poslednjih godina bimaksilarna hirurgija je široko prihvaćena kao efikasna hirurška procedura u korigovanju mandibularnog prognatizma. Cilj rada bio je da se utvrdi na koji način bimaksilarne hirurške korekcije menjaju skeletne dimenzije i odnose tipične za mandibularni prognatizam i mogućnost poređenja postoperativnih rezultata sa biometrijskim vrednostima tih dimenzija kod osoba sa normookluzijom. **Metode.** U studiju je bilo uključeno 50 ispitanika koji su bili podeljeni u dve grupe. Analiziranu grupu je činilo 20 ispitanika sa mandibularnim prognatizmom, prosečne starosti 19,8 ± 5,3 godine. Kontrolnu grupu je činilo 30 ispitanika sa I skeletnom klasom i normookluzijom, prosečne starosti 21,5 ± 3,5 godine. Rendgenkraniometrijska istraživanja su obavljena na 70 profilnih telerendgenskih snimaka glave načinjenih kod ispitanika analizirane grupe pre i nakon operacije i kod ispitanika kontrolne grupe. Pomoću kompjuterskog programa "Dr Ceph", na svakom snimku vrednovano je 30 linearnih i ugaonih skeletnih varijabli. U analiziranoj grupi upoređene su vrednosti ispitivanih varijabli pre i nakon operacije, a, takođe, te vrednosti su upoređene i sa vrednostima istih varijabli u kontrolnoj grupi. **Rezultati.** Bimaksilarne osteotomije su promenile većinu varijabli koje karakterišu mandibularni prognati

Correspondence to: Vladimir Sinobad, University of Belgrade, Faculty of Dental Medicine, Clinic for Maxillofacial Surgery, Dr Subotića 4, 11 000 Beograd, Serbia. E-mail: vladimir.sinobad@stomf.bg.ac.rs

zam. Promene u sagitalnim odnosima ogledale su se u značajnom povećanju uglova SNA (za 4°), ANB (za 6°) i značajnom smanjenju uglova SNB, ArGoMe, NGoMe, Bjorkovog poligona i ugla skeletnog konveksiteta lica NAPg. Promene u vertikalnim odnosima ogledale su se u značajnom smanjenju ukupne prednje visine lica N-Me (za 5 mm), donje prednje visine lica ANS-Me (za 4 mm), značajnom povećanju ukupne zadnje visine lica S-Go (oko 3 mm), donje visine lica PNS-Go (4 mm), značajnom smanjenju bazalnog ugla SpP/MP (5°) i ugla koji mandibularna ravan zaklapa sa prednjom kranijalnom bazom NS/MP (4°). Poređenje vrednosti ispitivanih varijabli u analiziranoj grupi nakon operacije sa istim vrednostima u kontrolnoj grupi pokazalo je da su se one značajno približile biometrijskim standardima. **Zaključak.** Bimaksilarne osteotomije značajno menjaju skeletne odnose i dimenzije lica tipične za mandibularni prognatizam i normalizuju skeletni profil kod operisanih pacijenata.

Ključne reči:
malokluzija, klase III; kefalometrija; hirurgija, oralna, procedure; hirurgija, ortognatska, procedure; lečenje, ishod.

Introduction

Mandibular prognathism is among the most serious genetic disorders of growth and development of the craniofacial skeleton. The deformity is manifested fully in the most sensitive age, the adolescent period, endangering the basic functions of the orofacial system, the appearance of the young persons, their psychological health, and quality of life. These are usually the basic motives why these patients seek orthognathic surgery.

Literature data indicate that severe forms of dentofacial deformities occur in 0.5% of people in the general population. The fact is, however, that of all patients requiring orthognathic surgery, 28%–34% are with mandibular prognathism [1].

Diagnosis and treatment of severe craniofacial disharmonies require a multidisciplinary approach and teamwork. The base of each treatment is a detailed analysis of the orofacial complex that provides objective information on the severity and phenotypic characteristics of the existing deformity. In the majority of cases, class III deformities are combined by maxillary retrognathia, mandibular prognathism, and varying degrees of vertical discrepancies [2–4].

During the past few decades, various surgical procedures have been advocated for the correction of these deformities. Until the 1980s, the surgical correction of mandibular prognathism has been mainly performed by isolated operations on the mandible [5–8]. Nowadays, it is clear that such operations, in most cases, cannot normalize the skeletal relationships and achieve the optimal aesthetic results [9–12]. Clinical experience and numerous scientific references suggest that correction of skeletal disharmonies, harmonization of occlusion, and correction of facial appearance in patients with severe mandibular prognathism can only be achieved by bimaxillary surgery, ie. by planned surgical reposition of both jaws [11–16].

The aim of this study was to determine to what extent and in what way bimaxillary surgical correction can change the skeletal dimensions and relations typical of mandibular prognathism and whether the postoperative results can be compared with biometric values of these dimensions in subjects with normal occlusion.

Methods

The sample of the study was comprised of two groups – the analyzed and the control group. The analyzed group consisted of 20 patients admitted to the Department of Maxillofacial Surgery, Faculty of Dental Medicine in Belgrade for surgical correction of mandibular prognathism from 2003–2013. There were ten female and ten male patients, mean age of 19.8 ± 5.3 years. The control group consisted of 30 young persons, mean age of 21.5 ± 3.5 years, with normal occlusion. For the purposes of cephalometric research, a total of 70 lateral cephalometric radiographs were made and divided into three groups: Group A consisted of 20 lateral cephalometric radiographs derived from the patients of the analyzed group before surgery and before orthodontic preparation; Group B consisted of 20 lateral cephalometric radiographs derived from the same patients of the analyzed group 6 months to a year after bimaxillary surgical correction of mandibular prognathism; Group C consisted of 30 lateral cephalometric radiographs made in the control group. This collection was selected from the files of our dental school (archive of the author).

Lateral cephalograms are made in the Plan-Meca Radiological Center and the Center for the Head and Neck Radiology at the Faculty of Dental Medicine in Belgrade with a special apparatus, "ORTOCEPH" (Siemens, Bensheim, Germany). The recordings were made by standard techniques at a voltage of 65 to 80 kV and a strength of 20 mA, and the exposure was from 1 to 1.5 sec. The recording was performed on the X-ray film 18 × 24 cm. All radiographs were scanned and transformed into digital form.

The choice of operative technique

Each patient of the analyzed group was subjected to special consultative review and selected for these investigations based on a precise analysis of the phenotypic characteristics of present deformity. The patients were sent to orthodontic preparation for a year and a half and then subjected to surgical correction. The surgical procedure was performed by a successive bimaxillary approach that involves LeFort I osteotomy of the maxilla and bilateral sagittal split ramus osteotomy of the mandible. The rigid fixation (mini titanium plates and screws) was used to fix the

bone fragments. A combination of solid and elastic intermaxillary immobilization was applied for 6–8 weeks after surgery [9, 17, 18].

Cephalometric research

All lateral cephalograms made in the analyzed group before and after surgery, as well as in the control group, were subjected to cephalometric analysis. For this purpose, a special computer program, "Dr. Ceph" (FYI Technologies, GA, USA, last revised edition, version 9.7), was used (Figure 1). This version allows the use of over thirty well-known cephalometric analyses, as well as adaptation of any analysis to the specific needs of the research. Using this program on each cephalogram of A, B, and C groups, the values of 30 linear and angular skeletal variables were recorded and evaluated.

Examined skeletal variables

a) Examined linear variables were (Figure 2): 1. N-Se – length of the anterior cranial base; 2. N-Me – total anterior face height; 3. N-ANS – upper anterior face height; 4. ANS-Me – lower anterior face height; 5. S-Go – total posterior face height; 6. S-PNS – upper posterior face height; 7. PNS-Go – lower posterior face height; 8. S-Ar – the length of the posterior cranial base; 9. Ar-Go – the length of the ramus; 10. Co-Go – the height of the ramus; 11. PNS-A – the length of the maxillary body; 12. Go-Me – the length of the mandibular body.

b) Examined proportions of linear variables were: 1. S-Go/N-Me – the relationship of anterior and posterior face heights; 2.N-ANS/ANS-Me – the ratio of upper and lower anterior face height; 3. N-ANS/N-Me – the ratio of the upper anterior face height to total anterior face height; 4. ANS-

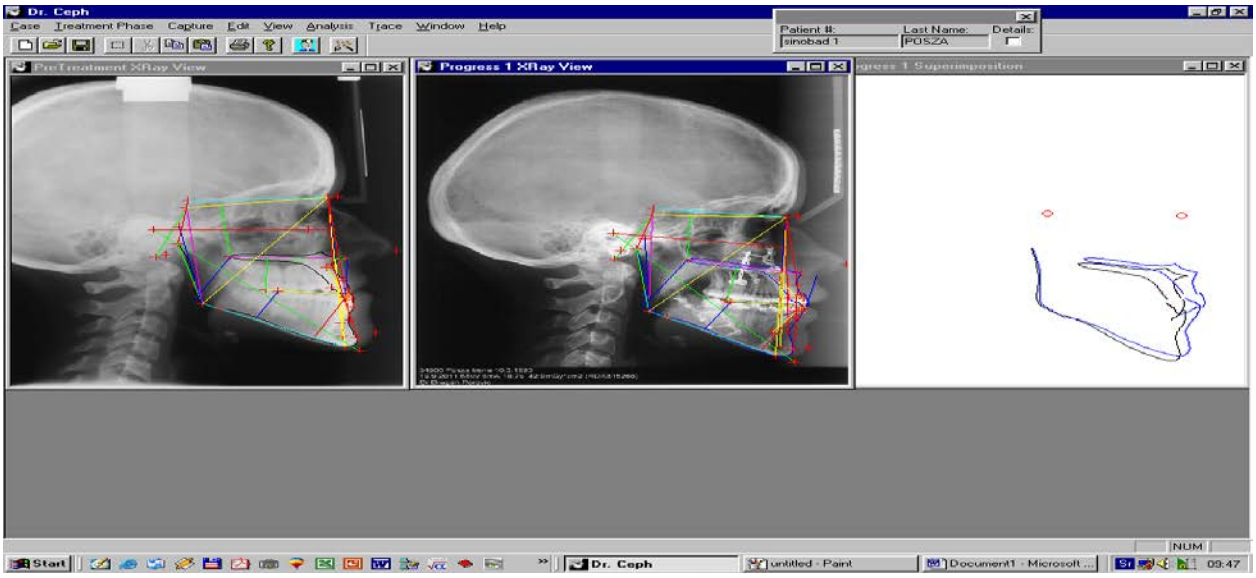


Fig. 1 – Cephalometric analysis of parameters by "Dr. Ceph" computer software.

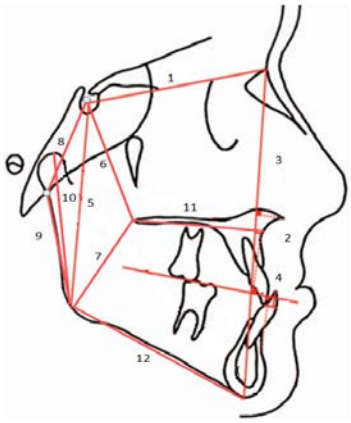


Fig. 2 – Examined linear skeletal variables.
1. N-Se – length of the anterior cranial base; 2. N-Me – total anterior face height;
3. N-ANS – upper anterior face height; 4. ANS-Me – lower anterior face height;
5. S-Go – total posterior face height; 6. S-PNS – upper posterior face height;
7. PNS-Go – lower posterior face height; 8. S-Ar – the length of the posterior cranial base;
9. Ar-Go – the length of the ramus; 10. Co-Go – the height of the ramus;
11. PNS-A – the length of the maxillary body; 12. Go-Me – the length of the mandibular body.

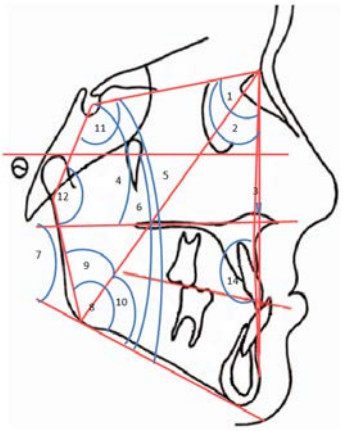


Fig. 3 – Examined angular skeletal variables.
1. SNA – anteroposterior position of the maxilla relative to the anterior cranial base;
2. SNB – anteroposterior position of the mandible relative to the anterior cranial base;
3. ANB – the relationship of the maxilla and mandible in the sagittal plane;
4. N-S/PP – the inclination of the maxilla to the anterior cranial base;
5. N-S/MP – the inclination of the mandible to the anterior cranial base;
6. FH/MP – the relationship between the Frankfurt plane and mandibular plane;
7. PP/MP – the relationship between the basic jaw planes;
8. ArGoMe – gonial angle by Bjork; 9. ArGoN – upper part of the gonial angle;
10. NGoMe – the lower part of the gonial angle; 11. NSAr – the angle of the saddle by Bjork;
12. SArGo – articular angle by Bjork.

Me/N-Me – the ratio of the lower anterior face height to the total anterior face height.

c) Examined angular skeletal variables were (Figure 3): 1. SNA – anteroposterior position of the maxilla relative to the anterior cranial base; 2. SNB – anteroposterior position of the mandible relative to the anterior cranial base; 3. ANB – the relationship of the maxilla and mandible in the sagittal plane; 4. N-S/PP – the inclination of the maxilla to the anterior cranial base; 5. N-S/MP – the inclination of the mandible to the anterior cranial base; 6. FH/MP – the relationship between the Frankfurt plane and mandibular plane; 7. PP/MP – the relationship between the basic jaw planes; 8. ArGoMe – gonial angle by Bjork; 9. ArGoN – upper part of the gonial angle; 10. NGoMe – the lower part of the gonial angle; 11. NSAr – the angle of the saddle by Bjork; 12. SArGo – articular angle by Bjork; 13. Bjork's sum – the sum of the angles NSAr, SarGo, and ArGoMe; 14. NAPg – the angle of facial skeletal convexity.

Numerical values of the examined skeletal variables were subjected to statistical analysis and compared. Due to surgical correction, the values of selected skeletal variables were compared before surgery and 6 months to a year after surgery to verify the changes in skeletal relationships.

The comparison of investigated variables between the analyzed group after surgery and the control group was used for objective evaluation of the success of bimaxillary surgery in correcting the mandibular prognathism.

Statistical analysis was performed using the computer programs MS Excel, MedCalc (MedCalc ver. 11.4 Software, Belgium), and SPSS ver. 18 (SPSS Inc, Chicago, IL). The comparison of two groups of independent data was performed using Student's *t*-test. Comparison of three sets of data was performed using the parametric analysis of variance (ANOVA) with Tukey-Snedecor *post hoc* test. The shape of data distribution was examined using the Kolmogorov-Smirnov test. This test showed that all variables had a normal distribution, and in the further course of data processing, they were portrayed as means, standard deviations, minimum and maximum values, and coefficients of variation (in %). The minimum requirement for a statistically significant difference was when the significance level (p) was less than or equal to 0.05.

Results

Comparison of values of linear skeletal variables in the analyzed group before and after surgery revealed a number of changes in their values. However, the only variables that showed significant differences between the situation before and after the operation were the following: N-Me, ANS-Me, Go-Me, PNS-A, S-Go, PNS-Go, S-Ar, and S-Go/N-Me (Table 1).

After surgery, the total anterior face height N-Me was reduced by 5 mm on average, the lower anterior face height ANS-Me by 4 mm on average, and the length of the mandible Go-Me for 3–3.5 mm. On the contrary, the values of the total posterior face height S-Go increased by 2.5–3 mm on average, and of the lower face height PNS-Go by 4 mm. The relationship between the posterior and anterior total face height changed in favor of the posterior face height. The effective maxillary length increased by 3–3.5 mm on average as a result of it shifting forward during surgery.

The surgery did not affect the length of the anterior cranial base N-S, nor the values of the anterior upper face

Table 1

The values of linear skeletal variables in the control group and the experimental group before and after surgery

Variables	Control group	Experimental group		*p*
		before operation	after operation	
N-Se	63.7 ± 6.37	66.8 ± 4.75*	66.8 ± 4.5*	<0.05
N-Me	114.9 ± 8.57	124.0 ± 6.89***	118.9 ± 7.83§§	<0.001
N-ANS	50.3 ± 4.62	53.0 ± 3.21*	52.1 ± 5.11	<0.05
ANS-Me	64.5 ± 5.79	71.0 ± 6.45***	66.7 ± 6.49§	<0.001
S-Go	78.5 ± 5.91	76.6 ± 5.20	79.3 ± 7.10§	< 0.05
S-PNS	44.0 ± 3,42	44.9 ± 3.72	44.7 ± 4.06	ns
PNS-Go	44.4 ± 4.15	38.9 ± 4.48***	42.8 ± 5.87§§	<0.001
S- Ar	36.1 ± 3.68	30.4 ± 5.59***	31.2 ± 5.07***, §§§	< 0.001
Ar-Go	46.5 ± 4.76	52.8 ± 6.49***	52.9 ± 5.24***	< 0.001
Co- Go	57.9 ± 5.03	61.8 ± 4.51**	62.0 ± 5.91*	< 0.001
S-Go/ N-Me	0.685 ± 0.0436	0.627 ± 0.05***	0.660 ± 0.06§§	< 0.001
N-ANS/ANS-Me	0.779 ± 0.0710	0.756 ± 0.10	0.773 + 0.10	ns
N-ANS/N-Me	0.438 ± 0.0256	0.430 ± 0.03	0.436 ± 0,03	ns
ANS-Me/ N-Me	0.562 ± 0.0256	0.571 ± 0.03	0.564 ± 0.03	ns
PNS-A	44.5 ± 3.43	43.6 ± 3.56	46.7 ± 3.95	ns
Go-Me	70.2 ± 5.57	77.6 ± 6.53***	74.7 ± 6.26**, §§	< 0.001

N-Se – length of the anterior cranial base; N-Me – total anterior face height; N-ANS – upper anterior face height; ANS-Me – lower anterior face height; S-Go – total posterior face height; S-PNS – upper posterior face height; PNS-Go – lower posterior face height; S-Ar – the length of the posterior cranial base; Ar-Go – the length of the ramus; Co-Go – the height of the ramus; PNS-A – the length of the maxillary body; Go-Me – the length of the mandibular body.
p* – *,**, *p* < 0.05, 0.01, 0.001 vs. control, §, §§, §§§ *p* < 0.05, 0.01, 0.001 vs. analyzed group before operation; ns – non significant (ANOVA test and *post hoc* Tukey test).**

height N-ANS, posterior upper face height S-PNS, length of ramus Ar-Go, and height of ramus mandible Co-Go.

Relations between the upper and lower anterior face height N-ANS/ANS-Me, the upper anterior and total anterior face height N-ANS/N-Me, and the relationship of the lower anterior to the total face height ANS-Me/N-Me were changed after the operation, but the differences were not significant.

Comparing linear skeletal variables in the analyzed group after surgery with the values of the same variables in the control group revealed that most linear variables after surgery returned to the level in controls (Table 1). This especially applied to the values of total anterior face height N-Me and the lower anterior face height ANS-Me which were significantly reduced by surgery, then to the values of the total posterior face height S-Go, the lower posterior face height PNS-Go, and their relationship, which significantly increased after surgery.

However, even after surgery, the posterior cranial base S-Ar remained considerably lower than in the control group, while the length and height of the ramus and even the length of the body of the mandible were significantly longer compared to their values in the control group.

Comparison of values of angular skeletal variables in the analyzed group before and after surgery revealed statistically significant differences in the following variables: SNA, SNB, ANB, NS/MP, FH/MP, PP/MP, ArGoMe, NGoMe, Bjork's sum, and NAPg (Table 2).

Due to maxillary advancement during Le Fort I osteotomy, the value of SNA angle increased to 4° on average. On the contrary, the values of the basic features of mandibular prognathism decreased significantly. The values of SNB angle decreased by an average of 3°, NS/MP angle by an average of 4°, FH/MP angle by an average of 4.7°, PP/MP angle by an average of 5°, ArGoMe angle by an average of 8°, NGoMe by an average of 6.2°, and Bjork's sum by an average of 7°.

The ANB angle with a high negative value before surgery (X = -4.7 ± 3.04°), became positive (X = 1.3 ± 1.22°) after surgery and significantly approached biometric standards (around ± 2°). The difference between the values of ANB angle before and after operation amounted to 6°.

The comparison of angular skeletal variables in the analyzed group after surgery with the values of the same variables in the control group showed that the majority of them approached the biometric norms (Table 2). This is especially true for angles SNA, SNB, NS/PP, NS/MP, FH/MP, PP/MP, ArGoN, and Bjork's sum. As the modified values of these angles are the main indicators of maxillary retrognathia and/or mandibular prognathism with a vertical type of growth, normalization of their values after surgery changed the progeny skeletal assembly in operated patients.

However, even after surgery in the analyzed group, the values of gonial angles ArGoMe and NGoMe and the angle of facial skeletal convexity NAPg remained significantly higher compared to their values in the control group, while the average value of the articular angle SarGo was significantly lower. The value of the ANB angle, which significantly increased after surgery (by 6° on the average), was still different from its value in the control group.

Table 2

The values of angular skeletal variables in the control group and the experimental group before and after surgery

Variables	Control group	Experimental group		*p*
		before operation	after operation	
SNA	81.4 ± 3.38	79.2 ± 4.66	83.7 ± 5.60§§§	ns
SNB	79.3 ± 3.06	84.0 ± 4.38 ***	82.7 ± 4.72§§	< 0.001
ANB	2.1 ± 1.30	4.7 ± 3.04 ***	1.3 ± 1.22$^{*, §§§}$	< 0.001
N-S/PP	8.2 ± 3.53	8.8 ± 4.68	9.2 ± 5.63	ns
N-S/MP	30.6 ± 5.56	37.1 ± 7.30**	33.3 ± 7.24§	< 0.001
FH/MP	23.3 ± 5.57	28.9 ± 7.81**	24.2 ± 6.44§§	< 0.01
PP/MP	22.9 ± 5.58	28.2 ± 8.00**	23.4 ± 8.77§§	< 0.01
ArGoMe	123.0 ± 5.91	135.5 ± 10.85***	127.5 ± 7.43$^{*, §§§}$	< 0.001
ArGoN	49.9 ± 3.20	51.3 ± 8.76	50.8 ± 5.61	ns
NGoMe	73.0 ± 4.58	82.4 ± 7.79***	76.6 ± 4.45$^{**, §§§}$	< 0.001
NSAr	123.5 ± 6.66	125.1 ± 10.83	125.3 ± 8.51	ns
SArGo	144.3 ± 6.32	138.3 ± 11.92*	139.3 ± 10.63*	< 0.05
Bjork's sum	390.9 ± 5.31	398.8 ± 9.91**	392.1 ± 5.97§§	< 0.001
NAPg	176.8 ± 1.86	172.0 ± 5.70 **	170.7 ± 6.39***	< 0.001

SNA – anteroposterior position of the maxilla relative to the anterior cranial base; SNB – anteroposterior position of the mandible relative to the anterior cranial base; ANB – the relationship of the maxilla and mandible in the sagittal plane; N-S/PP – the inclination of the maxilla to the anterior cranial base; N-S/MP – the inclination of the mandible to the anterior cranial base; FH/MP – the relationship between the Frankfurt plane and mandibular plane; PP/MP – the relationship between the basic jaw planes; ArGoMe – gonial angle by Bjork; ArGoN – upper part NSAr – the angle of the saddle by Bjork; SArGo – articular angle by Bjork; Bjork's sum – the sum of the angles NSAr, SarGo, and ArGoMe; NAPg – the angle of facial skeletal convexity. *p* – *,**,*** *p* < 0.05, 0.01, 0.001 vs. control, $^{§, §§, §§§}$ *p* < 0.05, 0.01, 0.001 vs. analyzed group before operation; ns – non significant (ANOVA test and *post hoc* Tukey test).

Discussion

The main objectives of the surgical treatment in patients with mandibular prognathism are to normalize the facial profile, harmonize the occlusion, and rehabilitate the basic functions of the orofacial system. Correction of the main skeletal parameters within the normal range of values is usually regarded as the main aim of the treatment.

Choosing a surgical technique is certainly one of the key factors for a successful realization of these objectives. Bearing in mind the extreme variability of the craniofacial morphology in patients with mandibular prognathism [2–4], it is clear that the modality of surgical treatment must be appropriate to the basic phenotypic characteristics of the present deformity [9–15].

The modality of surgical treatment in this study was determined after a detailed clinical and cephalometric analysis in each subject. In all subjects of the analyzed group, the Le Fort I maxillary advancement is associated with mandibular setback osteotomy [9, 17, 18].

The evaluation of certain skeletal variables in the experimental group before surgery revealed that 40% of subjects had a significantly decreased SNA angle in relation to biometric standards and that maxillary length was decreased in 55% of subjects. In 85% of subjects, the relationship of the mandible to the anterior cranial base (NS/MP angle) was typical of mandibular prognathism associated with vertical discrepancies. The average value of ANB angle in the analyzed group before the operation amounted to -4.7 ± 3.04°. In 75% of subjects in this group, the deformity was a combination of maxillary retrognathia and mandibular prognathism [9].

A comparative analysis of the selected skeletal variables in the analyzed group, 6 months to one year after surgery with the values of the same variables before surgery, showed that bimaxillary operations changed more linear and angular dimensions, characteristic for mandibular prognathism. This operative procedure significantly altered the position of the maxilla and mandible in the sagittal plane, and vertically the length of the mandible and its relation to the anterior cranial base. The total anterior and lower anterior face height were reduced by 5 mm on average. The specificity of this operation is a significant increase of total posterior and lower posterior face height (by 3–4 mm on average) and the length of the posterior cranial base S-Ar. These alterations normalized the relationship between the anterior and posterior face heights and led to the harmonization of facial dimensions in operated patients. These results are consistent with the results of numerous studies which indicate the significant harmonization of facial dimensions after bimaxillary operations [11–16, 19, 20].

The significant increase of posterior face height in operated patients, especially the increase of lower posterior face height, and the posterior cranial base is a result of the

anterior rotation of the proximal segment of the mandible during the bilateral ramus osteotomy, which is necessary in order to establish normal occlusal relationships.

Introducing Le Fort I osteotomy in the operative procedure significantly changed the values of SNA, SNB, ANB angles, and the angle of skeletal convexity NAPg. In that manner, bimaxillary surgery significantly altered the typical imbalance in anterior-posterior skeletal relationships in patients with mandibular prognathism. After the operation, the SNA angle increased by 4° on average, which is the specificity of bimaxillary surgical correction of mandibular prognathism. SNB angle after surgery was reduced on average by slightly more than 2° but is still higher than the biometric standard. The values of ANB angle in the analyzed group after operation increased by 6° on average, but they are still below optimum. Johnston et al. [19] also stated that values of SNA, SNB, and ANB angles after bimaxillary surgery showed significant improvement, but in 54% of treated patients, ANB angle values are still below the ideal, while 52% of patients still have great values of SNB angle.

Bimaxillary surgery also reduced the most vertical components of mandibular prognathism. Significant reduction of NS/MP, FH/MP, ArGoMe, ArGoN angles, and Bjork's sum normalized the positions of maxilla and mandible to the anterior cranial base and the mutual relation of the jaws vertically, as confirmed by other studies [14–16, 19, 20].

According to the literature, the efficiency of an operation has been expressed in the percentage of patients who have certain cephalometric dimensions brought into the framework of ideal or acceptable norms [19]. In the context of this study, the efficacy of bimaxillary surgery has been evaluated by comparing the tested skeletal parameters in the analyzed group after surgery with the values of these parameters in the control group.

These analyses revealed that values of most examined variables after surgery were significantly closer to their values in subjects of the control group. This is especially true for the values of the total anterior and posterior face heights, and the angles SNA, NS/PP, NS/MP, FH/MP, PP/MP, ArGoN, and Bjork's sum. These changes significantly altered the typical skeletal assembly of mandibular prognathism and contributed to the overall physiognomic effect in operated patients. Similar results were reported by Johnston et al. [19], Marsan et al. [15], Jakobsone et al. [16], Al-Gunaid et al. [14], and Aydemir et al. [20].

However, the operation did not remove all skeletal features of prognathism. The lengths of anterior and posterior cranial bases, the length of the ramus, and to some extent the length of the mandibular body after surgery are characteristic of mandibular prognathism. The values of the angles SNB, ANB, ArGoMe, NgoMe, and NS/MP even after surgery differ from their values in the control group. These findings are consistent with the results of Johnston et al. [19], Al-Gunaid et al. [14], and Sinobad et al. [9], who also found that surgical treatment did not lead to the full normalization of these skeletal dimensions.

Conclusion

Investigations in this study have confirmed that bimaxillary surgery significantly altered the large number of linear and angular dimensions that characterize mandibular prognathism. They normalized the overall anterior and posterior face heights in operated patients and their relationships. The maxillary advancement accompanied by mandibular setback osteotomy significantly altered the sagittal jaw relationship and normalized the overall skeletal facial convexity. The results of this study confirmed the reduction of most vertical components of mandibular prognathism. Reducing the angular values NS/MP, FH/MP, ArGoMe, ArGoN, and Bjork's sum normalized the positions of maxilla and mandible to the anterior cranial base and the mutual relation of the jaws vertically. After bimaxillary operations, the values of most linear and angular skeletal variables were significantly closer to or even completely identical with the values of these variables in patients with normal occlusion.

REFERENCES

1. *Proffit RW, White PR, Sarver MD.* Contemporary treatment of dentofacial deformity. St.Louis, Mo: Mosby Co; 2003,
2. *Bui C, King T, Proffit W, Frazier-Bowers S.* Phenotypic characterization of Class III patients. Angle Orthod 2006; 76(4): 564–9.
3. *Staudt CB, Kiliaridis S.* Different skeletal types underlying Class III malocclusion in a random population. Am J Orthod Dentofacial Orthop 2009; 136(5): 715–21.
4. *Vela KC.* Phenotypic characterisation of class C III malocclusion [thesis]. Iowa, US: University of Iowa's Institutional Repository; 2012.
5. *Ingervall B, Thüer U, Vuillemin T.* Stability and effect on the soft tissue profile of mandibular setback with sagittal split osteotomy and rigid internal fixation. Int J Adult Orthodon Orthognath Surg 1995; 10(1): 15–25.
6. *Aydil B, Özer N, Maryan G.* Bimaxillary surgery in Class III malocclusion: soft and hard tissue changes. J Craniomaxillofac Surg 2013; 41(3): 254–7.
7. *Wolford LM.* The sagittal split ramus osteotomy as the preferred treatment for mandibular prognathism. J Oral Maxillofac Surg 2000; 58(3): 310–2.
8. *Ghali GE, Sikes JW Jr.* Intraoral vertical ramus osteotomy as the preferred treatment for mandibular prognathism. J Oral Maxillofac Surg 2000; 58(3): 313–5.
9. *Sinobad V, Strajnić L, Sinobad T.* Skeletal changes in patients with mandibular prognathism after mandibular setback and bimaxillary surgery – a comparative cephalometric study. Vojnosanit Pregl 2020; 77(4): 395–40.
10. *Asada K, Motoyoshi M, Tamura T, Nakajima A, Mayahara K, Shimizu N.* Satisfaction with orthognathic surgery of skeletal Class III patients. Am J Orthod Dentofacial Orthop 2015; 148(5): 827–37.
11. *Ogasawara T, Kitagawa Y, Ogawa T, Yamada T, Nakamura M, Sano K.* Treatment of severe mandibular prognathism in combination with maxillary hypoplasia: case report. J Craniomaxillofac Surg 2002; 30(4): 226–9.

12. *Chew MT*. Soft and hard tissue changes after bimaxillary surgery in Chinese Class III patients. Angle Orthod 2005; 75(6): 959–63.
13. *Abeltins A, Jakobsone G, Urtane I, Bigestans A*. The stability of bilateral sagittal ramus osteotomy and vertical ramus osteotomy after bimaxillary correction of class III malocclusion. J Craniomaxillofac Surg 2011; 39(8): 583–7.
14. *Al-Gunaid T, Yamaki M, Takagi R, Saito I*. Soft and hard tissue changes after bimaxillary surgery in Japanese class III asymmetric patients. J Orthod Sci 2012; 1(3): 69–76.
15. *Marşan G, Cura N, Emekli U*. Soft and hard tissue changes after bimaxillary surgery in Turkish female Class III patients. J Craniomaxillofac Surg 2009; 37(1): 8–17.
16. *Jakobsone G, Stenvik A, Sandvik L, Espeland L*. Three-year follow-up of bimaxillary surgery to correct skeletal Class III malocclusion: stability and risk factors for relapse. Am J Orthod Dentofacial Orthop 2011; 139(1): 80–9.
17. *Trauner R, Obwegeser H*. The surgical correction of mandibular prognathism and retrognathia with consideration of genioplasty. I. Surgical procedures to correct mandibular prognathism and reshaping of the chin. Oral Surg Oral Med Oral Pathol 1957; 10(7): 677–89; contd.
18. *Turvey TA, White RP*. Maxillary surgery. In: *Proffit WR, White RP Jr, Sarver DM,* editors. Contemporary treatment of dentofacial deformity. St. Louis, Mo: Mosby Co; 2003. Chapter 9.
19. *Johnston C, Burden D, Kennedy D, Harradine N, Stevenson M*. Class III surgical-orthodontic treatment: a cephalometric study. Am J Orthod Dentofacial Orthop 2006; 130(3): 300–9.
20. *Aydemir H, Efendiyeva R, Karasu H, Toygar-Memikoğlu U*. Evaluation of long-term soft tissue changes after bimaxillary orthognathic surgery in Class III patients. Angle Orthod 2015; 85(4): 631–7.

Received on September 6, 2018.
Revised on March 11, 2019.
Accepted May 16, 2019.
Online First May, 2019.

ORIGINAL ARTICLE

UDC: 617.541:616.712/.713-007-053.2-071.3
https://doi.org/10.2298/VSP190430071K

How to assess chest wall deformity in children with *pectus excavatum* – evaluation of the agreement among methods

Kako izvršiti procenu deformiteta grudnog koša kod dece sa *pectus*-om *excavatum*-om – procena podudarnosti metoda

Marko Kostić*, Aleksandar Sretenović†, Milan Savić*, Marko Popović*, Sanja Kostić‡, Davor Stamenović§

Clinical Centre of Serbia, *Clinic for Thoracic Surgery, Belgrade, Serbia; †University Children's Hospital, Belgrade, Serbia; ‡University Hospital "Bežanijska Kosa", Belgrade, Serbia; §Clinic for Thoracic Surgery "ViDia Kliniken", Karlsruhe , Germany

Abstract

Background/Aim. *Pectus excavatum* (PE) is the most common deformity of the frontal aspect of the chest wall in children. A particular dilemma arises about the degree of deformity that should be subjected to surgical treatment. The aim of this study was to compare several morphological methods of evaluating the degree of deformity and determine the matching among them, as well as to determine the connection between the functional and morphological abnormalities of echocardiography. **Methods.** The study included 35 patients with PE, aged between 7 and 15 years. A noninvasive evaluation of chest deformity was carried out in all patients by photographic method (surrogate of clinical examination), native X-ray imaging, and computed tomography (CT), as well as by echocardiographic examination. **Results.** In our group of patients, males were more common (67.5%), as well as children with the Haller index (HI) > 3.5 [represented in most children (86.7%)]. A significant correlation of the index of the affected sternum segment (ASt) and the total length of the sternum (ASt/LSt) determined by the photographic method with that determined by the CT scan of the chest was established (p = 0.001). In addition, the correlation between HI, determined by the X-ray method and CT images was presented (p = 0.012). In contrast, despite the high frequency of echocardiographic abnormalities (69%), those were not mutually correlated with the degree of pronounced morphological deformities of the chest wall. **Conclusion.** A detailed clinical examination and photographic evaluation method, combined with the X-ray method, can determine the severity of deformity with a high degree of agreement with the CT chest findings. In this way, it is possible for children with PE, who are not candidates for surgical treatment, to be spared from repeated CT scans that are carried out in order to monitor the development of chest deformities with growth. Echocardiographic evaluation remains an integral part of the assessment of children with PE.

Key words:
child; echocardiography; funnel chest; methods; radiography; severity of illness index; tomography, x-ray computed.

Apstrakt

Uvod/Cilj. *Pectus excavatum* (PE) je najčešći deformitet frontalnog aspekta zida grudnog koša kod dece. Posebnu dilemu predstavlja stepen deformiteta koji treba da bude podvrgnut hirurškom lečenju. Cilj rada bio je da se uporedi više morfoloških metoda procene stepena deformiteta i utvrdi podudarnost između njih, kao i povezanost između funkcionalnih i morfoloških abnormalnosti na ehokardiografiji. **Metode.** Ispitivanjem je obuhvaćeno 35 bolesnika sa PE, uzrasta od 7 do 15 godina. Kod svih bolesnika je sprovedena neinvazivna procena deformiteta grudnog koša fotografskom metodom (surogat kliničkog pregleda), nativnom radiografijom (RTG) i kompjuterizovanom tomografijom (CT), kao i ehokardiografskim pregledom. **Rezultati.** U ispitanom uzorku češće je bio zastupljen muški pol (67,5%), kao i deca sa Halerovim indeksom (HI) > 3,5 (86,7% dece). U cilju procene podudarnosti metoda, utvrđena je značajna korelacija indeksa aficiranog segmenta sternuma (ASt) i ukupne dužine sternum LSt (ASt/LSt) određivanog na fotografiji sa onim koji je određivan na CT snimku grudnog koša (p = 0.001). Pored toga, pokazana je i korelacija između HI određenog putem RTG i CT snimka (p = 0.012). Suprotno navedenom, uprkos visokoj

Correspondence to: Marko Kostić, Clinical Centre of Serbia, Clinic for Thoracic Surgery, Koste Todorovića 26, 11 000 Belgrade, Serbia. E-mail: kostmarko@gmail.com

učestalosti ehokardiografskih abnormalnosti (69%) one nisu bile u korelaciji sa stepenom izraženosti morfoloških deformiteta zida grudnog koša. **Zaključak.** Detaljnim kliničkim pregledom i fotografskom metodom procene, zajedno sa RTG metodom, može se utvrditi težina deformiteta sa visokom podudarnošću u odnosu na CT grudnog koša. Na ovaj način, moguće je decu sa PE koja nisu kandidati za hirurško lečenje poštedeti od ponavljanja CT pregleda grudnog koša koji se sprovode u cilju praćenja razvoja deformiteta tokom rasta deteta. Ehokardiografska procena ostaje neophodni i sastavni deo procene stanja dece sa PE.

Ključne reči:
deca; ehokardiografija; pektus ekskavatum; metode; radiografija; bolest, indeks težine; tomografija, kompjuterizovana, rendgenska.

Introduction

Pectus excavatum (PE) is the most common deformity of the frontal aspect of the chest wall [1]. It is characteristic of the expressed depression of the sternum, as well as the lower costal cartilage, which disrupts the human figure and consequently leads to a person's withdrawal and development of complexes. With its complexity, PE deformity leaves its pressure and effects on the heart. It could also reduce the volume of the chest, which, as a consequence, has an impact on the respiratory system. Thus, the most common application for treatment of the deformity is surgical, which is the most acceptable solution for its correction [2].

The key problem in solving PE is in defining the morphology and severity of the deformity, after which it is necessary to determine precise indications for treatment and assess the risk.

The aim of this study was to describe the dysmorphia and anatomical deformity of PE, comparing the different diagnostic methods that are currently being applied.

Methods

Patients

Thirty-five patients with anatomical deformity of PE, referred to our Clinic in the period between March 2008 and March 2016, were included in the study regarding the evaluation aimed at the optimal approach to treat this deformity.

The study protocol was approved by the Ethics Committee of the University Children's Hospital – Tiršova, Belgrade, Serbia, and written informed consent was obtained prior to study engagement from each patient's legal representative (in all cases, they were parents or closest relatives).

The overall evaluation of this category of patients included history, physical examination, photographs, but also particularly important procedures of thoracic imaging – native chest X-ray, computed tomography (CT) chest scan, and echocardiographic examination [3].

Photographs were made by a digital camera at a distance of 1 m in order to indicate the median or sternal line of the chest, the vertical length of the affected sternum (ASt), the total vertical length of the sternum (LSt), and the vertical deformation length (LDEF) (Figure 1).

CT chest scans were conducted by Siemens Somatom Emotion 16-slice, with a mediastinal and bone window (on 5 mm display) with reconstructions and 3D display.

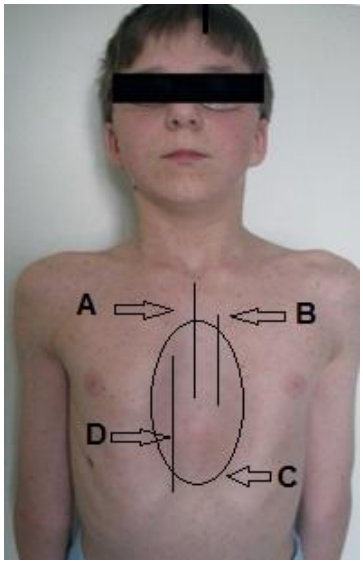
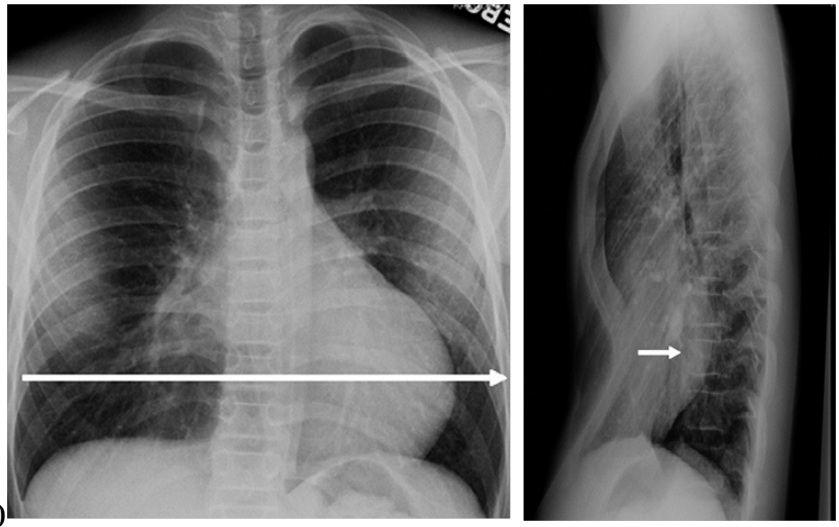


Fig. 1 – Photographic evaluation of chest wall deformity.
A - medial sternal line, length of sternum (LSt);
B - the length of the affected sternum (ASt)
C - a form of deformity; D - length of deformity (LDEF).

Echocardiographic examinations were performed by the Philips Sonos 7500. Standard echocardiographic techniques were applied, including two-dimensional echocardiography (2D mod), M-mode, and Color Doppler echocardiography. The echocardiographic analysis included the detection of any change in the morphology and function of the heart, particularly the valves, a type of syndrome prolapse, mitral valve dysplasia, mitral valve regurgitation, pulmonary artery dilatation truncus. The values of the longitudinal movements of the mitral annular (MAPSE) and the tricuspid valve (TAPSE) were also determined by the M-mode technique.

The description of dysmorphology in our patients was largely based on Cartoski et al. [4] (Nuss's group, Children's Hospital of King's Daughters, London, UK). Thus, the following indices were calculated: from photographs – ASt/LSt index = B/A × 100; symmetry index left = [L/(L+R) × 100]; symmetry index right = [R/(L+R) × 100]; ASt/LDEF index = B/D × 100; LSt/ LDEF index = A/D × 100). Then, from native X-ray (Figure 2) following indices were calculated: the Haller index (Hi) = T/A (right-side view); Hi = T/A (left-side view). And finally, from CT scans (Figure 3) following indices of sternal angle were calculated (at the level of the measured



A) **B)**

Fig. 2 – Native chest radiograph: illustrative case of a patient with *pectus excavatum*:
A) transversal diameter (arrow);
B) the shortest distance between the sternum and the front end of vertebrae (arrow).

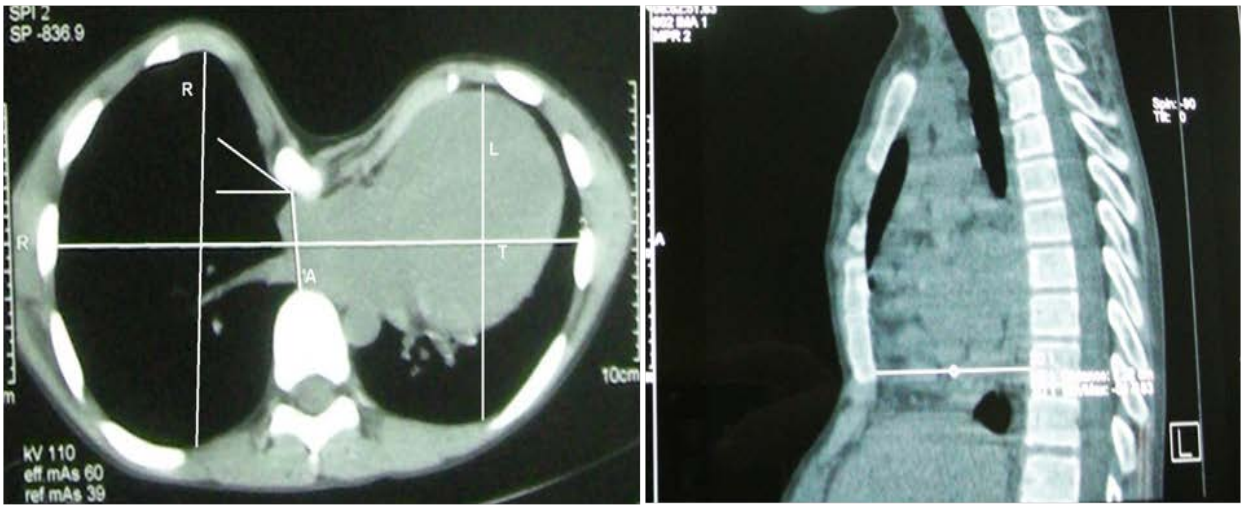


Fig. 3 – Computed tomography (CT) chest scan of a child with *pectus excavatus* deformity:
A - the shortest distance between the sternum and the front end of vertebra; T – transversal diameter Sternal angle; R – the largest distance of the right hemi-thorax in the anterior-posterior direction; L – the largest distance of the left hemi-thorax in the anterior-posterior direction; LSt - vertical length of sternum; ASt – length of affected sternum.

Hi): Hi = T/A; asymmetry index left = (L/R x 100); asymmetry index right = (R/L x 100); chest shape index (right-side view) = T/R x 100; chest shape index (left-side view) = T/L x 100; ASt/ LSt index.

The severity of pectus deformity was determined in accordance with the HI, according to which deformity levels are divided into: mild < 3.20; moderate: between 3.21 and 3.50; and severe: > 3.51. Normal HI considered values between 2.5–2.7 [5].

Concerning the degree of sternal torsion severity, which is usually graded as large, i.e., pronounced (> 30°) or small (<30°), we decided, based on the clinical judgment, to expand this division introducing the medium degree of this deviation (transitional form), in order to provide additional precision. Therefore, our division in this study implied three levels of sternal torsion severity: small (< 20°), medium (20–29°), as a transitional form, and large (> 30°).

Statistical analysis

Descriptive statistics were generated for all variables. The correlation of the findings with the use of different evaluation techniques was evaluated by the Pearson's correlation coefficient. The Student's *t*-test and Mann-Whitney *U*-test were used to test the differences when appropriate; significance level was set at the $p < 0.05$ level. The SPSS for Windows (release 16.0; SPSS, Chicago, IL, USA) was used to perform the statistical analysis.

Results

During the study, we evaluated 35 children with anatomical deformity of PE, at the age of 12.5 ± 2.4 years (range 7–15). According to age, in order to evaluate the growth effect on the development of PE deformities, patients

were further divided into age groups of 7–9 (19.4%), 10–12 (22.6%), and 13–15 (58.1%) years. In our sample, the male group was almost twice as big as the female group (65.7% males vs. 34.3% females).

Clinical and diagnostic characteristics of PE chest deformities are shown in Table 1.

Table 1

Clinical and diagnostic characteristics of *pectus excavatum* chest deformity

Parameter	Mean ± SD
Photographic method	
ASt/LSt index	47.11 ± 7.68
symmetry index R	48.34 ± 5.14
symmetry index L	51.62 ± 5.11
ASt/LDEF index	60.71 ± 9.56
LSt/LDEF index	131.44 ± 26.75
Radiographic method	
HI R	4.55 ± 1.30
HI L	4.47 ± 1.08
Computed tomography	
sternal angle	22.17 ± 11.74
HI	4.93 ± 1.69
asymmetry index R	101.43 ± 10.44
asymmetry index L	99.55 ± 9.99
chest shape index (right-side view)	176.31 ± 23.58
chest shape index (left-side view)	177.36 ± 17.56
ASt/LSt index	46.04 ± 12.50

ASt – affected sternum; LSt – length of sternum; L – left; R – right; LDEF – length of deformity; HI – Haller Index; SD – standard deviation.

According to the HI value, one patient (3.3%) had a mild degree of chest deformity (< 3.2), 3 patients (10%) had a moderate degree (3.2–3.5), and 26 patients (86.7%) had a severe degree of deformity (> 3.5).

In the present sample, we determined a significantly higher incidence of patients, 86.7% of subjects (p = 0.004), with a severe degree of chest wall deformity (according to the HI), which is the expected distribution for tertiary health centers.

Further analysis within the group of patients with the most severe degree of chest deformity (HI > 3.5) showed that most patients belong to the age group from 13–15 years (61.5%).

The angle of sternal torsion was changed in a similar way as the age-related increase of the HI, thus a high degree of rotation was observed in all subjects with the HI > 3.5 in the age group from 13–15 years.

Comparing various methods of evaluating PE deformities, it was found that the ASt/LsT index, determined by the photographic method, significantly correlated with the same index (r = 0.608, p = 0.001) when determined with CT.

Furthermore, considering the HI as the most widely accepted indicator of chest deformity, a consistent correlation was shown between the index determined by the X-ray method (right profile) and the one obtained by measuring by the CT scan (r = 0.528, p = 0.012) (Figure 4).

Similar correlation was established between the X-ray-determined HI (right profile) and ASt/LSt index measured by CT scan (r = 0.536, p = 0.012).

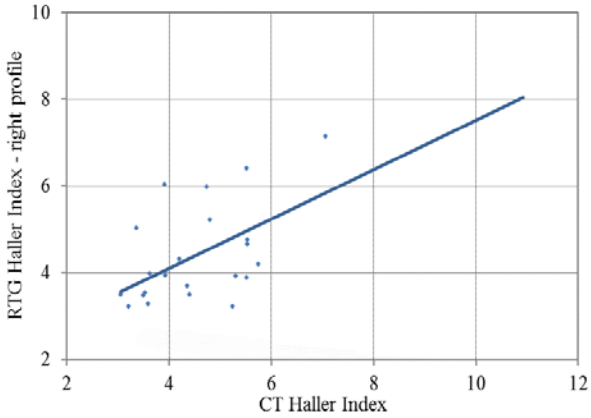


Fig. 4 – Line graph shows correlation between the Haller index measured by computed tomography (CT) scan and native radiography of the chest (right profile) in children with *pectus excavatus* deformity.

Since the asymmetry of chest cavity forms is a very common finding in children with PE deformity, defining the correlation is of particular importance. Therefore, if the irregularity of the chest cavity is considered, the right-side chest shape index (CT parameter) showed a high correlation with the asymmetry indices for the right (r = -0.618, p = 0.001) and left half (r = 0.696, p = 0.001) of the chest, CT measured as well (Figure 5).

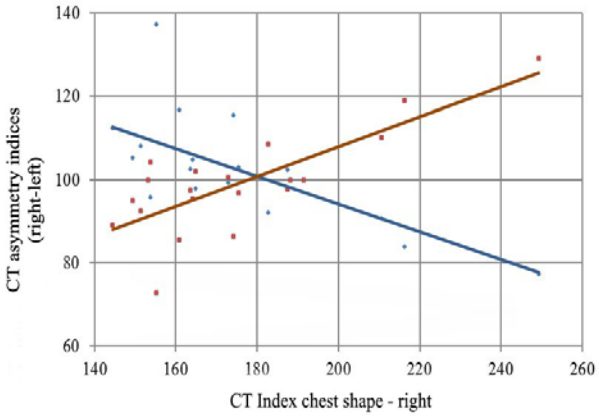


Fig. 5 – Line graphs show correlation of asymmetry indices and chest shape index (right profile blue, left profile red) in children with *pectus excavatum* deformity [both measured by computed tomography (CT) scan].

The right asymmetry indices were in a negative correlation, which means that the higher index value corresponded to the lower values of the right side of the chest shape index and *vice versa*. Contrary to that, the left asymmetry index was in a high positive correlation, which means that the higher index value corresponded to the higher values of the right side of the chest shape index.

Echocardiographic examinations in our sample of patients showed a high incidence of abnormalities (69%). The most common abnormalities were elongation, dysplasia, and mitral valve prolapse, with very frequent findings of

Table 2

Echocardiographic findings in children with *pectus excavatum* chest deformity

Variables	Children n (%)
Elongation of the mitral valve	14 (56.0)
Dysplasia of the mitral valve	6 (24.0)
Prolapse of the mitral valve	6 (25.0)
Combined prolapse and dysplasia of the mitral valve	8 (33.3)
Pressure	3 (12.5)
Regurgitation	11 (45.8)
Combined prolapse and dysplasia of the mitral valve with regurgitation	10 (41.7)

combined abnormalities (Table 2). Contrary to that, the least common disorder in our group of subjects was the pressure on the right ventricle or the atrial septum (12.5%).

Comparison of morphological abnormalities evaluated by different photographic or radiological parameters with functional findings defined by echosonography revealed the existence of only one relevant connection; both indices of asymmetry (photographically determined), right-sided ($r = -0.412$; $p = 0.045$) and left-sided ($r = 0.420$; $p = 0.040$), showed an association with the findings of a mitral valve prolapse in PE patients.

Discussion

The key findings of this study indicate that there is a significant correlation in the application of clinical assessment by the photographic method with radiological methods (X-ray and CT scan) of the chest dysmorphism in children with PE.

The distribution of the male sex in our sample was almost twice greater than the female sex (65.7% vs. 34.3%), which is common in this type of chest wall deformity.

In our study, the male sex was prevalent, but not with a statistically significantly higher incidence of PE. However, studies of other authors show that the incidence of this disease is higher (3– 5 times) in males [6–9], and according to some authors, even 80% of patients are male [10]. Therefore, the comparability of data is limited due to the specificity of our sample.

The chronological course of the disease shows that the symptoms are less pronounced in early childhood, but they increase with age [11]. The prenatal diagnosis of PE was seldom reported [12]. PE is more frequently present at birth, but in large series, only one-third of patients have deformities clearly expressed in early childhood [6, 13]. In most cases, greater deformation is observed in puberty, when otherwise there is a rapid increase.

In our patient sample, the correlation between the degree of PE deformity, expressed by the HI, concerning the subject's age did not show statistical significance, even though the HI in the majority of patients (16/18), in the oldest age group (13–15 years), was higher than 3.5. In contrast, the angle of rotation of the sternum showed a positive correlation with age in our sample of patients. All the remaining CT parameters applied in this study did not show a significant linear connection either with age or with the HI value, resulting in a dilemma about the significance of these indices in the preoperative assessment of patients.

However, as we already stated, the high degree of agreement of the HI measured on CT scan images and photographic analysis of radiographic images from the profile are even more important for the concept of this research.

In terms of the cut-off HI values from which the indication for operative treatment is derived, Kilda et al. [14] state that changes in the HI values, comparing the values before and after the surgery, were not observed if the preoperative value was 3.12. From this observation, they suggest that surgical interventions are conducted in children whose degree of deformity measured by the HI exceeds 3.1.

Furthermore, Potts [15] believes that the surgical attitude for surgical treatment of PE is somewhere between the two extremes, from having to operate on the vast majority of children with PE to not having to operate on any child at all. Patients selected for surgical treatment should have at least two or more defined criteria, which include the HI. Yoshida et al. [16] suggest that the progression of asymmetry on the right side occurs in children aged 10–12 years and that after 13 years, half of the children with PE have serious asymmetries. Based on these results, they decide upon the optimal period for performing the surgical treatment. However, several authors suggest that the time for surgery is problematic in younger children. They advocate that deformation correction should be carried out at a later stage of teenage growth, allowing the patient to complete growth and reduce the possibility of recurrence or damage [17–21]. Young children with severe cardiopulmonary symptoms may also be candidates for the implementation of an operative procedure. However, a corrective surgery at premature age may result in an inappropriate growth of the chest wall and other complications, including recurrence [22–24].

Many patients with PE have noticed decreased physical abilities, as well as minor chest pains, which is one of the indications for these major thoracic surgeries, for ensuring normal cardiac function [17, 25].

Cardiological examination of patients with PE is important because of the presence of a significant percentage of patients with right ventricular compression as well as mitral valve prolapse. In different studies, mitral valve prolapse was present in 17%–65% of patients, in contrast to the normal population where it was present in only 1% of patients [13, 26, 27]. Therefore, prolapse

of the mitral valve in PE cases could be a direct consequence of compression. Cardiac conduction disorders, such as first-degree atrioventricular block, right bundle branch block, and Wolff-Parkinson-White syndrome, were present in up to 16% of patients [28].

The majority of children in our study had some form of cardiac abnormality with the highest incidence of elongation, dysplasia, and prolapse of the mitral valve. In an attempt to determine the relationship between indicators of morphological abnormalities of the chest wall and echocardiographically defined disturbances, the only correlation was found between the index of symmetry obtained by the photographic method and the frequency of mitral valve prolapse.

Conclusion

According to our experience and the results of this study, it can be suggested that the optimal algorithm evaluation, that takes into account the rationality and reliability of diagnostic approaches, includes detailed clinical examination. The examination is further documented by standardized photographs of the chest, followed by either unavoidable native X-ray, while the CT scan is reserved for children for which the possibility of operative treatment is considered with a high level of suspicion. In our opinion, due to the high incidence of abnormal findings and because of the noninvasive examination, the echocardiographic examination should be an integral and indispensable part of the evaluation of this category of patients.

R E F E R E N C E S

1. *Shamberger RC.* Congenital chest wall deformities. Curr Probl Surg 1996; 33(6): 469–542.
2. *Abid I, Ewais MM, Marranca J, Jaroszewski DE.* Pectus Excavatum: A Review of Diagnosis and Current Treatment Options. J Am Osteopath Assoc 2017; 117(2): 106–13.
3. *Emil S.* Current Options for the Treatment of Pectus Carinatum: When to Brace and When to Operate? Eur J Pediatr Surg 2018; 28(4): 347–54.
4. *Cartoski MJ, Nuss D, Goretsky MJ, Proud VK, Croitoru DP, Gustin T,* et al. Classification of the dysmorphology of pectus excavatum. J Pediatr Surg 2006; 41(9): 1573–81.
5. *Daunt SW, Cohen JH, Miller SF.* Age-related normal ranges for the Haller index in children. Pediatr Radiol 2004; 34(4): 326–30.
6. *Blanco FC, Elliott ST, Sandler AD.* Management of congenital chest wall deformities. Semin Plast Surg 2011; 25(1): 107–16.
7. *Fokin AA, Steuerwald N, Ahrens WA, Allen KE.* Anatomical, histologic, and genetic characteristics of congenital chest wall deformities. Semin Thorac Cardiovasc Surg 2009; 21(1): 44–57.
8. *Park JM, Varma SK.* Pectus excavatum in children: diagnostic significance for mitral valve prolapse. Indian J Pediatr 1990; 57(2): 219–22.
9. *Sabiston JH Jr.* Congenital deformities of the chest wall. In: *Sabiston JH*, editor. Textbook of Surgery. Philadelphia: WB Sauders Co.; 1997. p. 1888–96.
10. *Nowak H.* Dieerblische Trichterbrust. Duetsche Med Wchnschr 1936; 62: 2003. (German)
11. *Jaroszewski DE, Fonkalsrud EW.* Repair of pectus chest deformities in 320 adult patients: 21 year experce. Ann Thorac Surg 2007; 84(2): 429-33.
12. *Salamanca A, Girona A, Padilla MC, Sabatel RM, Gonzales-Gomez F.* Prenatal diagnosis of pectus excavatum and its relation to Down's syndrome. Ultrasound Obstet Gynecol 1992; 2(6): 446–7.
13. *Shamberger RC, Welch KJ, Sanders SP.* Mitral valve prolapse asso-ciated with pectus excavatum. J Pediatr 1987; 111(3): 404–7.
14. *Kilda A, Basevicius A, Barauskas V, Lukosevicius S, Ragaisis D.* Radiological assessment of children with pectus excavatum. Ind J Pediatr 2007; 74 (2): 143–7.
15. *Potts WJ.* The Surgeon and the Child. Philadelphia, PA: W.B.Saunders, 1959.
16. *Yoshida A, Uemura S, Yamamoto M, Nouso H, Kuyama H, Muta Y.* Correlation of asymmetric chest wall deformity and growth in patients with pectus excavatum, J Pediatr Surg 2013; 48(4): 771–5.
17. *Kelly RE Jr.* Pectus excavatum: historical background, clinical picture, preoperative evaluation and criteria for operation. Semin Pediatr Surg 2008; 17(3): 182–93.
18. *Fonkalsrud EW.* Current management of pectus excavatum. World J Surg 2003; 27(5):502–8.
19. Colombani PM. Preoperative assessment of chest wall deformities. Semin Thorac Cardiovasc Surg 2009; 21(1): 58–63.
20. *Davis JT, Weinstein S.* Repair of the pectus deformity: results of the Ravitch approach in the current era. Ann Thorac Surg 2004; 78(2): 421–6.
21. *Nuss D, Kuhn M.* Our approach: minimally invasive surgical repair of pectus excavatum. Contemp Surg 2007; 63: 444–51.
22. *Haller JA Jr, Colombani PM, Humphries CT, Azizkhan RG, Loughlin GM.* Chest wall constriction after too extensive and too early operations for pectus excavatum. Ann Thorac Surg 1996; 61(6): 1618–24; discussion 1625.
23. *Kim DH, Hwang JJ, Lee MK, Paik HC.* Analysis of the Nuss procedure for pectus excavatum in different age groups. Ann Thorac Surg 2005; 80(3): 1073–7.
24. *Pilegaard HK, Licht PB.* Routine use of minimally invasive surgery for pectus excavatum in adults. Ann Thorac Surg 2008; 86(3): 952–6.
25. *Lawson ML, Cash TF, Akers R, Vasser E, Burke B, Tabangin M,* et al. A pilot study of the impact of surgical repair on disease-specific quality of life among patients with pectus excavatum. J Pediatr Surg 2003; 38(6): 916–8.
26. *Saint-Mezard G, Duret JC, Chanudet X, Larrue J, Bonnet J, Bricaud H.* Mitral valve prolapse and pectus excavatum. Fortuitous association or syndrome? Presse Med 1986; 15(9): 439. (French)
27. *Warth DC, King ME, Cohen JM, Tesoriero VL, Marcus E, Weyman AE.* Prevalence of mitral valve prolapse in normal children. J Am Coll Cardiol 1985; 5(5): 1173–7.
28. *Nuss D, Croitoru DP, Kelly RE.* Congenital chest wall deformities. In: *Ashcraft KW, Holcomp GW 3rd, Murphy JP*, editors. Pediatric Surgery. 4th ed. Philadelphia, PA: Elsevier Saunders, 2005. p. 245–63.

Received on April 30, 2019.
Accepted on May 29, 2019.
Online First June, 2019.

ORIGINAL ARTICLE

UDC: 616.31-092.9:616.31-74/-77
https://doi.org/10.2298/VSP190405069M

Use of calcium hydroxyapatite and growth factors in endodontic therapy

Primena kalcijum-hidroksiapatita i faktora rasta u endodontskoj terapiji

Aleksandar Mitić*, Milan Živković†, Dušan Živković†, Lidija Popović‡, Zorana Veličković†, Milan Miladinović†, Ljiljana Šubarić†, Dragan Marjanović†, Andrijana Cvetković†

Unoversity of Niš, Faculty of Medicine, *Dental Clinic, Niš, Serbia; University of Priština/Kosovska Mitrovica, †Faculty of Medicine, Kosovska Mitrovica, Serbia; ‡Institute for Psychophysiological Disorders and Speech Pathology "Prof. Dr. Cvetko Brajović", Belgrade, Serbia

Abstract

Background/Aim. Hydroxyapatite (HAp) is one of the most commonly used calcium phosphate bioceramics with osteoconductive properties. Growth factors are capable of directly inducing morphological and functional differentiation of neodontoblasts. The aim of this study was to investigate the effectiveness of HAp-based biomaterial in combination with transforming growth factor-β1 (TGF-β1) in the creation of new dentine and obturation of the root canal apex in the teeth of an experimental animal model. **Methods.** Rodent (rabbit) teeth were used as the experimental animal model. After pulp removal with a pulp extirpator in vital pulpectomy, the biomaterial was applied using a Lentulo spiral in the apex portion at the level of the physiological *foramen apicale*. The experiment was performed in general anesthesia. Animals were kept alive for 3, 6, and 12 months after the treatment. The extracted teeth were analyzed by scanning electron microscopy (SEM). **Results.** Using SEM, it was found that the number of teeth with newly created dentine and apex canal obturation was greater 12 months after the treatment. **Conclusion.** Apex obturation of the dental root canal with newly created dentine took place in our experimental groups treated by biomaterial with or without TGF-β1.

Key words:
odontoblasts; endodontics; dentin; biocompatible materials; transforming growth factors; tooth root; rabbits.

Apstrakt

Uvod/Cilj. Hidroksiapatit (HAp) je jedna od najčešće korišćenih kalcijum fosfatnih biokeramika koja ispoljava osteokonduktivna svojstva. Faktori rasta direktno indukuju morfološku i funkcionalnu diferencijaciju neodontoblasta. Cilj rada je bio ispitivanje efikasnosti biomaterijala na bazi HAp u kombinaciji sa faktorom rasta-β1 (TGF-β1) u stvaranju novog dentina i zatvaranju apeksa kanala korena na zubima eksperimentalnog modela. **Metode**. Kao eksperimentalni animalni model korišćeni su zubi glodara (zečeva). Nakon uklanjanja pulpe zuba pulpekstirpatorom kod vitalne pulpektomije, u apeksnom delu na nivou fiziološkog apikalnog foramena, lentulo spiralom aplikovan je biomaterijal. Eksperiment je obavljen u opštoj anesteziji. Životinje su održavane u životu 3, 6 i 12 meseci posle tretmana. Ekstrahirani zubi su analizirani pomoću *scanning* elektronske mikroskopije. **Rezultati**. *Scanning* elektronskom mikroskopijom dokazano je da je broj zuba sa novostvorenim dentinom i apeksnom opturacijom kanala veći 12 meseci nakon tretmana. **Zaključak**. Utvrđeno je da je u eksperimentalnim grupama tretiranim biomaterijalom, sa ili bez TGF-β1, došlo do apeksne opturacije kanala korena zuba novostvorenim dentinom.

Ključne reči:
odontoblasti; endodoncija; dentin; biokompatibilni materijali; faktori rasta, transformišući; zub, korenski kanal; zečevi.

Introduction

A diagnosis of the pulp inflammation degree is essential in the attempts to preserve pulp vitality by appropriate strategies whenever possible.

It is generally accepted that the prognosis of dental root canal treatment largely depends on the quality of the root canal filling [1]. The apical third of the canal deserves special attention in the mechanical preparation since it is the most sensitive zone that communicates with the vital tissue, which

Correspondence to: Milan Miladinović, University of Priština/Kosovska Mitrovica, Faculty of Medicine, Dental Clinic, Kosovska Mitrovica, Anri Dinana b.b., 38 220 Kosovska Mitrovica, Serbia. E-mail: milanbetter@gmail.com

is most important for the healing process. A provision of biologically acceptable sealing of the apical portion of the root canal before the definitive filling has urged many authors to consider the issue of apical barrier formation during the root canal treatment. This would prevent material crossing over during the obturation on the one hand and provide high quality, compact, and airtight canal filling on the other [2]. An apical plug would prevent the occurrence of adverse effects associated with the material used for definitive canal filling in the periapical area [3].

Teodorović [1] has combined hydroxyapatite powder with 35% of calcium sulphate and successfully used it for the formation of apical plugs in endodontically treated teeth with completed root growth.

There are a few materials capable of inducing the creation of hard tissues, especially the cement tissue, and even able to stimulate bone reparation if large defects have occurred [4].

Over the years, various materials have been investigated as potential therapeutic agents in vital pulpectomy. Calcium hydroxide has been most extensively studied and used. Despite all of its positive properties, it is not an ideal biological material for apical sealing, and many authors think that its stimulative effects have not been sufficiently elucidated yet [1–5].

Gollmer [6] was the first author who created an apical plug out of dentine chips, believing that successful healing after pulpectomy could not be expected without an effective, biocompatible apical "sealant", which would prevent an irritative contact of the used filling materials with periapical tissue.

Dianat et al. [7] used dentine plugs to create an apical seal in their experiment conducted on monkey teeth. The results of their study demonstrated solid tissue (osteodentine) creation at the interface of dentine powder and the remaining vital pulp stump after vital pulpectomy. The studies by Jacobsen et al. [8] contested the positive results obtained by the use of dentine plugs on account of significant apical "leakage" after such obturation. Recent studies in both medicine and dentistry have attempted to identify synthetic materials which would not act as antigens and which would, at the same time, be able to successfully replace the bone tissue, i.e. have an osteoconductive effect [9, 10]. Ceramic biomaterials based on hydroxyapatite (HAp) or three-calcium phosphate are most similar to inorganic bone tissue components by their chemical composition and structure [10]. Some studies have investigated the use of biomaterials such as hydroxyapatite for pulp therapy within the techniques of direct pulp capping [11–13], amputation of the coronal portion of the pulp [14], and for endodontic treatment of the teeth with completed root growth as a material for apical barrier formation [15]. Hydroxyapatite is one of the most commonly used calcium phosphate bioceramic materials with osteoconductive properties [14–17]. As a potentially good growth factor delivery vehicle (scaffold), calcium phosphate-based materials have been suggested, whose porous structure enables gradual release and diffusion of growth factors [18].

Based on the aforementioned studies, Pissiotis and Spangberg [19] concluded that due to tissue reaction, predictability, and stability of both HAp and its mixture with collagen, the "plug", created by the compression of crystals of these materials, could represent an optimal solution for apical plug formation. These authors also suggested that clinical problems associated with the manipulation and application of HAp crystals into the apical third of the dental root canal warranted further extensive studies.

According to Grossman [20], the ultimate goal of dental root canal therapy is an airtight (hermetic) filling of the canal space.

Sugawara et al. [21] have investigated *in vivo* the use of calcium phosphate ceramic as a material for the definitive dental root canal filling on canine teeth. They proved that the material was compatible with periapical tissue and capable of binding and forming a solid mass in the presence of tissue fluids.

Mongiorgi et al. [22] have studied the new alloplastic bioceramic material formulations, but with a new cement composition for the definitive dental root canal filling (Proendo, Vebas, Italy). Based on the obtained results, the authors concluded that the material was biocompatible, osteoconductive, non-toxic, with good adhesive properties, and that it provided good apical sealing. The sealing prevented percolation and transit of both bacteria and their products along the endodontically processed/prepared and definitively filled dental root canals.

There have been attempts of using hydroxyapatite and growth factors for the same purpose, although with very low success rates. These studies are still very attractive, though.

The results of the above studies have shown that using some of the growth factors, especially transforming growth factor-beta (TGF-β), can stimulate odontoblast differentiation and induce the release of endogenous growth factors contained in the organic dentine matrix, which additionally stimulates dentinogenesis [23]. Recent insights into the role of growth factors in dental tissue reparation, whether it is reactive or reparative dentinogenesis, could represent the basis for a different approach to pulp treatment. Naturally, nowadays, therapeutic procedures involving teeth with incomplete root growth are being rationalized, and the time required for a therapeutic procedure is getting increasingly shorter [24].

Some clinical studies have shown that the use of platelet-rich plasma has beneficial effects on the reparation processes, while other studies do not report such an effect. These conflicting data can be perhaps explained by different methods of preparation and, consequently, different PRP concentrations. In fact, the issue of PRP concentration, which is optimal for tissue reparation and regeneration, is still unresolved [25].

The aim of this study was to investigate the effectiveness of calcium HAp and growth factors as medicaments on the creation of new dentine and apical dental root canal obturation in vital pulp extirpation on the teeth of our experimental model.

Methods

The study was performed at the Institute for Biomedical Research, Faculty of Medicine in Niš, and the Faculty of Medicine in Kosovska Mitrovica, with the approval of the Ethics Committee of the Faculty of Medicine in Niš.

Three 6-month-old chinchilla rabbits, 3–4 kg of body weight (BW) each, were included in the experiment. The animals were anesthetized by intramuscular administration of combination of toletamine and zolazepam (Zoletil 100®, Virbac S.A., France) at a dose of 10 mg/kg BW and ketamine hydrochloride (Ketlar®, Pfizer, UK) at a dose of 1–4.5 mg/kg BW. After pulp space trepanation, in samples for vital extirpation, the pulp was removed using a pulp extirpator, and the biomaterial was applied with Lentulo spiral up to the level of the physiological apical foramen. FlexoFile® endodontic files (Maillefer, Switzerland) were used for biochemical canal processing. Definitive canal obturation was performed with Lentulo spiral again, with an AH Plus® (combination of calcium tungstate and zirconium oxide) root canal sealer and gutta percha points. All the cavities were definitively capped with a glass ionomer cement (GIC) and dental amalgam.

In this study, we used calcium hydroxyapatite/poly(lactide-co-glycolide) – HAp/PLGA and recombinant human TGF-β1.

The teeth were divided into three groups. The first experimental group (n = 15), composed of lower jaw teeth on the left side (incisors, premolars, molars), into which calcium HAp/PLGA biomaterial was applied. The second experimental group (n = 15), composed of upper jaw teeth on the right side (incisors, premolars, molars), into which calcium HAp/PLGA biomaterial was applied, combined with TGF-β. Calcium HAp/PLGA biomaterial served as the delivery vehicle, 80 : 20 (0.5 g) (product of the Institute of Technical Sciences of the Serbian Academy of Sciences and Arts, Belgrade). The third group (n = 15) composed of intact teeth in the left upper jaw and right lower jaw (incisors, premolars, molars) from the same sacrificed animals (control group).

After this initial part of the study, the animals were kept alive for 3, 6, and 12 months, and after that, they were sacrificed with a lethal dose of Ketalar®. Jawbones were disarticulated, and each tooth was extracted separately. Material preparation involved the storage of teeth in sterile saline at 40°C, without any fixing agents.

All the samples were processed by a single operator. Occlusal 2–3 mm thick surfaces (dental crowns) were circularly cut with the finest fissure diamond burs. Dental roots were incised longitudinally with dental separator discs in order to provide adequate separation into the oral and vestibular halves. Each half of the sample was mounted onto an appropriate stand, and thus the samples fixated were gold evaporated in a vacuum evaporator and observed under a scanning electron microscope (SEM) JEOL-JCM-5300.

Data entry and tabular data representation were done using the MS Office Excel software, and calculations were made using the 2007 SPSS, version 15.0.

The differences between the parameters of interest among the groups, as well as within the groups, were established using the Mantel-Haenszel chi-squared test or Fisher's test of the exact probability of the null hypothesis (when some of the expected frequencies were below 5).

Results

Obtained results are presented in Tables 1 and 2. In total, 45 teeth from 3 sacrificed experimental animals (chinchilla rabbits) were included in the investigation. Vital pulp extirpation (VPE) was performed in both experimental groups on the same number of teeth (15 teeth from each group). In the control group, there were no teeth with dental root canal obturation. Comparing the groups with TGF-β1 + HAp/PLGA and HAp/PLGA after 12 months, the greatest difference in the number of teeth with obturated canals was found, but the difference did not reach the level of statistical significance ($p = 0.30$).

Table 1

Total number of treated teeth in experimental groups and the control group

Animal number	Number of treated teeth		
	Control	TGF-β1+HAp/PLGA	HAp/PLGA
I	5	5	5
II	5	5	5
III	5	5	5
Total number of teeth	15	15	15

TGF-β1– transforming growth factor- β;
HAp/PLGA – hydroxyapatite/poly (lactide-co-glycolide)

Table 2

Dental root canal capping during vital pulp extirpation in experimental groups and the control group

Group	n	Period		
		3 months	6 months	12 months
		n (%)	n (%)	n (%)
(TGF-β + HAp/PLGA)	15	3 (20.0)	3 (20.0)	4 (26.7)
(HAp/PLGA)	15	0 (0)	1 (6.7)	1 (6.7)
(Control)	15	0 (0)	0 (0)	0 (0)

For abbreviations see under Table 1.

The results obtained 3, 6, and 12 months after applying TGF-β1 + HAp/PLGA and HAp/PLGA by SEM microscopy for vital pulp extirpation (VPE) are shown in Figures 1–8.

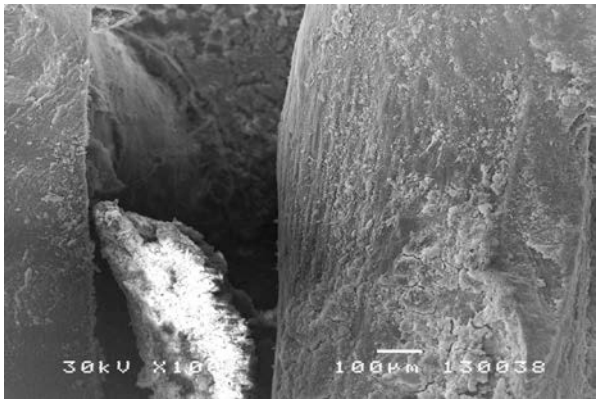


Fig. 1 – TGF-β1 (scanning electron microscopy): Incomplete apical sealing with newly formed dentine (3-month observation period).
For abbreviations see under Table 1.

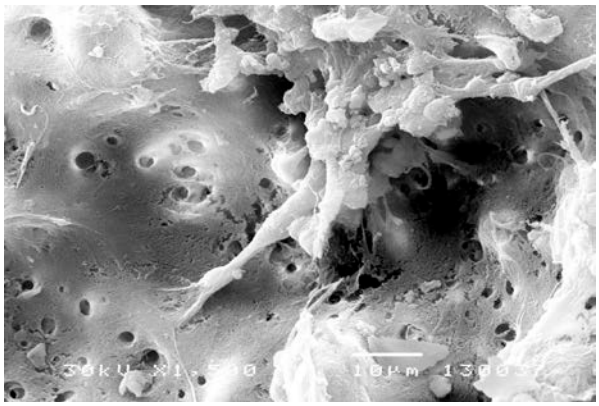


Fig. 2 – HAp/PLGA (scanning electron microscopy): Incomplete apical sealing with newly formed dentine (3-month observation period).
For abbreviations see under Table 1.

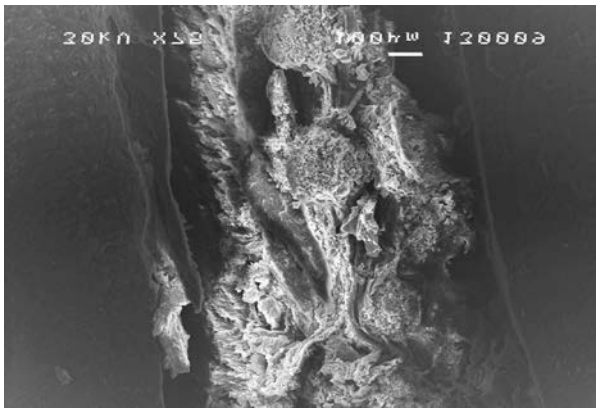


Fig. 3 – TGF-β1+ HAp/PLGA (scanning electron microscopy): Complete root canal obturation with newly formed dentine (6-month observation period).
For abbreviations see under Table 1.

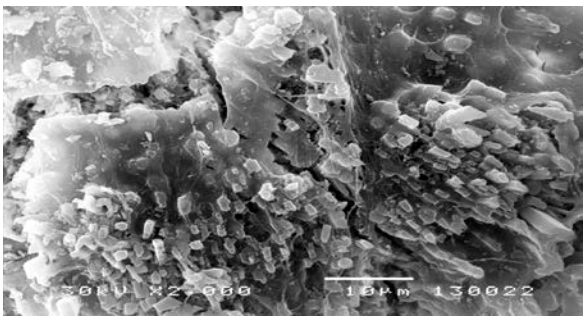


Fig. 4 – HAp/PLGA (scanning electron microscopy): Irregular dentine (6-month observation period).
For abbreviations see under Table 1.

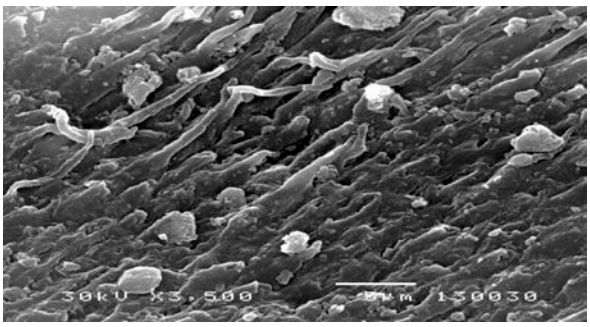


Fig. 5 – TGF-β1+HAp/PLGA (scanning electron microscopy): Complete apical obturation with newly formed dentine (12-month observation period).
For abbreviations see under Table 1.

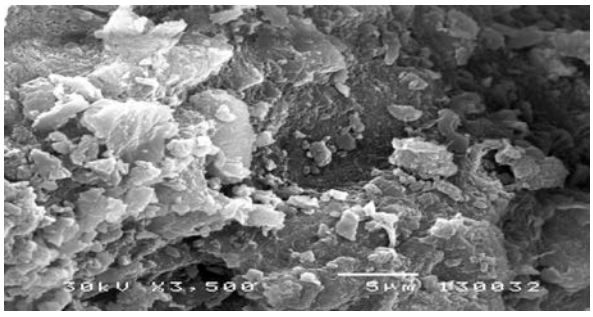


Fig. 6 – HAp/PLGA (scanning electron microscopy): Apical obturation with newly formed dentine (12-month observation period).
For abbreviations see under Table 1.

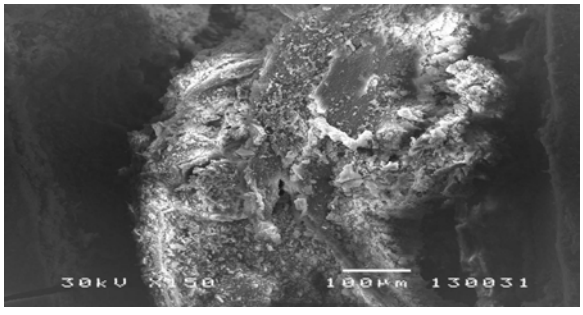
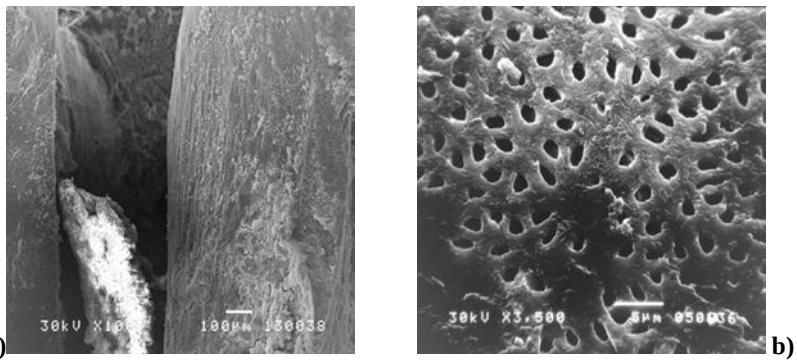


Fig. 7 – TGF-β1 +HAp/PLGA (scanning electron microscopy): Complete apical obturation with newly formed dentine (12-month observation period).
For abbreviations see under Table 1.



a) b)

Fig. 8 – Control group – intact tooth (scanning electron microscopy): a) open dental root canal apex; b) regular dentine.

Discussion

The results of the study showed that apical obturation of dental root canal occurred in our experimental groups.

In order to objectively evaluate and interpret these results clinically in a valid way, we should review the methodology employed in the study. Rodent (rabbit) teeth were used as an animal model, although frequently regarded as inappropriate for such experiments due to their specificity reflected in constant growth and wear (which refers especially to front teeth) [25]. Nevertheless, many authors dispute such an attitude, emphasizing that the rodent pulp-dentine complex has a significant potential for studying many aspects of reactive dentinogenesis [26], as well as for observing pulp reactions to bioactive molecules [27].

The interaction between the material and the injured pulp tissue, as well as the pathways of initiation and progression of healing and regeneration processes, are still insufficiently understood. There are numerous hypotheses about that, but the latest studies have paid significant attention to growth factors and their roles in angiogenesis, progenitor cell mobilization, differentiation, and, finally, biomaterial-supported mineralization [28].

In all samples studied, the application of HAp and growth factors produced complete apical dental root canal obturation with newly formed dentine in the period of 12 months.

Many authors have also noticed the difficulties in clinical manipulation, application, and retention of material at the application site, especially with high pulp amputations and deeper material placements into the root canal. These authors, therefore, recommended a collagen-HAp combination, with satisfactory results in laboratory animals and pre-prosthetic preparation of the alveolar process [19].

Calcium phosphate vehicles/scaffolds for growth factors have also been suggested as potentially good (which agrees with our study). They enable gradual release and diffusion of growth factors due to their porous structure. In our study, HAp was a good growth factor delivery vehicle.

Our results also corroborate other authors' findings in studies with dogs, which demonstrated stability and osteoconduction using calcium phosphate ceramic as a definitive filling material [29].

Petrović et al. [4], using synthetic HAp in their study (with an average particle size of 100 µm) on laboratory animals – dogs, applied the material on the pulp of young teeth with incomplete root growth. In one part of the study, in addition to the tested material (HAp), they also applied an autogenic growth factor originating from platelet-rich plasma in amputations and high amputations of the pulp. All the samples were radiographically controlled and compared to contralateral untreated teeth. Based on the analysis of dental X-rays, it was found that root apex formation continued in all the studied samples.

Teodorović and Martinović [30] combined HAp powder with 35% of calcium sulphate and used it successfully as a paste for the formation of apical plugs in endodontically treated teeth with completed root growth. In addition to biocompatibility, the studies have shown that HAp is a stable and osteoconductive material. The results of the histological analysis showed adequate stability, evidenced by the presence of HAp in the period of 24 experimental weeks without any signs of resorption. Furthermore, other authors' results agree with these results, demonstrating stability and osteoconduction in their experiments on dogs, using calcium phosphate ceramic as a definitive root canal filling material. The studies have shown that all the reactions between hard tissues (dentine, cement) and HAp take place at their interface (contact surfaces).

As some studies have demonstrated, HAp is applicable in clinical practice in the formation of apical plugs, but care should be taken regarding the type of material for definitive root canal filling, which covers the placed biological plug. At the end of the 12-month observation period, the results were identical for both samples, those treated with HAp and those treated with HAp and platelet-rich plasma. This suggests that growth factors influenced more rapid healing, i.e. dentine bridge creation and complete apical dental root canal obturation with newly formed dentine [31], which agreed with our findings.

In recent years, much attention has been paid to growth factors and their role in the initiation of reparation processes in pulp damage, which constituted a part of our study as well. These bioactive molecules promote proliferation and differentiation of cells, matrix synthesis, and angiogenesis. Very attractive are also the studies, both preclinical and

clinical, whose results indicate that the use of growth factors can provide a favorable prognosis regarding bone, periodontium, and cement regeneration [7].

The results by Tziafa et al. [26] have shown that the use of some of the growth factors, especially TGF-β, is able to stimulate odontoblast differentiation and lead to the release of endogenous growth factors contained in the dentine organic matrix, which additionally stimulates dentinogenesis.

A strictly applied contemporary conception of the root canal treatment enables and facilitates healing processes in the apical periodontium, which is the principal goal of a successful endodontic treatment [29]. Apical barrier formation during the treatment is especially important in specific clinical situations. The barrier, i.e. the apical "plug", plays multiple roles, opposing toxic actions at the interface of the definitive filling material and vital periapical tissue and enabling high quality and complete, airtight dental root canal filling [29]. Apical plug formation and "microleakage" problems have not been solved by the attempts with calcium hydroxide, nor with the use of dentine chips [15].

Sugawara et al. [21], Teodorović [1, 15], and Teodorović and Martinović [30] have reported that ceramic biomaterials are capable of binding and forming a solid mass in the presence of tissue fluids following a definitive root canal filling.

In the era of regenerative endodontics, the introduction of new procedures and materials is expected to take place as both biological treatment and for the purpose of tooth revitalization [31].

Conclusion

Based on the facts stated above, a conclusion may be drawn that new dentine was indeed created and apical root canal closure has occurred in the experimental groups. HAp/PLGA was shown to be a good growth factor delivery vehicle.

REFERENCES

1. *Teodorović N.* Hydroxyapatite-based ceramic materials in endodontic therapy of dental root canals. Monograph. Belgrade: University of Belgrade, Faculty of Dentistry; 2004. (Serbian)
2. *Tronstad L.* Tissue reactions following apical plugging of the root canal with dentin chips in monkey teeth subjected to pulpectomy. Oral Surg Oral Med Oral Pathol 1978; 45(2): 297–304.
3. *Teodorović N.* Research in adhesive performances of three canals sealers – SEM stady. 7th Congress of the Balcan Stomatological Society (BaSS); 2002 March 28–30; Kushadasi, Turkie 2002.
4. *Petrović V.* Modalities of use of hydroxyapatite in apexogenesis [dissertation]. Belgrade: University of Belgrade, Faculty of Dentistry; 2007. (Serbian)
5. *Taha NA, Abdulkhader SZ.* Full Pulpotomy with Biodentine in Symptomatic Young Permanent Teeth with Carious Exposure. J Endod 2018; 44(6): 932–7.
6. *Gollmer L.* The Use of Dentin Debris as a Root Canal Filling. Int J Orthod 1937; 23: 101–2.
7. *Dianat O, Mashhadi Abas F, Paymanpour P, Eghbal MJ, Haddadpour S, Bahrololumi N.* Endodontic repair in immature dogs' teeth with apical periodontitis: blood clot vs plasma rich in growth factors scaffold. Dent Traumatol 2017; 33(2): 84–90.
8. *Jacobsen EL, Bery PF, BeGole EA.* The effectiveness of apical dentin plugs in sealing endodontically treated teeth. J Endod 1985; 11(7): 289–93.
9. *Popović-Bajić M.* Impact of amelogenin, growth factors and new nanostructural materials based on calcium silicate cements on pulp regeneration [dissertation]. Belgrade: University of Belgrade, Faculty of Dentistry; 2015. (Serbian)
10. *Besinis A, van Noort R, Martin N.* Remineralization potential of fully demineralized dentin infiltrated with silica and hydroxyapatite nanoparticles. Dent Mater 2014; 30(3): 249–62.
11. *Jalan AL, Warhadpande MM, Dakshindas DM.* A comparison of human dental pulp response to calcium hydroxide and Biodentine as direct pulp-capping agents. J Conserv Dent 2017; 20(2): 129–33.
12. *Hegde S, Sowmya B, Mathew S, Bhandi SH, Nagaraja S, Dinesh K.* Clinical evaluation of mineral trioxide aggregate and biodentine as direct pulp capping agents in carious teeth. J Conserv Dent 2017; 20(2): 91–5.
13. *Li Z, Cao L, Fan M, Xu Q.* Direct pulp capping with calcium hydroxide or mineral trioxide aggregate: A meta-analysis. J Endod 2015; 41(9): 1412–7.
14. *Bimstein E, Rotstein I.* Cvek pulpotomy - revisited. Dent Traumatol 2016; 32(6): 438–42.
15. *Teodorovic, N.* Use of ceramic biomaterials in dental root canal treatment canals [dissertation]. Belgrade: University of Belgrade, Faculty of Dentistry; 1998. (Serbian)
16. *Djordjevic M.* Comparative study of hydroxyapatie and calcium hydroxyde as the materials for direct and indirect pulp capping [dissertation]. Belgrade: University of Belgrade, Faculty of Dentistry; 2004. (Serbian)
17. *Melih I.* Experimental and clinical evaluation of adhesion of different materials for the root canal obturation [dissertation]. Pančevo: Faculty of Dentistry, University of Economics Academy in Novi Sad; 2015. (Serbian)
18. *Brizuela C, Ormeño A, Cabrera C, Cabezas R, Silva CI, Ramírez V,* et al. Direct Pulp Capping with Calcium Hydroxide, Mineral Trioxide Aggregate, and Biodentine in Permanent Young Teeth with Caries: A Randomized Clinical Trial. J Endod 2017; 43(11): 1776–80.
19. *Pissiotis E, Spangberg LS.* Biological evaluation of collagen gels containing calcium hydroxide and hydroxyapatite. J Endod 1990; 16(10): 468–73.
20. *Grossman L.* Short-cuts in endodontic practice: are they worth the risks? Oral Health 1976; 66(12): 9–10.
21. *Sugawara A, Chow LC, Takagi S, Chohayeb H.* In vitro evaluation of the sealing ability of a calcium phosphate cement when used as a root canal sealer-filler. J Endod 1990; 16(4): 162–5.
22. *Mongiorgi R, Prati C, Bertocchi G, Monti S.* Chemistry and structure of a new canal sealer showing a dynamical behaviour. Boll Soc Ital Biol Sper 1993; 69(6): 415–22.
23. *Melin M, Joffre-Romeas A, Farges JC, Couble ML, Magloire H, Bleicher F.* Effects of TGFbeta1 on dental pulp cells in cultured human tooth slices. J Dent Res 2000; 79(9): 1689–96.
24. *Santos SCNDS, Sigurjonsson ÓE, Custódio CA, Mano JFCDL.* Blood Plasma Derivatives for Tissue Engineering and Regenerative Medicine Therapies. Tissue Eng Part B Rev 2018; 24(6): 454–62.
25. *Verstraete FJM, Osofsky A.* Dentistry in pet rabbits. Comp Cont Educ Pract 2005; 27(9): 671–84.
26. *Tziafa C, Koliniotou-Koumpia E, Papadimitriou S, Tziafas D.* Dentinogenic responses after direct pulp capping of miniature swine teeth with Biodentine. J Endod 2014; 40(12): 1967–71.

27. *Orhan EO, Maden M, Senguüven B.* Odontoblast-like cell numbers and reparative dentine thickness after direct pulp capping with platelet-rich plasma and enamel matrix derivative: a histomorphometric evaluation. Int Endod J 2012; 45(4): 317–25.
28. *Laurent P, Camps J, About I.* Biodentine(TM) induces TGF-β1 release from human pulp cells and early dental pulp mineralization. Int Endod J 2012; 45(5): 439–48.
29. *Girish K, Mandava J, Chandra RR, Ravikumar K, Anwarullah A, Athaluri M.* Effect of obturating materials on fracture resistance of simulated immature teeth. J Conserv Dent 2017; 20(2): 115–9.
30. *Teodorović N, Martinović Ž.* Significance of apico-coronal technique of dental root canal preparation in endodontic therapy using hydroxyapatite-based sealants. Vojnosanit Pregl 2005; 62(6): 447–52. (Serbian)
31. *Zhang L, Li QL, Cao Y, Wang Y.* Regenerating a monoblock to obturate root canalsvia a mineralising strategy. Sci Rep 2018; 8(1): 13356

Received on April 5, 2019.
Accepted May 30, 2019.
Online First June, 2019.

ORIGINAL ARTICLE

UDC: 617.3:616.718.5-08
https://doi.org/10.2298/VSP190110070T

Segmental tibial fractures treated with Ilizarov circular fixator

Segementni prelomi tibije lečeni fiksatorom po Ilizarov-u

Slavko Tomić*†, Nemanja Slavković*†, Goran Tulić†‡, Andreja Baljozović*, Želimir Jovanović*, Milan Mirković*, Stanislav Rajković*, Nikola Bogosavljević*, Dušan Šaponjski§, Sladjana Mihajlović‖, Danilo Jeremić*

***Institute for Orthopaedic Surgery "Banjica", Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, Belgrade, Serbia; Clinical Centre of Serbia, ‡Clinic for Orthopaedic Surgery and Traumatology, §Centre for Radiology and MRI, Belgrade, Serbia; University Hospital "Dragiša Mišović", ‖Clinic for Gynecology, Belgrade, Serbia**

Abstract

Background/Aim. Segmental fractures represent complex tibial injuries, featuring a unique fracture type that is most commonly caused by high-energy trauma. These fractures are considered to be a treatment challenge for orthopaedic surgeons due to their sporadic presentation, wide zone of soft tissue injury, and increased rate of complications. They are characterized by a highly unstable intermediary segment and a high rate of open fractures. The method of Ilizarov with its characteristics could offer many advantages over the existing operative techniques. This method, using a percutaneous approach, minimizes the intraoperative trauma and avoids the additional compromising of the biological environment at the fracture site. The aim of this study was to evaluate the results of the Ilizarov fixator in the treatment of segmental tibial fractures. **Methods.** We analyzed 30 patients treated with the Ilizarov fixator between 2012 and 2017. The average age of patients was 36 years (from 24 to 65). The most common mechanism of injury was a road traffic accident. Open fractures were noted in 22 cases. All fractures were reduced using indirect percutaneous techniques with a great focus on achieving the correct length, rotation, and axial alignment of fragments. All patients were advised to bear weight as tolerated from the second postoperative day. Bone healing and functional results were evaluated according to the criteria established by the Association for the Study and Application of the Method of Ilizarov. **Results.** Bone healing was achieved in all patients. The average time to union was 25 weeks (19 to 36 weeks). Bone results were excellent in 23 patients, good in five, and fair in two patients. Functional results were excellent in 22 cases, good in 5, and fair in three cases. Eight patients had minor pin-tract infections, successfully treated with oral antibiotics. Patients were without any major complications. **Conclusion.** The Ilizarov method is a safe and efficient treatment modality for segmental tibial fractures.

Key words:
tibial fractures; external fixators; ilizarov technique; recovery of function.

Apstrakt

Uvod/Cilj. Segmentni prelomi tibije predstavljaju kompleksne povrede koje se karakterišu složenim obrascem preloma i uglavnom su posledica sila visokog intenziteta. Ove povrede predstavljaju izazov u izboru načina lečenja usled svoje sporadične prezentacije, obimne zone značajno oštećenog mekog tkiva i povećane stope komplikacija. Odlikuju se izuzetno nestabilnim intermedijarnim fragmentom i visokom učestalošću otvorenih preloma. Ilizarovljev metod svojim karakteristikama nudi mnoge prednosti nad ostalim postojećim hirurškim tehnikama. Ovaj metod, svojim perkutanim pristupom, značajno smanjuje traumu operativnog polja, čime se izbegava dodatno narušavanje biološke sredine na mestu preloma. Cilj ovog rada je analiza rezultata lečenja segmentnih preloma tibije metodom Ilizarova. **Metode.** Analizirali smo podatke od 30 bolesnika lečenih metodom Ilizarova u periodu od 2012. do 2017. godine. Prosečna starost bolesnika iznosila je 36 godina (24 do 65 godina). Najčešći mehanizam povređivanja bio je saobraćajni traumatizam. Otvorene prelome imalo je 22 bolesnika. Prelomi su reponirani perkutanom tehnikom, sa velikom pažnjom na uspostavljanje adekvatne dužine, rotacije i osovine potkolenice. Svim bolesnicima je bio dozvoljen oslonac od drugog postoperativnog dana. Rezultati su procenjivani prema kriterijumima Asocijacije za proučavanje i primenu metode Ilizarova. **Rezultati.** Koštano zarastanje ostvareno je kod svih bolesnika. Prosečno trajanje primene aparata po Ilizarovu iznosilo je 25 nedelja (19 do 36 nedelja). Koštani rezultati bili su odlični kod 23

Correspondence to: Dušan Šaponjski, Clinical Centre of Serbia, Centre for Radiology and MRI, Cara Dušana 31, 11 080 Belgrade, Serbia. E-mail: saponjski.d@gmail.com

bolesnika, dobri kod pet i umereni kod dva bolesnika. Funkcionalni rezultati bili su odlični kod 22 bolesnika, dobri kod pet i umereni kod tri bolesnka. Kod osam bolesnika konstatovana je infekcija oko igala aparata, koja je uspešno tretirana oralnom antibiotskom terapijom. Nisu uočene druge značajne komplikacije. **Zaključak.** Metoda po Ilizarovu predstavlja bezbedan i efikasan modalitet lečenja segmentnih preloma tibije.

Ključne reči:
tibija, prelomi; fiksatori, spoljni; ilizarov tehnika; funkcija, povratak.

Introduction

Segmental tibial fractures are very complex injuries. They are characterized by a unique fracture pattern with an intermediate fragment separated from the rest of the tibia with two different fracture lines [1]. In some cases, bone comminution is present, which additionally contributes to the severity of the injury [2]. Although tibia fractures are a frequent topic of scientific papers, little attention is given to treating exclusively segmental fractures of this bone [3]. This type of fracture is often caused by high-energy trauma. It mostly occurs in road traffic accidents, of which the most serious injuries are among motorcyclists [4].

Due to low incidence, specific fracture pattern, extensive soft tissue damage, and a significant degree of complications, these fractures represent a great challenge in treatment [5]. Out of many operating techniques in the literature, most attention is given to intramedullary nailing [6]. However, due to the unstable intercalary segment, fracture reduction is mainly the main concern of this method and may require the use of additional implantation material [3]. Many authors suggest high complication rates following intramedullary nails, including osteomyelitis (up to 47%), nonunion (up to 29%), and subsequent amputation (up to 7%). Despite numerous studies, there is no consensus on the question of surgical treatment, and the optimal treatment modality of these injuries remains the topic of debate [7].

The anatomical localization of the tibia and its subcutaneous position with a poor soft tissue cover on the medial side are the cause of the high incidence of open fractures [8, 9]. The naturally scarce vascularization of the tibia, in particular at the juncture of its distal and medial third, is additionally disrupted in the case of fracture. The effort to maintain vascularisation of fracture fragments represents a determining factor in the healing of these fractures. The exposure of the fracture site leads to disturbance of the biological environment which has a negative effect on the bone union. All of the above-mentioned factors lead to a greater tendency towards the onset of the infection, dehiscence of the wound, compartment syndrome, and nonunion [10, 11]. The complications are often not isolated, they consist of a combination of infection, nonunion, bone and soft tissue defects [12].

The method of Ilizarov with its characteristics could offer many advantages over the existing operative techniques. This method, using a percutaneous approach, minimizes the intraoperative trauma and avoids the additional compromising of the biological environment at the fracture site. Such surgical doctrine aims to utilize the complete potential of bone and soft tissue in the attainment of angiogenesis and induction of osteogenesis [13]. Indirect closed reduction is made possible by using a circular frame with thin tensioned wires. An important advantage is the possibility of achieving multiplanar and multilevel stability [7]. The sturdy construct, provided by such an external fixator, enables, at the same time, dynamic functional axial loading of the injured leg. The importance of the early weight-bearing and the initiation of movements in adjacent joints are well established in the literature [10]. One of the essential characteristics of this method is its modularity which allows the construction of a frame specific to each patient and according to each fracture pattern. This modularity also enables treatment for potential bone defects, as well as possible leg length discrepancies [14].

The aim of this study was to evaluate the results of the Ilizarov fixator in the treatment of segmental tibial fractures.

Methods

This retrospective study was conducted at the Institute for Orthopaedic Surgery "Banjica" on 30 patients with segmental tibial fractures treated with the Ilizarov external fixator from 2012–2017. The age range was 24 to 65 years with an average of 36 years; 6 patients were female, and 24 were male. All fractures analyzed in this study were defined as tibial fractures with intermediary fragments separated by two completely distinct fracture lines, type 42-C2 according to the AO/Orthopaedic Trauma Association (AO/OTA) classification [15]. Segmental fractures of the tibia were further classified according to Melis et al. [16]. There were no multisegmental fractures with four or more segments in our patient sample.

The mechanism of injury was a road traffic accident in 23 cases, falling from height in 4, and direct trauma in 3 cases. The left leg was injured in 11 and the right limb in 19 patients. Eight patients had closed fractures, and the remaining 22 patients had open tibial fractures. The open fractures were Gustilo–Anderson [17] type I in three cases, type II in four, type IIIa in eleven, and type IIIb in four cases. Five out of 8 closed fractures had grade II, and three were classified as grade I of soft tissue injuries according to the classification of Oestern and Tscherne [18].

Calcaneal skeletal traction was applied to the injured leg in order to maintain leg length and alignment of bony fragments until definitive surgery was performed. All open injuries were initially treated with meticulous debridement and irrigation followed by plastic surgical management, if needed, in order to achieve appropriate soft tissue closure.

The soft tissue condition had a crucial role in planning the definitive operation moment. The Ilizarov frame

application was dependent on soft tissue swelling degree and healing of the soft tissue reconstruction in conjunction with the plastic surgery department. Prophylactic first generation cephalosporin antibiotics were administered intravenously in all cases for three days, with additional aminoglycoside antibiotics, in cases of open fractures for five days. All operations were performed by the same surgical team under spinal or general anaesthesia. Thromboprophylaxis with low-molecular-weight heparin was carried out for four weeks after the initial trauma. The fractures were reduced with the indirect percutaneous technique with a great focus on gaining the correct length, rotation, and axial position of the fragments. The Fracture was stabilized with one or two rings *per* segment depending on its length in a 'near-far' pattern. The intermediate fragment was reduced and fixated using the opposed olive wires technique.

The postoperative care consisted of daily performed pin-site dressing and passive knee and ankle motion exercises in addition to isometric quadriceps strengthening. All patients were advised to bear weight as tolerated from the second postoperative day. The patients were evaluated clinically and radiographically using standing radiographs once a week for the first six weeks and then in one-month intervals. The external fixator was removed when mature bridging callus on anteroposterior and lateral radiographs was established, and patients achieved painless full-weight bear walking. An example is illustrated in Figure 1. Bone healing and functional results were evaluated according to the criteria established by the Association for the Study and Application of Method of Ilizarov (ASAMI) [19].

For data description, we use a measure of central tendency (arithmetic mean) and measures of variability (minimum and maximum value).

Results

The patients were followed up for 14 months on average (range from 12 to 21 months). The time that passed from the injury until surgery was 7 days in most cases (4 to 12). The individual preoperative details and surgery outcomes for all patients in this study are shown in Table 1.

Twenty-nine fractures healed completely following the initial application of the Ilizarov frame without the necessity for any bone healing stimulating procedure. One patient that

Fig. 1 – A 46-year-old man with closed segmental tibial fracture: (a) Initial anteroposterior and lateral radiographs; (b) After application of the Ilizarov fixator; (c) Radiographic and (d) clinical appearance six months after the removal of the frame showing complete bone union with fragments in residual recurvatum and varus position.

Table 1

Preoperative patient details and surgery outcome

Case	Gender/ age (years)	Injury	Type (Gustilo)	Melis class.	Fixator time (weeks)	ASAMI score	
						bone	function
1	M/61	RTA	IIIa	II	30	Excellent	Good
2	M/33	DT	Close	III	24	Excellent	Excellent
3	F/24	RTA	I	II	25	Excellent	Excellent
4	M/49	Fall	IIIb	I	36	Fair	Fair
5	M/52	RTA	Close	I	25	Excellent	Excellent
6	M/27	RTA	IIIa	IV	27	Excellent	Excellent
7	M/48	RTA	IIIa	II	26	Good	Excellent
8	M/26	RTA	II	I	21	Excellent	Excellent
9	F/42	DT	IIIb	I	30	Good	Excellent
10	M/28	RTA	Close	I	19	Excellent	Excellent
11	M/31	Fall	IIIa	IV	26	Excellent	Good
12	M/24	RTA	I	I	20	Excellent	Excellent
13	F/36	RTA	Close	II	21	Excellent	Excellent
14	M/43	RTA	IIIa	II	25	Good	Good
15	M/34	RTA	II	III	23	Excellent	Excellent
16	M/40	RTA	IIIa	II	29	Excellent	Excellent
17	M/35	DT	Close	II	22	Excellent	Excellent
18	M/29	RTA	I	I	25	Excellent	Excellent
19	M/65	RTA	IIIb	II	30	Good	Fair
20	F/34	Fall	IIIa	II	27	Excellent	Excellent
21	M/26	RTA	II	IV	25	Excellent	Excellent
22	M/25	RTA	IIIa	II	29	Excellent	Good
23	M/46	RTA	Close	I	19	Excellent	Excellent
24	F/31	RTA	IIIa	I	28	Excellent	Excellent
25	M/26	RTA	IIIb	II	30	Good	Fair
26	M/27	RTA	II	III	19	Excellent	Excellent
27	M/40	Fall	Close	IV	22	Excellent	Excellent
28	F/27	RTA	IIIa	II	25	Fair	Good
29	M/29	RTA	Close	III	20	Excellent	Excellent
30	M/45	RTA	IIIa	IV	29	Excellent	Excellent

M – male; F – female; ASAMI – Association for the Study and Application of the Method of Ilizarov; RTA –road traffic accident; DT – direct trauma.

had open type IIIb fracture showed no signs of the union after a prolonged period of time due to frame loosening and required new frame application. The patient was successfully treated as stiff nonunion with distraction-compression osteogenesis [20] at the fracture site with the eventual union after 36 weeks. The bone healing time was defined as the time from operation to the removal of the fixator. In our study, bone union time was 25 weeks on average (19 to 36 weeks). Proximal callus formation was observed between the second and fifth week. The time needed for distal callus formation was a little longer and ranged from 3 to 7 weeks.

The open fracture wounds were all localized in the anterior aspect of the lower leg. Five patients had partial-thickness soft tissue defects without exposed bone. They were all treated by a plastic surgeon with a split-thickness skin graft. Four patients had exposed bone with full-thickness soft tissue defects that needed coverage with adequate flaps.

No major complications, such as osteomyelitis or amputation, occurred in any of the patients. None of the patients required blood transfusion. In our study, there were no cases of pulmonary embolism, deep vein thrombosis, soft tissue necrosis, or palsy of the peroneal nerve. There were no signs of compartment syndrome in any patient before or after the surgery. Three patients had breakage of the frame wire that required replacement, and eight patients had minor pin-tract infections, successfully treated with oral antibiotics. Signs of deep infection were not observed in any patient secondary to pin-track infection. Patients analyzed in this study did not have any other skeletal injuries or injury to other organs or organ systems.

An adequate tibial alignment was noted on standing radiographs in 28 cases. Two patients had residual valgus and antecurvatum of 15°. No rotational deformities were observed. None of the patients required treatment for tibial axis malalignment.

The bone results were excellent in 23 cases, good in five, and fair in two cases. Out of two patients with fair bone results, one had shortening of the lower leg with additional valgus deformity more than 7°, and one patient had shortening of the lower leg with associated antecurvatum deformity of more than 7°. The functional results were excellent in 22 patients, good in 5, and fair in three patients. Among the three patients with fair functional results, one patient had knee pain and reduced ankle dorsiflexion with consequent limping, and the other two patients had knee pain with extension deficit and limping.

Discussion

Segmental tibial fracture is described by many studies as a unique type of injury associated with a high complication rate [6]. Woll and Duwelius [2] described segmental tibial fractures as "an extremely high-risk injury". They noted postoperative complications more frequently than in any other category of the tibia fracture.

The optimal treatment of segmental tibial fractures represents a very complex surgical problem. Reduction and fixation pose a significant challenge with fracture patterns featuring great axial and rotational instability [21]. The results of conservative treatment are negligible to have any value and be further considered in the treatment of these types of fractures. Intramedullary stabilization is the most frequently analyzed method in previous stud-ies [6, 22]. Many disadvantages and problems were reported by various authors using this method. Both reaming and non-reaming techniques have their drawbacks, such as decreasing cortical circulation of an intermediary fragment, endosteal necrosis, and increased infection rate when used in open fractures [23, 24]. The Ilizarov external fixator is a commonly used method for treating complications after failed internal fixation of the tibia [6].

High-energy mechanisms of injury are commonly followed by severe soft tissue defects. As a result of the damage to the surrounding soft tissues, approximately 53–80% of segmental tibia fractures are open injuries [25]. Following trauma, both endosteal and periosteal blood supply of the intermediate segment are damaged. As this circulation is naturally precarious, any sustained damage additionally compromises the healing potential [5]. The ideal treatment of the fracture should avoid additional disturbance to the soft tissues and bone, strive to preserve the remaining blood supply, and provide a structured environment that stimulates osseous biological processes toward bone union [6]. Taking these aims into consideration, the Ilizarov method should meet all those requirements [26].

All patients in this study with closed fractures show excellent bone results. In contrast, most of the patients with open injuries had lower grades of final bone and functional results. Only one of four patients in this study with type IIIb open fractures achieved excellent bone results. Patients with closed and open fractures were analyzed in the same sample group. The reason was that the aim of the research was not to compare these two types in terms of treatment methods and outcomes, which is one of the drawbacks of the study.

In our study, functional results were excellent in 22, good in 5, and fair in 3 patients, which corresponds to the findings of Oztürkmen et al. [6]. The physiological bone alignment was shown on all radiological evaluations, except in two patients with valgus and antecurvatum of no more than 15°.

All patients in our study achieved bone union. One of the possible reasons for such a high healing rate is the preservation of the local blood supply and initial biological environment. Only one patient required additional surgery in terms of reapplication of fixator after aseptic loosening. A similar union rate is reported in other smaller series [6, 27, 28]. Three studies that analyze the value of the Ilizarov method in treating segmental tibia fractures have shown consistently high rates of the primary union of at least 90%, with very low rates of complication [6, 27, 28]. The series described by Oztürkmen et al. [6] demonstrated that 22 out of 24 cases healed without further intervention; Tilkeridis et al. [27] reported 30 out of 33, and Giotakis et al. [28] 18 out of 20 patients achieving satisfactory union.

In the research of Foster et al. [7], the average time to union was 23 weeks, and Giotakis et al. [28] present their results with the median time to union of 21.7 weeks (range 12.8–31 weeks). Our findings correlate highly with those studies. In our research, we saw that the distal callus took an extended time to be formed (3–7 weeks) compared to the proximal fracture site, where the time range was 2–5 weeks. Oztürkmen et al. [6] propose that the fixation at the distal fracture site be as firm as possible because the distal tibial shaft has a natural tendency of showing prolonged union. As opposed to this, Giotakis et al. [28] reported no difference in bone union time observed between the proximal and distal fracture levels.

In order to promote fracture healing, modifications of the fixator are possible at any point of treatment. Trauma-induced bone defects, concomitant rotational or angular malalignment can be easily corrected by frame adjustments. This advantage of the Ilizarov fixator reduces the need for possible additional operations used to correct the resulting deformities. One of the significant problems is the achievement of adequate rotational stability of the distal part of the tibia. According to Audigex et al. [29], the distal fracture site is usually more unstable than the proximal one. Even in cases where the distal fragment is short, the Ilizarov method ensures its stable fixation [26].

Most patients with severe soft tissue injury require reconstructive surgical techniques, such as skin grafts or flaps, which may prolong the time until definitive bone fixation [7]. In our series, the maximal waiting time for the operation was twelve days which did not decrease reduction ability and the bone union potential. By utilizing bone compression and distraction, the Ilizarov method has the effect on increasing the bone and the surrounding soft tissue blood supply [6]. On routine radiographic follow-up, we noticed callus being formed earlier on the posterolateral aspect than on the anteromedial cortex of the tibia. These observations emphasize the significance of soft tissue coverage and blood supply preservation.

Pin-track infections and patient intolerance to wearing the external device are some of the most common drawbacks of this method. Despite this, pin-track infection is easily treated with regular pin-site dressing and oral antibiotics [7]. Eight patients in our study had minor pin-tract infections successfully treated with oral antibiotics, without any serious complications in terms of osteomyelitis.

Conclusion

The Ilizarov method is a safe and efficient treatment modality for segmental tibial fractures. This surgical technique could provide a high rate of bone union with predictable functional outcomes. Low incidence of soft tissue complications, early mobilization, and weight-bearing, as well as good functional recovery, all correlate amiably with other literature results and advocate that the Ilizarov external fixator should be one of the first treatment options for these complex injuries.

REFERENCES

1. *Zhang J, He X, Li M, Yu Y, Zhu L.* Treatment of segmental tibial fractures with supercutaneous plating. Orthopaedics 2014; 37(8): 712–6.
2. *Woll TS, Duwelius PJ.* The segmental tibial fracture. Clin Orthop Relat Res 1992; (281): 204–7.
3. *Reynders P.* Open acute segmental tibial fracture fixation using the less invasive stabilization system (LISS): study of 23 consecutive cases. Injury 2009; 40(4): 449–54.
4. *Miller NC, Askew AE.* Tibia fractures. An overview of evaluation and treatment. Orthop Nurs 2007; 26(4): 216–23; quiz 224–5.
5. *Mcmurtry J, Mounasamy V.* Segmental Tibia Fractures. Ann Orthop Rheumatol 2015; 3(3): 1051.
6. *Oztürkmen Y, Karamehmetoğlu M, Karadeniz H, Azboy I, Canikli-oğlu M.* Acute treatment of segmental tibial fractures with the Ilizarov method. Injury 2009; 40(3): 321–6.
7. *Foster PA, Barton SB, Jones SC, Morrison RJ, Britten S.* The treatment of complex tibial shaft fractures by the Ilizarov method. J Bone Joint Surg Br 2012; 94(12): 1678–83.
8. *Webb LX, Bosse MJ, Castillo RC, MacKenzie EJ.* Analysis of surgeon-controlled variables in the treatment of limb-threatening type-III open tibial diaphyseal fractures. J Bone Joint Surg Am 2007; 89(5): 923–8.
9. *Wani N, Baba A, Kangoo K, Mir M.* Role of early Ilizarov ring fixator in the definitive management of type II, IIIA and IIIB open tibial shaft fractures. Int Orthop 2011; 35(6): 915–23.
10. *Bari MM, Islam S, AHMA R, Rahman M.* Management of Segmental Fracture Tibia by Ilizarov Technique. MOJ Orthop Rheumatol 2015; 3(3): 00097.
11. *Shadgan B, Pereira G, Menon M, Jafari S, Darlene Reid W, O'Brien PJ.* Risk factors for acute compartment syndrome of the leg associated with tibial diaphyseal fractures in adults. J Orthop Traumatol 2015; 16(3): 185–92.
12. *El Rosasy MA.* Appraisal of the role of external skeletal fixation in the management of sequelae of open tibial fractures. Indian J Orthop 2008; 42(4): 420–5.
13. *Ilizarov GA.* The tension-stress effect on the genesis and growth of tissues. Part II. The influence of the rate and frequency of distraction. Clin Orthop Relat Res 1989; (239): 263–85.
14. *Mkize S, Ferreira N.* Outcome of bilateral circular fixators in complex lower limb fractures. SA Orthop J 2017; 16(3): 51–4.
15. *Court-Brown CM.* Fractures of the tibia and fibula. In: *Bucholz RW, Heckman JD, Court-Brown CM*, editors. Fractures in adults. 6th ed. Philadelphia: Lippincott; 2006. p. 2079–146.
16. *Melis GC, Sotgiu F, Lepori M, Guido P.* Intramedullary nailing in segmental tibial fractures. J Bone Joint Surg Am 1981; 63(8): 1310–8.
17. *Gustilo RB, Mendoza RM, Williams DN.* Problem in the management of type III (severe) open fractures: a new classification of type III open fractures. J Trauma 1984; 24(8): 742–6.
18. *Oestern HJ, Tscherne H.* Pathophysiology and Classification of Soft Tissue Injuries Associated with Fractures. In: *Tscherne H, Gotzen L*, editors. Fractures with Soft Tissue Injuries. Berlin, Heidelberg: Springer; 1984. p. 1–9.
19. *Shahid M, Hussain A, Bridgeman P, Bose D.* Clinical outcomes of the Ilizarov method after an infected tibial non union. Arch Trauma Res 2013; 2(2): 71–5.
20. *Tomić S.* Pseudoarthrosis and bone defects, Ilizarov method. Belgrade: Želnid; 2001. p. 214–35. (Serbian)
21. *Claes L, Augat P, Schorlemmer S, Konrads C, Ignatius A, Ehrnthaller C.* Temporary distraction and compression of a diaphyseal osteotomy accelerates bone healing. J Orthop Res 2008; 26(6) 772–7.
22. *Teraa M, Blokhuis TJ, Tang L, Leenen LP.* Segmental tibial fractures: an infrequent but demanding injury. Clin Orthop Relat Res 2013; 471(9): 2790–6.
23. *French B, Tornetta P 3rd.* High-energy tibial shaft fractures. Orthop Clin North Am 2002; 33(1):211-30, ix.
24. *Sohn HS, Chung JY, Song HK.* Analysis of complications and clinical outcomes in the treatment of segmental tibial fractures according to the method of internal fixation. Asian J Surg 2019; 42(7): 740–5.
25. *Weiss RJ, Montgomery SM, Ehlin A, Al Dabbagh Z, Stark A, Jansson KA.* Decreasing incidence of tibial shaft fractures between 1998 and 2004: information based on 10,627 Swedish inpatients. Acta Orthop 2008; 79(4): 526–33.
26. *Abdelsatar T, Elsany M, Zayda A, Samy A.* Management of segmental tibial fractures by an Ilizarov external fixator. Menoufia Med J 2016; 29(3): 680–4.
27. *Tilkeridis K, Owen AJ, Royston SL, Dennison MG, Vinsent M, Vashista G.* The Ilizarov method for the treatment of segmental tibial fractures. Injury Extra 2009; 40: 228.
28. *Giotakis N, Panchani SK, Narayan B, Larkin JJ, Al Maskari S, Nayagam S.* Segmental fractures of the tibia treated by circular external fixation. J Bone Joint Surg Br 2010; 92(5): 687–92.
29. *Audigex L, Griffin B, Bhandari M, Kellam J, Rüedi TP.* Path analysis of factors for delayed healing and nonunion in 416 operatively treated tibial shaft fractures. Clin Orthop Relat Res 2005; 438: 221–32.

Received on April 22, 2019.
Revised on May 28, 2019.
Accepted on June 6, 2019.
Online First June, 2019.

ORIGINAL ARTICLE

UDC: 616.379-008.64:616.5
https://doi.org/10.2298/VSP190226073U

Association between skin manifestations and glycemic control in patients with type 2 diabetes mellitus

Povezanost između kožnih manifestacija i glikemijske kontrole kod bolesnika sa dijabetesom melitusom tipa 2

Sanja Umičević Šipka*, Jagoda Balaban*, Radojka Bijelić†

University Clinical Centre of the Republic of Srpska, *Skin and Venereal Diseases Clinic, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; Health Center, †Family Medicine Service, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

Abstract

Background/Aim. Diabetes mellitus (DM) can be associated with numerous skin diseases. This study aimed to determine the pattern and incidence of skin manifestations in patients with type 2 DM and their link to glycemic control. **Methods.** This cross-sectional study was conducted at the Skin and Venereal Diseases Clinic, University Clinical Centre of the Republic of Srpska in Banja Luka, Bosnia and Herzegovina, from January 2016 to January 2018. Adult patients of both genders suffering from type 2 DM and cutaneous manifestations participated in the study. Glycemic control was assessed according to the values of glycated hemoglobin (HbA1c) of 7%. **Results**. The mean age of 105 study participants (46% male and 54% female) was 68.4 ± 10 years, while the mean HbA1c was 8.3 ± 1.6%. Unsatisfactory glycemic control was found in 74.3% of patients with the mean HbA1c at 8.9 ± 1.4%, while satisfactory glycemic control was found in 25.7% of patients, with the mean HbA1c at 6.7 ± 0.2% ($p < 0.001$). Infections were the most frequent skin diseases (43.9%). Bacterial infections were most common (26.7%), followed by fungal infections (24.8%), xerosis (17.1%), psoriasis (15.2%), fibroma molle (14.3%), diabetic ulcer (7.7%), prurigo (6.7%), and stasis dermatitis (5.7%). Other skin manifestations were found at a lower rate. A significant association was found between unsatisfactory glycemic control and skin infections ($p = 0.009$). **Conclusion.** The most common skin manifestations in patients with type 2 diabetes are infections. They occurred more often in patients with unsatisfactory glycemic control.

Key words:
diabetes mellitus, type 2; skin manifestation; blood glucose; bosnia and herzegovina; infection.

Apstrakt

Uvod/Cilj. Dijabetes mellitus (DM) može biti udružen sa brojnim kožnim bolestima. Cilj naše studije bio je da se ustanovi uzorak i frekvencija kožnih manifestacija kod bolesnika sa DM tipa 2 i njihova povezanost sa glikemijskom kontrolom. **Metode.** Ova studija preseka sprovedena je na Klinici za kožne i polne bolesti, Univerzitetskog Kliničkog centra Republike Srpske, Banja Luka, Bosna i Hercegovina, u periodu od januara 2016. do januara 2018. godine. U studiju su bili uključeni odrasli bolesnici oba pola koji su imali kožne bolesti i DM tip 2. Glikemijska kontrola posmatrana je prema ciljnoj vrijednosti glikolizirano hemoglobina (HbA1c) od 7%. **Rezultati.** U studiju je bilo uključeno 105 bolesnika (46% muškaraca i 54% žena), srednje dobi od 68,4 ± 10 godina, sa prosečnom vrednosti HbA1c od 8,3 ± 1,6%. Nezadovoljavajuća glikemijska kontrola utvrđena je kod 74,3% bolesnika koji su imali prosečnu vrednost HbA1c 8,9 ± 1,4%, a zadovoljavajuća kod 25,7% bolesnika sa prosečnom vrednosti HbA1c 6,7 ± 0,2% ($p < 0,001$). Među kožnim manifestacijama najzastupljenija je bila infekcija (43,4%). Bakterijska infekcija je bila najčešća (26,7%), a zatim gljivična infekcija (24,8%), kseroza kože (17,1%), psorijaza (15,2%), meki fibromi (14,3%), dijabetično stopalo (7,7%), prurigo (6,7%) i stazni dermatitis (5,7%). Druge kožne manifestacije uočene su sa manjom zastupljenošću. Utvrđena je značajna povezanost nezadovoljavajuće glikemijske kontrole sa infekcijama kože ($p = 0,09$). **Zaključak.** Najčešće kožne manifestacije kod bolesnika sa DM tipa 2 su infekcije. One su češće kod bolesnika sa nezadovoljavajućom glikemijskom kontrolom.

Ključne reči:
dijabetes melitus, insulin-nezavisni; koža, manifestacije; glikemija; bosna i hercegovina; infekcija.

Correspondence to: Sanja Umičević Šipka, University Clinical Centre of the Republic of Srpska, Skin and Venereal Diseases Clinic, Dvanaest beba, 78 000 Banja Luka, Bosnia and Herzegovina. E-mail: sanjaumicevic84@gmail.com

Introduction

Diabetes mellitus (DM) is the most common endocrine disorder with a broad spectrum of cutaneous manifestations [1]. Increased serum glucose causes damage to a wide range of cell types, including endothelial, neuron, and renal cells, but also keratinocytes and fibroblasts [2]. The overall prevalence of skin disorders in both types of DM varies from 51% to 97 % in different regions worldwide [3]. Most documented studies have shown that the incidence of cutaneous disorders associated with diabetes is between 30% and 71% [4].

Skin diseases can appear as the first sign of diabetes or may develop at any time in the course of the illness [5]. In dermatology, a great number of findings on skin diseases have been associated with diabetes, some demonstrating a stronger connection than others. Some of them have various health implications ranging from those that are concerning from the aesthetic point of view to those that may be life-threatening [6]. Awareness of cutaneous manifestations of DM can provide an insight into the present or prior metabolic status of patients. Recognition of such findings may aid in diagnosing diabetes or may be monitored as a marker of glycemic control [7]. Good metabolic control may prevent some of these manifestations and support treatment [8]. There is considerable uncertainty around the pathogenesis of many cutaneous conditions affecting diabetic patients because of insufficient understanding of the metabolic basis of DM itself [9]. There is no strict classification of skin lesions related to DM, but academic literature usually classifies them into four categories: skin lesions with strong-to-weak association with diabetes (necrobiosis lipoidica, diabetic dermopathy, diabetic bullae, yellow skin, eruptive xanthomas, perforating disorders, acanthosis nigricans, oral leucoplakia, lichen planus), infections (bacterial, fungal), cutaneous manifestations of diabetic complications (microangiopathy, macroangiopathy, neuropathy), and skin reactions to diabetic treatment (sulphonylurea or insulin). Some authors also add endocrine syndrome with skin changes and diabetes as the fifth group [7, 10].

Glycated hemoglobin (HbA1c) has been used as an objective marker of average glycemic control for many years. Recommendations for clinical practice by the American Diabetes Association (ADA) suggest that maintaining HbA1c value closer to normal levels may be beneficial for patients. The target value for prevention of microvascular complications is < 7% [11]. Since dermatological patients often have diabetes, the aim of this study was to determine the pattern and incidence of skin manifestations in patients with type 2 DM and their link with glycemic control.

Methods

This cross-sectional study included 105 adult patients of both genders treated at the Skin and Venereal Diseases Clinic, University Clinical Centre of the Republic of Srpska in Banja Luka, Bosnia and Herzegovina, from January 2016 to January 2018. The study was approved by the Ethics Committee University Clinical Centre of the Republic of Srpska in Banja Luka.

Patients with skin diseases and diabetes mellitus were referred to a hospital or outpatient treatment by their family doctor. Referral factors for outpatients included adult patients of both genders with type 2 DM diagnosed by an endocrinologist and with the value of HbA1c tested within the last two weeks. Glycated hemoglobin test for hospital patients was carried out during the diagnostic examination. Based on the ADA recommendations for the target value of HbA1c of 7% for glycemic control, patients were divided into two groups. The first group included patients with satisfactory and the second group included patients with unsatisfactory glycemic control [11]. Satisfactory glycemic control was defined as HbA1c $\leq$ 7%. Unsatisfactory glycemic control was defined as HbA1c $\geq$ 7%. Detailed medical history was obtained from the study participants including diabetes duration and treatment method for diabetes. The diabetes duration was observed over four time periods: 1) less than one year, 2) between 1 and 9 years, 3) between 10 and 19 years, and 4) 20 years and over. The treatment method for diabetes was observed based on insulin dependency, i.e. insulin-dependent and insulin-independent.

Clinical diagnosis of dermatological findings was established after a detailed general, systemic and cutaneous examination. In addition to clinical findings, this study used relevant laboratory blood tests, bacteriological, mycological, immunological, or other necessary laboratory investigations where required in order to confirm the diagnosis of skin diseases. The study respondents were divided into three groups based on skin manifestations. The first group included patients with skin manifestations related to diabetic complications (i.e. complications of infectious, microangiopathy, or neuropathic origin). The second group included patients with skin diseases known as commonly associated with diabetes. The third group involved patients with other skin manifestations which are not commonly associated with diabetes.

Statistical analyses were carried out using SPSS 22 software package. The data were described by mean values and standard deviations (SD) for continuous variables and incidence and percentages (%) for categorical variables. The differences between subgroup mean values were analyzed by the *t*-test and the one-way analysis of variance (ANOVA) depending on the number of groups. The chi-squared test (χ^2) was used to determine whether there was a significant difference between the incidences of categorical variables. *P*-values lower than 0.05 were considered significant.

Results

The study included 105 adult patients with different skin diseases and type 2 DM. There were 54.2% of women and 45.8% of men at the mean age of 68.4 ± 10.7 years and with the mean HbA1c 8.3 ± 1.6%. Participants were divided into two groups according to their glycemic control. Satisfactory glycemic control (HbA1c 6.7 ± 0.2%) was observed in 25.7% of patients (mean age 70.4 ± 8.1 years). Unsatisfactory glycemic control (HbA1c 8.9 ± 1.4%) was observed in

74.3% of patients (mean age 67.6 ± 11.4 years). Most patients in this study had unsatisfactory glycemic control ($p < 0.001$). There was no statistically significant difference in relation to gender ($p = 0.547$) or the mean age ($p = 0.192$) between the two groups. Most participants with unsatisfactory glycemic control (77%) had a long duration of diabetic disease (between 10 and 19 years). Even 95% of patients with diabetes duration > 20 years had unregulated glycemic control. Duration of diabetes differed significantly between the two groups ($p < 0.001$) (Table 1).

(16.2%). Other bacterial infections such as impetigo, furunculous, erysipelas, erythrasma, and folliculitis had low incidence. Fungal infections caused by *Candida* spp. were found in 15.2% of patients, while infections caused by dermatophytes were found in 9.5% of patients. The mean HbA1c in patients with these skin disorders was 8.9 ± 1.8%. The highest value of HbA1c was found in patients with cellulitis (10 ± 1.9%), while the lowest one was in patients with Schamberg's disease, i.e. 7.5 ± 1.3%. Satisfactory glycemic control had 25.9% of patients, while 55.1% of patients had unsatis-

Table 1

Characteristics of the study population

Variables	Total patients n (%)	Glycemic control		*p*-value
		satisfactory n (%)	unsatisfactory n (%)	
	105 (100)	27 (25.7)	78 (74.3)	0.000
Age (years), mean ± SD	68.36 ± 10.72	70.44 ± 8.08	67.64 ± 11.45	0.192
Gender, n (%)				
male	48 (45.8)	11 (22.9)	37 (77.1)	0.547
female	57 (54.2)	16 (28.1)	41 (71.9)	
HbA1c (%), mean ± SD	8.32 ± 1.57	6.67 ± 0.21	8.88 ± 1.43	0.000
Duration of diabetes (years)				
< 1	8 (7.6)	3 (37.5)	5 (62.5)	0.480
1–9	33 (31.4)	13 (39.4)	20 (60.6)	0.223
10–19	44 (41.9)	10 (22.7)	34 (77.3)	0.000
≥ 20	20 (19.0)	1 (5.0)	19 (95.0)	0.000
Insulin dependency, n (%)				
dependent	60 (57.1)	11 (18.3)	49 (81.7)	0.000
independent	45 (42.9)	16 (35.6)	29 (64.4)	0.053

HbA1c – glycated hemoglobin; SD – standard deviation.

Table 2

Skin manifestations related to diabetic complications and glycemic control

Skin manifestations	Total patients (n = 105) n (%)	HbA1c (%) mean ± SD	Glycemic control		*p*-value
			satisfactory (n = 27) n (%)	unsatisfactory (n = 78) n (%)	
All skin infections	46 (43.9)	8.98 ± 1.86	6 (22.2)	40 (51.3)	0.009
Bacterial infections	28 (26.7)	9.30 ± 2.03	5 (18.5)	23 (29.4)	0.267
cellulitis	17 (16.2)	10.07 ± 1.92	1 (3.7)	16 (20.6)	0.029
impetigo	3 (2.8)	7.13 ± 0.47	1 (3.7)	2 (2.6)	
furunculous	3 (2.8)	9.50 ± 2.36	1 (3.7)	2 (2.6)	
erysipelas	2 (1.9)	9.10 ± 0.28	0 (0.0)	2 (2.6)	
erythrasma	2 (1.9)	6.60 ± 0.00	2 (7.4)	0 (0.0)	
folliculitis	1 (0.9)	8.00 ± 0.00	0 (0.0)	1 (1.3)	
Fungal infections	26 (24.8)	9.00 ± 1.62	1 (3.7)	25 (32.0)	0.003
candidiasis	16 (15.2)	9.48 ± 1.80	0 (0.0)	16 (20.6)	0.197
dermatophytosis	10 (9.5)	8.71 ± 1.47	1 (3.7)	9 (11.6)	
Viral infections	2 (1.9)	8.85 ± 1.34	0 (0.0)	2 (2.6)	0.401
Diabetic foot ulcer	8 (7.7)	8.28 ± 0.89	0 (0.0)	8 (10.2)	0.083
Schamberg's disease	2 (1.9)	7.55 ± 1.34	1 (3.7)	1 (1.3)	0.428
Total patients	50 (47.6)	8.91 ± 1.82	7 (25.9)	43 (55.1)	0.009

For abbreviations see under Table 1.

Skin disorders related to diabetic complications were found in 47.6% of patients. The most common disorders in this group were skin infections (43.9%). Bacterial infections were found in 26.7% of cases and fungal in 24.8% of cases. Foot ulcers were found in 7.7% of participants, while viral infections and Schamberg's disease were low (both by 2.1%). The most common bacterial infection was cellulitis

factory glycemic control. The difference in the occurrence of skin manifestations related to diabetic complications between patients with unsatisfactory and satisfactory glycemic control was statistically significant ($p = 0.009$) (Table 2).

In this study, 51.4% of participants had aggravated or skin diseases commonly associated with diabetes. The most recurrent skin disease in this group was xerosis (17.1%),

followed by psoriasis (15.2%) and fibroma molle (14.3%). Other diseases detected with a lower incidence were dermopathia diabeticorum (8.6%), pruritus (6.7%), granuloma annulare (5.7%), and scleredema diabeticorum (2.8%). The mean HbA1c in this group was 8.4 ± 1.7%. The highest HbA1c value of 10.1 ± 2.7% was found in patients with scleredema diabeticorum and the lowest value of 7.6 ± 1.2% was found in patients with annular granuloma. Satisfactory glycemic control had 41.8% and unsatisfactory 52.6% of these patients. There was no significant difference in the occurrence of skin diseases aggravated or commonly associated with diabetes according to glycemic control between the two groups (Table 3).

Other different skin manifestations either not commonly associated or unassociated with diabetes were found in 48.5% of patients. The most common was seborrheic keratosis (20%). Other skin diseases detected in a lower incidence in the descending order included the following: prurigo (6.7%), stasis dermatitis (5.7%), urticaria/angioedema (4.8%), drug-induced exanthema (3.3%), pemphigus (3.3%), parapsoriasis (2.8%), erythroderma (2.8%), and bullous pemphigoid (1.2%). The mean value of HbA1c in this group of skin disorders was 8.3 ± 1.7%. The highest value of HbA1c was in patients with nodular prurigo (10 ± 2.4%). The lowest value was in patients with exanthema (6.9 ± 0.7%) and urticaria/angioedema (6.9 ± 0.5%). There was no significant difference in the occurrence of skin diseases not commonly associated with diabetes between the two groups according to glycemic control (Table 4).

Discussion

The study found that patients with type 2 diabetes had a wide range of different skin manifestations. Among them, the most common were skin infections of bacterial and fungal origin. Skin infections were more frequent in patients with unsatisfactory glycemic control.

There was no gender difference among the study participants (p = 0.547). The result was similar to the report presented by Bhat et al. [12]. However, some authors reported that dermatological manifestations were more common in women since a higher number of women visit doctors, which indicates a higher disease burden and health awareness among the females [13]. On the other hand, some authors have shown a preponderance among men [14, 15]. The mean age of the study participants was 68 ± 11 years. This result was higher than in the findings by various similar studies in which the age of patients with type 2 DM and skin manifestations was usually between 50 and 60 years [13–16]. The mean age for DM presentation indicated that the majority of patients had longstanding diabetes, and the study confirmed this with the findings on diabetes duration, which was between 10 and 19 years in 42% of patients and more than 20 years in 20% of patients. Diabetes lasted longer ($p < 0.001$) in patients with poor glycemic control. Nevertheless, in similar studies, some authors found diabetes duration < 10 years among a higher number of respondents [4, 12]. The majority of patients (74%) had poorly controlled diabetes, with the mean HbA1c at 8.9 ± 1.4%. This was considerably higher than the target value recom-

Table 3

Skin manifestations aggravated or commonly associated with diabetes and glycemic control

Skin manifestations	Total patients (n = 105) n (%)	HbA1c (%) mean ± SD	Glycemic control		*p*-value
			satisfactory (n = 27) n (%)	unsatisfactory (n = 78) n (%)	
Xerosis	18 (17.1)	8.97 ± 2.08	4 (14.8)	14 (17.9)	0.710
Psoriasis	16 (15.2)	7.80 ± 1.04	5 (18.5)	11 (14.1)	0.582
Fibroma molle	15 (14.3)	8.87 ± 1.99	3 (11.1)	12 (15.4)	0.584
Diabetic dermopathy	9 (8.6)	9.45 ± 2.15	0 (0.0)	9 (11.6)	0.065
Pruritus	7 (6.7)	9.00 ± 1.83	1 (3.7)	6 (7.7)	0.474
Granuloma annulare	6 (5.7)	7.66 ± 1.21	2 (7.4)	4 (5.1)	0.660
Scleredema diabeticorum	3 (2.8)	10.13 ± 2.67	0 (0.0)	3 (3.8)	0.301
Total patients	54 (51.4)	8.45 ± 1.68	13 (48.1)	41 (52.6)	0.692

For abbreviations see under Table 1.

Table 4

Skin manifestations not commonly associated with diabetes and glycemic control

Skin manifestations	Total patients (n = 105) n (%)	HbA1c (%) mean ± SD	Glycemic control		*p*-value
			satisfactory (n = 27)	unsatisfactory (n = 78)	
Keratosis seborrhoica	21 (20.0)	8.57 ± 1.47	4 (14.8)	17 (21.8)	0.435
Prurigo	7 (6.7)	10.08 ± 2.40	1 (3.7)	6 (7.7)	0.474
Stasis dermatitis	6 (5.7)	7.88 ± 1.16	2 (7.4)	4 (5.1)	0.660
Urticaria/angioedema	5 (4.8)	6.96 ± 0.54	3 (11.1)	2 (2.6)	0.072
Exanthema	4 (3.8)	6.95 ± 0.76	3 (11.1)	1 (1.3)	0.021
Pemphigus	4 (3.8)	8.15 ± 1.76	0 (0.0)	4 (5.1)	0.230
Parapsoriasis	3 (2.8)	7.50 ± 0.84	1 (3.7)	2 (2.6)	0.759
Erythroderma	3 (2.8)	7.20 ± 0.51	2 (7.4)	1 (1.3)	0.100
Pemphigoid bullous	2 (1.2)	7.50 ± 0.84	1 (3.7)	1 (1.3)	0.428
Total patients	51 (48.5)	8.38 ± 1.70	14 (51.8)	37 (47.4)	0.692

For abbreviations see under Table 1.

mended by the ADA [11]. However, the results of this study corresponded to the findings of Furquana et al. [13], who found that 68% of patients with unsatisfactory glycemic control had the mean HbA1c at 8.6 ± 1.5%. Foss et al. [17] found HbA1c at 12.7% in type 2 diabetic patients with inadequate metabolic controls. Ahmed et al. [18] reported an incidence of 93% of uncontrolled diabetes in a similar series of patients. In this study, 60% of patients were on insulin therapy very often in combination with oral antidiabetics. A higher number of patients with unsatisfactory glycemic control were insulin-dependent. The results in the incidence of insulin dependency vary from one study to another [13, 18, 19]. This probably depended on a number of factors, including diabetes duration and patients' age.

Skin infections, as disorders related to diabetic complications, were the most common skin manifestation (44%) found in the study. The study findings were consistent with the academic literature data, according to which the overall incidence of skin infections varied between 20–50% [20–22]. The data depended on the study design, eligibility criteria of the involved patients, and regional affiliation [3]. Many patients with infections had poorly regulated glycemic control. Bacterial infections were found in 27% of the study participants, with the mean HbA1c at 9.3 ± 2.0%. The most common bacterial infection was cellulitis (16.2%). The study participants with cellulitis had the highest mean value of HbA1c (10 ± 1.9%) of all patients with infections. Other bacterial infections such as impetigo, furunculus, erysipelas, erythrasma, and disseminate folliculitis had a low incidence. Furquana et al. [13] have reported similar results (26%). Other authors found a higher incidence of bacterial infections, while there were studies in which bacterial infection had a much smaller incidence [23, 24]. Fungal infections were found in 24.8% of patients. Almost all of these patients had unsuccessful diabetic control ($p = 0.003$). The average value of HbA1c in these patients was 9.0 ± 1.6%. Among fungal infections, 15% of the study participants had candidiasis. Mucocutaneous infections with *Candida* spp. are considered to be an early indicator of an undiagnosed DM or inadequately controlled glycemia [25]. Dermatophytosis was found in 9% of respondents. Some authors considered that fungal infections in patients with type 2 DM were more prevalent than bacterial [3]. This was confirmed by studies with a high incidence of dermatophytosis [17, 23, 24]. Viral infections had a very low incidence in the study. This study has registered only two patients with herpes zoster. Otherwise, viral infections in diabetic patients or with low participation in similar studies were rarely mentioned [3].

We found diabetic foot ulcers in 7.7% of patients. All patients had unsuccessful diabetic control, and the mean HbA1c at 8.3 ± 0.8%. The diabetic neuropathic ulcer was the most frequently recognized complication in diabetics. Zhang et al. [26] found that global diabetic foot ulcer prevalence was 6.3%, while this value was 5.1% in Europe. Some studies from different parts of the world present different data. Foss et al. [17] and Yosipovitch et al. [27] cited a very low incidence of HbA1c at 0.7% and 0.8%. In a community-based study in the Northwestern United Kingdom, the incidence of active foot ulcers identified at screening among persons with diabetes was 1.7% [28]. Some Indian authors found a higher incidence of diabetic foot ulcers [29].

We found a small incidence of Schamberg's disease (progressive pigmentary dermatosis). Only two patients (1.9%) with the average value of HbA1c at 7.5 ± 1.3% had a progressive type of this disease. Results of this study were consistent with the data according to which Schamberg's disease is rare and usually associated with diabetes mellitus, rheumatoid arthritis, or systemic lupus erythematosus [30, 31].

In the group of skin manifestations commonly associated with DM, the most frequent were xerosis, psoriasis, and fibroma molle. Other diseases were less frequent. There was no significant difference between skin manifestations and glycemic control in this group ($p > 0.05$). Xerosis had an incidence of 17% among patients in this study, with the mean HbA1c at 9.0 ± 2.0%. Xerosis was reported in several studies, and rates showed high heterogeneity. Bhardwaj et al. [24] have reported an incidence of 10.3%, while in the study of Goyal et al. [4], xerosis accounted for the most common skin manifestation (44 %).

Psoriasis was found in 15% of the study participants, with the mean HbA1c at 7.8 ± 1%. Studies by Cvitanović et al. [10] and Sasmaz et al. [31] identified 11% of patients with psoriasis vulgaris. Vahora et al. [20] found a lower incidence of psoriasis (3%). On the other hand, some authors indicated that psoriasis was not associated with diabetes [32]. However, it is known that psoriasis, as a multisystemic inflammatory disease, is related to an increased cardiometabolic risk and that DM is a major contributor to cardiovascular morbidity and mortality [33]. Some authors supported a view that psoriasis had the strongest association with metabolic syndrome among all skin diseases [34]. Several studies have shown that psoriasis is associated with diabetes and its complications. Khalid et al. [35], in a Danish nationwide cohort study, concluded that psoriasis was associated with increased incidence rates of the new-onset type 2 DM. Armstrong et al. [36] also noted that psoriasis was associated with an increased prevalence and incidence of diabetes and that association may be the strongest among the patients with severe psoriasis.

Fibroma molle (skin tags, acrochordons) as a feature of diabetes was also found in 14.3% of the study participants, and they had the mean HbA1c at 8.9 ± 1.9%. Since fibroma molle is highly prevalent among the general population, increasing in incidence with patient's age, this study considered only multiple forms (more than 30 skin tags). Vahora et al. [20] have reported a similar result (13.3%). The possible association of skin tags with DM was first mentioned in 1951. Since then, a few clinical studies have been conducted in order to examine this hypothesis and they have come up with conflicting results. Multiple skin tags have been associated with abnormalities in the glucose metabolism, specifically type 2 diabetes, hyperinsulinemia, and insulin resistance [37].

Diabetic dermopathy had an incidence of 8.6% among patients who participated in this study. All patients had unsatisfactory glycemic control, with the mean HbA1c at 9.4 ± 2.1%. Furquana et al. [13] came up with the same result. It has

been reported that diabetic dermopathy occurs in variable percentages between 9% and 55% of patients with diabetes [38]. However, some authors found a very low incidence of this disease in diabetics, like Morgan and Schwartz [39] (0.2%) and Foss et al. [17] (1.2%). This distribution may result from variations among sample sizes and ethnicities of the study groups.

As a feature of diabetes, pruritus was also found in 6.7% of the study participants. All patients except one had poor glycaemic control with the mean HbA1c at 9.0 ± 1.8%. Five of seven patients had localized, while two had generalized pruritus. Pruritus is well known to be associated with diabetes mellitus, as reported by the past academic literature. The findings of this study correspond to the results by Cvitanović et al. [10] (7%), but other authors have found a higher incidence in a similar study [40].

Granuloma annulare (GA) was found in 5.7% of the study participants, with the mean HbA1c at 7.6 ± 1.2%. Three patients had a localized, and three patients had a disseminated form of the disease. GA may be an idiopathic entity. However, GA is persistently described within the setting of a variety of systemic diseases. DM and hyperlipidemia are most commonly reported. Some papers support while others disprove the existence of an association between GA and DM. George and Walton [41] had reported that this association is 4%, while others found a lower incidence of GA among diabetic patients [10]. Nobari et al. [42] emphasized that in this type of skin lesion, particularly in disseminated forms, the clinicians are supposed to carry out a diabetic evaluation of all patients, even those without symptoms. On the other hand, Cheng et al. [43] considered that an association between GA and DM remains controversial. Nebesio et al. [44] did not find an association between type 2 diabetes mellitus and GA.

Scleredema diabeticorum was found in 2.8% of the study participants. All three patients had strikingly poor glycemic control, and the mean HbA1c at 10.1 ± 2.6%. Scleredema diabeticorum is a rare cutaneous manifestation of DM. Results of this study were in accordance with the data presented by Draznin et al. [45]. A few studies reported an incidence of scleredema diabeticorum between 2.5% and 14% [46].

In the group of skin diseases unassociated with diabetes, this study found various skin manifestations mainly occurring at an older age. The most frequent skin manifestation in this group was seborrheic keratosis (20%). The result corresponded with the data according to which seborrheic keratosis is the most common benign cutaneous neoplasm occurring in at least 20% of older adults [47].

As a feature of diabetes, prurigo nodularis was also found in 6.7% of the study participants. These patients had the highest mean value of HbA1c in the group of skin manifestations (10.0 ± 2.4%). Foss et al. [17] came to a similar result, while Sasmaz et al. [31] found prurigo among 9.9% of patients in their study. There is a deficiency in epidemiological data regarding the incidence and prevalence of prurigo nodularis, but it seems that elderly people are most frequently affected, usually as patients with chronic kidney disease [48]. We found stasis dermatitis in 5.7% of patients. Stasis dermatitis is also most common in people > 50 years old, with an overall disease prevalence of 6–7% [49]. Urticaria was found in 4.8% of the study participants. All of them had satisfactory glycemic control. One of five patients had urticaria associated with non-steroidal anti-inflammatory drugs. Two patients had isolated angioedema caused by antihypertensive drugs from the group of angiotensin-converting enzyme (ACE) inhibitors, and two patients had chronic idiopathic urticaria. Other skin diseases found in low incidence were the following: exanthema, pemphigus, parapsoriasis, erythroderma, and bullous pemphigoid. In this group of skin diseases, there was no significant difference between patients with satisfactory and unsatisfactory glycemic control ($p > 0.05$), except for exanthema ($p = 0.021$). Other similar studies found extremely different skin manifestations which are either not commonly associated or are associated with diabetes, such as actinic degeneration, pigmentation disorders, benign skin tumors, eczemas, nail dystrophy, and peripheral hypotrichia [3]. The pattern of skin manifestations depends on regional affiliation, study design, age of study respondents, and diabetes duration.

Conclusion

Skin infections of bacterial and fungal origin are the most frequent skin manifestations in patients with type 2 DM. Other different skin disorders are comparatively less common. This study confirmed that skin infections in type 2 DM highly correlate with unsuccessful diabetic control. Achieving appropriate glycemic control in patients with diabetes can reduce skin infections and other skin manifestations related to diabetic complications. Early detection and adequate treatment of not only elevated glycemia but also of skin disorders in diabetics may reduce morbidity, complications, and hospital visits and improve the quality of life of diabetics. Since skin manifestations in diabetics are common and easily visible, dermatologists have to emphasize the importance of the multidisciplinary and team approach to diabetes.

REFERENCES

1. *Farshchian M, Farshchian M, Fereydoonnejad M, Yazdanfar A, Kimyai-Asadi A.* Cutaneous manifestations of diabetes mellitus: a case series. Cutis 2010; 86(1): 31–5.
2. *Lima AL, Illing T, Schliemann S, Elsner P.* Cutaneous Manifestations of Diabetes Mellitus: A Review. Am J Clin Dermatol 2017; 18(4): 541–53.
3. *De Macedo GMC, Nunes S, Barreto T.* Skin disorders in diabetes mellitus: an epidemiology and physiopathology review. Diabetol Metab Syndr 2016; 8: 63.
4. *Goyal A, Raina S, Kaushal SS, Mahajan V, Sharma NL.* Pattern of cutaneous manifestations in diabetes mellitus. Indian J Dermatol 2010; 55(1): 39-41.

5. *Duff M, Demidova O, Blackburn S, Jay Shubrook J.* Cutaneous Manifestations of Diabetes Mellitus. Clin Diabetes 2015; 33(1): 40–8.
6. *Han G.* A new appraisal of dermatologic manifestations of diabetes mellitus. Cutis 2014; 94(1): E21–6.
7. *Rosen J, Yosipovitch G.* Skin Manifestations of Diabetes Mellitus. [updated 2018 Jan 4]. In: *Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A,* et al, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000. Available from: http://www.ncbi.nlm.nih.gov/books/NBK481900/
8. *Van Hattem S, Bootsma AH, Bing Thio H.* Skin manifestations of diabetes. Review. Cleve Clinic Med 2008; 75(11): 772, 774, 776–7, 780–7.
9. *Sreeedevi C, Car N, Pavlić-Renar I.* Dermatologic lesions of diabetes mellitus. Review. Diabetol Croat 2002; 31(3): 147–59.
10. *Cvitanović H, Jančić E, Knežević E, Kuljanac I.* Skin changes in patients with diabetes mellitus in Karlovac county. Acta Med Croat 2009; 45(4): 370–80.
11. *Kojić Damjanov S, Đerić M, Eremić Kojić N.* Glycated hemoglobin A1c as a modern biochemical marker of glucose regulation. Med Pregl 2014; 67(9–10): 339–44.
12. *Bhat YJ, Gupta V, Kudyar RP.* Cutaneous manifestations of diabetes mellitus. Int J Diabetes Dev Ctries 2006; 26: 152–5.
13. *Furquana N, Bashir F, Shams N, Shaikh Z, Ahmed I.* Cutaneous manifestations of diabetes mellitus type 2: prevalence and association with glycemic control. J Pak Assoc Dermatol 2016; 26 (1): 4–11.
14. *Phulari YJ, Kaushik* V. Study of cutaneous manifestations of type 2 diabetes mellitus. Int J Res Dermatol 2018; 4(1): 8–13.
15. *Furqan S, Kamani L, Jabbar A.* Skin manifestations in diabetes mellitus. J Ayub Med Coll Abbottabad 2014; 26(1): 46–8.
16. *Ghos K, Das K, Ghos S, Chakraborty S, Jatua SK, Bhattacharya A,* et al. Prevalence of skin changes in diabetes mellitus and its correlation with internal diseases: a single center observational study. Indian J Dermatol 2015; 60(5): 465–9.
17. *Foss NT, Polon DP, Takada MH, Foss-Freitas MC, Foss MC.* Skin lesions in diabetic patients. Rev Saude Publica 2005; 39(4): 677–82. (Portoguese)
18. *Ahmed K, Muhammad Z, Qayum I.* Prevalence of cutaneous manifestations of diabetes mellitus. J Ayub Med Coll Abbottabad 2009; 21(2): 76–9.
19. *Mahajan S, Koranne RV, Sharma SK.* Cutaneous manifestation of diabetes mellitus. Indian J Dermatol Venereol Leprol 2003; 69(2): 105–8.
20. *Vahora R, Thakkar S, MarfatiaY.* Skin, a mirror reflecting diabetes mellitus: A longitudinal study in a tertiary care hospital in Gujarat. Indian J Endocr Metab 2013; 17(4): 659–64.
21. *Chatterjee N, Chattopadhyay C, Sengupta N, Das C, Sarma N, Pal SK.* An observational study of cutaneous manifestations in diabetes mellitus in a tertiary care hospital of Eastern India. Indian J Endocr Metab 2014; 18(2): 217–20.
22. *Demirseren DD, Emre S, Akoglu G, Arpacı D, Arman A, Metin A,* et al. Relationship between skin diseases and extracutaneous complications of diabetes mellitus: clinical analysis of 750 patients. Am J Clin Dermatol 2014; 15(1): 65–70.
23. *Vani G, Reddy VN.* A clinical study of diabetic dermatological manifestations at a tertiary care hospital in South India. Int J Res Dermatol 2018; 4(3): 293–7.
24. *Bhardwaj N, Roy S, Jindal R, Ahmad S.* Cutaneous manifestations of diabetes mellitus: a clinical study. Int J Res Dermatol 2018; 4(3): 352–6.
25. *Piérard GE, Seité S, Hermanns-Lê T, Delvenne P, André Scheen, Piérard-Franchimont C.* The skin landscape in diabetes mellitus. Focus on dermocosmetic management. Clin Cosmet Investig Dermatol 2013; 6: 127–35.
26. *Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y.* Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis (†). Ann Med 2017; 49(2): 106–6.
27. *Yosipovitch G, Hodak E, Vardi P, Shraga I, Karp M, Sprecher E,* et al. The prevalence of cutaneous manifestations in IDDM patients and their association with diabetes risk factors and microvascular complications. Diabetes Care 1998; 21(4): 506–9.
28. *Armstrong DG, Boulton AJM, Bus SA.* Diabetic Foot Ulcers and Their Recurrence. N Engl J Med 2017; 376(24): 2367–75.
29. *Chandrashekar SM, Suraj Muralidhar S.* A study on the prevalence of risk factors and presence of diabetic foot ulcers in T2DM patients in K. R. Hospital. Int Surg J 2017; 4(9): 2983–6.
30. *Hussain SM, Ahmed SI.* Schamberg Purpura - A Rare Complication of Skin associated with Type 2 Diabetes Mellitus. Journ Rawalp Med College (JRMC) 2014; 18(1): 163.
31. *Sasmaz S, Buyukbese M, Cetinkaya A, Celik M, Arican O.* The Prevalence of Skin Disorders in Type-2 Diabetic Patients. Int J Dermatol 2004; 3: 1.
32. *Casagrande SS, Menke A, Cowie CC.* No Association between Psoriasis and Diabetes in the U.S. Population. Diabetes Res Clin Pract 2014; 104(3): e58–60.
33. *Dinić MŽ, Zečević RD, Hajduković Z, Mijušković M, Djurić P, Jović Z,* et al. Psoriasis is the independent factor for early atherosclerosis: A prospective study of cardiometabolic risk profile. Vojnosanit Pregl 2016; 73(12): 1094–101.
34. *Karadag AS, Ozly E, Lavery MJ.* Cutaneous manifestations of diabetes mellitus and the metabolic syndrome. Clin Dermatol 2018; 36(1): 89–3.
35. *Khalid U, Hansen PR, Gislason GH, Lindhardsen J, Kristensen SL, Winther SA,* et al. Psoriasis and new-onset diabetes: a Danish nationwide cohort study. Diabetes Care 2013; 36(8): 2402–7.
36. *Armstrong AW, Harskamp CT, Armstrong EJ.* Psoriasis and the risk of diabetes mellitus: a systematic review and meta-analysis. JAMA Dermatol 2013; 149(1): 84–91.
37. *Maluki AH, Abdullah AA.* Metabolic Associations with Skin Tags. Int J Dermatol Clin Res 2016; 2(1): 3–11.
38. *George MS, Walton S.* Diabetic dermopathy. Br J Diabetes Vasc Dis 2014; 14(3): 95–7.
39. *Morgan AJ, Schwartz RA.* Diabetic dermopathy: A subtle sign with grave implications. J Am Acad Dermatol 2008; 58(3): 447–51.
40. *Babakinejad P, Walton S.* Diabetes and pruritus. Br J Diabetes 2016; 16: 154–5.
41. *George MS, Walton S.* Granuloma annulare. Br J Diabetes 2016; 16: 58–61.
42. *Nobari NN, Ghalamkarpour F, Gheisari M, Iranmanesh B.* Multiple granuloma annulare as presenting sign of asymptomatic diabetes mellitus in a child. J Dermatol Dis 2018; 5: 274.
43. *Cheng YW, Tsai WC, Chuang FC, Chern E, Lee CH, Sung CH* et al. A retrospective analysis of 44 patients with granuloma annulare during an 11-year period from a tertiary medical center in south Taiwan. Dermatol Sin 2016; 34(3): 121–5.
44. *Nebesio CL, Lewia C, Chuang TZ.* Lack association between granuloma annulare and type 2 diabetes mellitus. Br J Dermatol 2002; 146(1): 122–4.
45. *Draznin M, Eison R, Maverakis E, Huntley A.* Cutaneus manifestations of diabetes mellitus. In: *Bowker J, Pfeifer M,* editor. Levin and O'Neal's The Diabetic Foot with CD-ROM. 7th ed. Mosby 2008. P. 185–97.
46. *Shrestha B, Sharma E, Mukhtar O, Kaler J, Thapa S, Khalid M.* Scleredema Diabeticorum with Superimposed Cellulitis and

Abscess Formation. Case Rep Endocrinol 2018: 2018: 9513768.

47. *Del Rosso JQ*. A Closer Look at Seborrheic Keratoses: Patient Perspectives, Clinical Relevance, Medical Necessity, and Implications for Management. J Clin Aesthet Dermatol 2017; 10(3): 16–25.
48. *Mettang T, Vonend A, Raap U*. Prurigo nodularis: its association with dermatoses and systemic disorders. Hautarzt 2014; 65(8): 697–703. (German)
49. *Sundarsen S, Migden MR, Silapunt S*. Stasis dermatitis: Pathophysiology, Evaluation and Management. Am J Clin Dermatol 2017; 18(3): 389–90.

Received on February 26, 2019.
Revised on May 17, 2019.
Accepted June 17, 2019.
Online First June, 2019.

ORIGINAL ARTICLE

UDC: 616.314-089.23
https://doi.org/10.2298/VSP190303074I

Aesthetic components of index of orthodontic treatment need in Serbian adolescents

Estetska komponenta indeksa potrebe za ortodontskom terapijom kod adolescenata u Srbiji

Jana Ilić*, Dragana Daković†‡, Margareta Lekić†‡, Tatjana Lemić†‡, Tatjana Čutović†‡

***The Health Center of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; Military Medical Academy, †Clinic for Dentistry, Belgrade, Serbia; University of Defence, ‡Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia**

Abstract

Background/Aim. The biggest motivating factor for undertaking orthodontic treatment is poor dental aesthetics as a consequence of occlusal abnormalities. The aim of this study was to determine the need for orthodontic treatment, based on the aesthetic components of the Index of Orthodontic Treatment Need (IOTN), to compare the degree of aesthetic component of IOTN and the subjective perception of individuals about their dental aesthetics, but also to compare their evaluation of the aesthetic component of IOTN in comparison with the evaluation of the therapist. **Methods.** The study was conducted on a sample of 316 students aged 15–19 years who did not have an orthodontic treatment prior to the survey. The research was carried out using the IOTN. The IOTN consists of dental and aesthetic components based on which the need for therapy was determined. The aesthetic component of the index was noted by the therapist (specialist of orthodontics) as well as the subject. **Results.** According to the grades of subjects, the need for orthodontic treatment was present in 0.38% of male subjects and 2% of female subjects. According to the evaluations of the therapists, the need for orthodontic treatment was present in 7.52% of male subjects and 8% of female subjects. Observing all subjects, the need for orthodontic treatment was present in 0.63% of subjects, and according to the therapist, the need for orthodontic treatment was present in 7.59% of subjects. The mentioned difference was statistically significant. **Conclusion.** Obtained results show that there is a significant difference in evaluation of dental aesthetics and the need for orthodontic treatment between the subjects and therapists. This can be a cause for concern because patients who are not aware of their orthodontic abnormality can limit the need for further treatment.

Key words:
adolescent; aesthetics, dental; malocclusion; orthodontics, corrective.

Apstrakt

Uvod/Cilj. Najveći motivacioni faktor za preduzimanje ortodontske terapije je loša dentalna estetika, nastala kao posledica okluzalne nepravilnosti. Cilj rada bio je da se odredi potreba za ortodontskom terapijom na osnovu estetske komponente Indeksa potrebe za ortodontskom terapijom (IOTN), da se uporedi stepen estetske komponente IOTN-a i subjektivne percepcije ispitanika o njegovoj dentalnoj estetici, kao i da se uporedi evaluacija estetske komponente IOTN ispitanika u odnosu na terapeuta. M**etode.** Istraživanje je sprovedeno na uzorku od 316 učenika, uzrasta 15–19 godina koji pre istraživanja nisu bili ortodontski lečeni. Na osnovu IOTN izvršena je procena potrebe za ortodontskom terapijom. Indeks IOTN sastoji se iz dve komponente, dentalne i estetske. Estetska komponenta indeksa zabeležena je od strane terapeuta, specijaliste ortopedije vilica, kao i od samog ispitanika. **Rezultati.** Prema ocenama ispitanika potreba za ortodontskom terapijom bila je prisutna kod 0.38% ispitanika muškog pola, i 2% ispitanika ženskog pola. Prema ocenama terapeuta, potreba za ortodontskom terapijom bila je prisutna kod 7.52% ispitanika muškog pola i 8% ispitanika ženskog pola. Posmatrajući sve ispitanike, potreba za ortodontskom terapijom bila je prisutna kod 0.63% ispitanika, a prema mišljenju terapeuta potreba za ortodontskom terapijom bila je prisutna kod 7.59% ispitanika. Pomenuta razlika je bila statistički značajna. **Zaključak.** Dobijeni rezultati ukazuju na to da postoji značajna razlika u pogledu zahteva za estetiku zuba i potrebe za ortodonstskom terapijom između ispitanika i terapeuta. To može biti razlog za zabrinutost, jer pacijenti koji nisu svesni svoje ortodontske nepravilnosti, mogu ograničiti potrebu za daljim lečenjem.

Ključne reči:
adolescenti; estetika, stomatološka; malokluzija; ortodoncija, korektivna.

Correspondence to: Tatjana Čutović, Military Medical Academy, Clinic for Dentistry, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: mafi649@gmail.com

Introduction

Malocclusion represents a variation regarding the normal dental and skeletal characteristics [1]. Disturbed facial appearance, as a direct consequence of occlusal abnormalities, is the most common reason why patients require orthodontic treatment.

The patient's perception of the impact of dental variations on his/her self-image depends on many factors, such as religious, social, cultural, and others. Some patients are not even aware of their irregularities, while others complain of a lot fewer irregularities [2–4]. As a result, numerous indices have been developed in order to determine the need for treatment more objectively. The purpose of most occlusal and orthodontic indexes is to assess the anatomical characteristics of malocclusion without assessing the patient's subjective perception of orthodontic anomalies and their impact on the self-esteem and quality of life of the patient. The first index that includes the patient's perception of dental aesthetics is the Index of Orthodontic Treatment Need (IOTN) (its aesthetic component).

In 1989, Brook and Shaw [5] described the index of the need for orthodontic treatment – IOTN, which consists of two components: Dental Component (DHC) and Aesthetic Component (AC).

DHC includes various occlusal traits divided into five categories (degrees) depending on the severity. The first and second degree do not indicate the need nor a slight need for orthodontic treatment, the third degree indicates the borderline need for therapy, and the fourth and fifth degree indicate a great need for orthodontic treatment [2, 3, 6–9]. While determining this component of the index, not every alteration is marked, but the worst determined occlusal trait is the one that defines the highest degree of the need for therapy [1].

The aesthetic component of the index consists of ten intraoral photographies depicting various malocclusions graded according to aesthetic appeal – from the most attractive to the most unattractive dental look [2, 3, 6, 7]. By using this index component, it can be evaluated how much facial appearance is disturbed with the present orthodontic irregularity.

Several studies have shown the validity of the IOTN. It is a reliable, reproducible, accurate, and efficient way to subjectively and objectively assess treatment needs [5, 10, 11].

The greatest limitations of AC of the IOTN are that it is subjective and it does not measure occlusal traits. AC of the IOTN assesses the aesthetic aspects of the malocclusion only in the frontal view and emphasizes the subjective nature of it [12].

There is also a modified form of this index that simplifies identifying people in the need of treatment. The modified IOTN has two categories – definite need for treatment and no definite need for treatment [12, 13].

In many countries, studies on the use of the IOTN are conducted, for example, in Saudi Arabia [14, 15], Nigeria [16, 17], France [18], Italy [19], Iran [20], Spain [21], and Serbia [22].

The aim of this study was to determine the need for orthodontic treatment based on AC of the IOTN, to compare the degree of AC of the IOTN and subjective perception of individuals about their dental aesthetics, but also to compare the evaluation of an individual about AC of the IOTN in comparison with the evaluation of a therapist.

Methods

The study included 316 army students (226 boys and 50 girls) of the Military Gymnasium in Belgrade, Serbia. Students were 15–19 years old, and up to the moment of the research, they were not subjected to orthodontic treatment. Before inclusion, written informed consent was obtained from each participant. Students were examined as part of the Oral Health project of the Military Medical Academy in Belgrade that had been approved by the institutional Ethics Committee.

Clinical examination was performed by one dentist, a specialist in orthodontics, at the Clinic for Dentistry, Military Medical Academy. During the examination of students, AC of the IOTN was noted by the therapist as well as by each student.

AC of the IOTN consists of ten intraoral photos that are graded according to the aesthetic appeal of the teeth. The first photo represents the most attractive and the tenth the least attractive degree. The aesthetic scale is divided into three categories, according to the need for treatment: the first – no need for therapy (Figure 1, 1–4); the second – borderline required therapy (Figure 1, 5–7); the third – great need for therapy (Figure 1, 8–10).

Color photographs were used for the clinical determination of the index. The attractiveness of the teeth was rated according to AC, and the grade was the number that standed next to the photo. Students were shown an AC of 10 photographs, and then a photograph most similar to their tooth appearance was selected.

During the examination, the appearance of the students' teeth was compared with the photos and classed in one of the suitable degrees, both by the therapist and by the subjects as well.

Students with cognitive disorders, chronic illnesses, craniofacial anomalies, and students who had previously undergone orthodontic therapy were excluded from the study. Patients who were not given their consent or were undergoing orthodontic therapy were also excluded from the study.

Statistical analysis

For statistical analysis of the data, software IBM SPSS Statistics 21.0 for Windows was used. The values in which $p < 0.05$ were taken as statistically significant.

Data were evaluated by using the χ^2-test, Mann-Whitney test, Kolmogorov-Smirnov test, and Spearman's correlation.

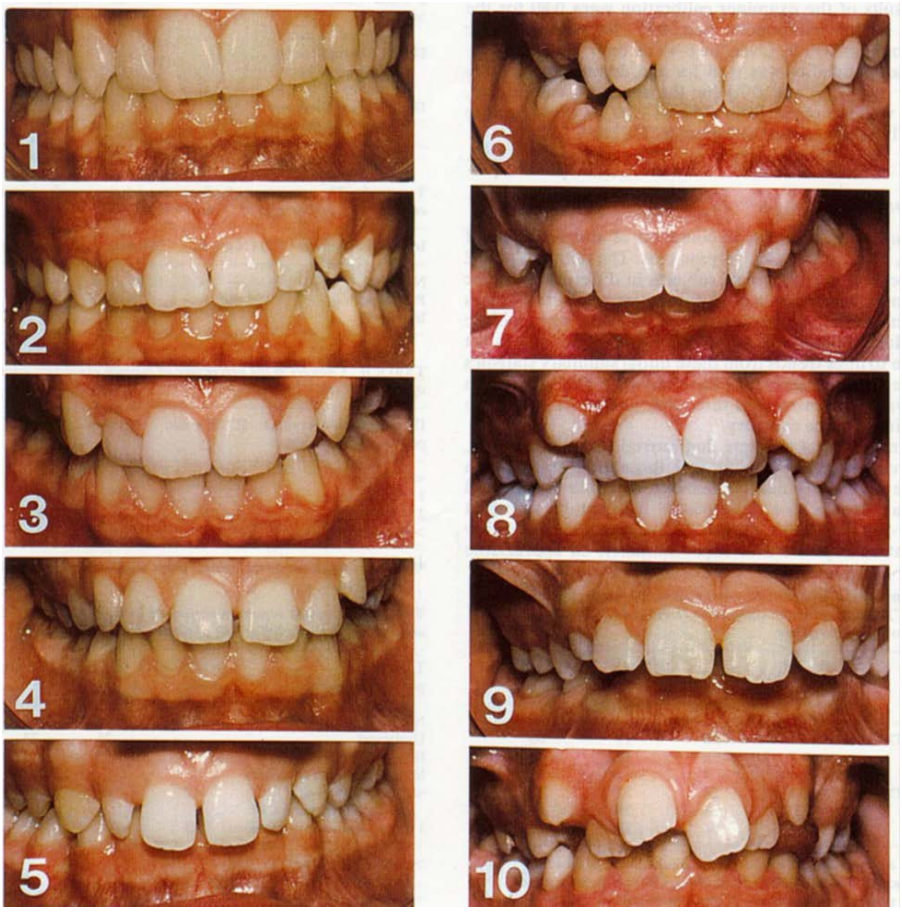


Fig. 1– Aesthetic component of the Index of Orthodontic Treatment Need (IOTN): 1-4 – no need for therapy; 5-7 – borderline required therapy; 8-10 – great need for therapy.

Results

The need for orthodontic treatment based on subjects' evaluation of AC of the IOTN is shown in Table 1. According to AC of the IOTN, 95.25% of subjects had no need for orthodontic treatment (grades 1-4), 4.11% had borderline need (grades 5-7), and 0.63% had a great need for orthodontic treatment (grades 8-10).

Table 1

Distribution of subjects by gender and the need for orthodontic treatment (assessment of all subjects)

Need for orthodontic treatment	Subjects, n (%)		
	male	female	total
No	254 (95.49)	47 (78.00)	301 (95.25)
Borderline	11 (4.14)	2 (14.00)	13 (4.11)
Great	1 (0.38)	1 (8.00)	2 (0.63)
Total	266 (100.00)	50 (100.00)	316 (100.00)
Significance	$\chi^2 = 1.766$; Df = 2; $p = 0.414$		

There were no statistically significant differences between the sexes during the AC grading within the IOTN.

The need for orthodontic treatment based on evaluation of AC of the IOTN by the therapist is shown in Table 2. According to the therapist's evaluation of AC of the IOTN, 83.23% of subjects had no need for therapy (grades 1-4), 9.18% had borderline need (grades 5-7), while 7.59% of subjects had a great need for orthodontic treatment (grades 8-10).

Table 2

Distribution of subjects by gender and the need for orthodontic treatment(assessment of therapist)

Need for orthodontic treatment	Subjects, n (%)		
	male	female	total
No	224 (84.21)	39 (78.00)	263 (83.23)
Borderline	22 (8.27)	7 (14.00)	29 (9.18)
Great	20 (7.52)	4 (8.00)	24 (7.59)
Total	266 (100.00)	50 (100.00)	316 (100.00)
Significance	$\chi^2 = 1.713$; Df = 2; $p = 0.425$		

There were statistically significant differences in determining AC of the IOTN between the subjects and the therapist.

If we consider all subjects (n = 316), the grades of subjects and the therapist were in a statistically significant medium-strong positive correlation (Spearman's correlation coefficient: R = 0.463; $p = 000$).

By observing all subjects, the average grade of subjects was 2.21, while the average grade of the therapist was 3.00. This difference was statistically significant.

By observing all subjects, 0.63% of them needed orthodontic treatment, while according to the therapist, 7.59% of subjects needed orthodontic treatment. There was a statistically significant difference (Table 3).

Table 3
Distribution of all subjects by the need for orthodontic treatment (assessment of all subjects and therapist)

Need for orthodontic treatment	Subjects n (%)	Therapist n (%)
No	301 (95.25)	263 (83.23)
Borderline	13 (4.11)	29 (9.18)
Great	2 (0.63)	24 (7.59)
Total	316 (100.00)	316 (100.00)
Significance	$\chi^2 = 27.271$; Df = 2; $p < 0.001$	

If we consider only male subjects (n = 266), the grades of participants and the therapist were in a statistically significant medium-strong positive correlation (Spearman's correlation coefficient: R = 0.452; $p < 0.001$).

The average grade of male subjects was 2.20, while the average grade of the therapist was 3.00. This mentioned difference was statistically significant.

According to the male subjects, 0.38% of them needed orthodontic treatment, while according to the therapist, 7.52% of subjects needed orthodontic treatment. There was a statistically significant difference (Table 4).

Table 4
Distribution of male subjects by the need for orthodontic treatment (assessment of male subjects and therapist)

Need for orthodontic treatment	Subjects n (%)	Therapist n (%)
No	254 (95.49)	224 (84.21)
Borderline	11 (4.14)	22 (8.27)
Great	1 (0.38)	20 (7.52)
Total	266 (100.00)	266 (100.00)
Significance	$\chi^2 = 22.740$; Df = 2; $p < 0.001$	

If we look at the female subjects (n = 50) , the grades of the participants and the therapist were in a statistically significant correlation (Spearman's correlation coefficient: R = 0.530; $p < 0.001$).

The average grade of female subjects was 2.28, while the average grade of the therapist was 3.00. This mentioned difference was statistically significant.

According to the female subjects, 2% of subjects needed orthodontic treatment, while according to the therapist, 8.00% of subjects needed orthodontic treatment. There was a statistically significant difference (Table 5).

Table 5
Distribution of female subjects by the need for orthodontic treatment (assessment of female subjects and therapist)

Need for orthodontic treatment	Subjects n (%)	Therapist n (%)
No	47 (94.00)	39 (78.00)
Borderline	2 (4.00)	7 (14.00)
Great	1 (2.00)	4 (8.00)
Total	50 (100.00)	50 (100.00)
Significance	$\chi^2 = 5.322$; Df = 2; $p = 0.070$	

Discussion

In recent years, the demand for orthodontic treatment, together with enhanced general awareness about aesthetics, has increased in many countries. In our country, children with small aesthetic imperfections and children with serious occlusal anomalies have the same right for orthodontic treatment. Introduction of indexes in orthodontic practice would eliminate the defects of traditional orthodontic diagnosis, which is subjective, and priority should be given to patients in whom therapy is necessary [23].

Besides patients' appearance, psychosocial circumstances significantly affect the determination of the need for orthodontic treatment. Therefore, it is difficult to determine just based on the analysis of plaster models and X-rays for whom the therapy is necessary and for whom it is not [3]. One of the main reasons why patients require orthodontic treatment is the reduction of psychosocial problems related to the appearance of the teeth and face. Not only are these problems aesthetic, but they can also significantly affect the quality of life [3]. It has been confirmed by some studies in Spain that the aesthetic appearance of teeth and the smile significantly affects patients' self-confidence, especially in the student population [24].

According to this survey, 8% of subjects had a great need for orthodontic treatment based on the analysis of AC of the IOTN with a significant difference in the assessments of the subjects and the therapist. Similar to our results, Janošević et al. [22] found out that 15.3% of subjects had malocclusions that needed treatment from an aesthetic viewpoint.

As part of research among children aged 9 to 12 years in France, Souames et al. [18] gave similar assessments based on the analysis of AC of the IOTN. According to that study, 7% of children had a great need for orthodontic treatment. There were no significant differences in the aesthetic evaluation between boys and girls.

Our study showed a significant difference between the grades of the subjects and the therapist, which was also the case with Nobile et al. [19].

Nobile et al. [19] conducted a study in Italy among children aged 11 to 14 years in which they compared AC of the index grades between the examiner and the examined children. Therefore, they obtained the following results: the therapist found that therapy was necessary for 8.6% of subjects, while subjects found that therapy was necessary for 3.2% of subjects. Based on these results, they came to a conclusion that the therapist's expert opinion is significantly more critical than the views of subjects concerning the disruption of the face aesthetic with present orthodontic abnormalities.

Same as Nobile et al. [19], Manzanera et al. [21] and Hedayati et al. [20] found reduced need for orthodontic treatment based on the analysis of AC of the IOTN.

Contrary to our study, a study in Shiraz found a slightly statistical correlation between the grades of subjects and examiners. The aim of that study was to assess the need for orthodontic treatment in children aged 11 to 14 years. Subjects were assessed based on AC of the IOTN, according

to which 4.11% of students had a great need for orthodontic treatment. Therapists also gave similar ratings according to which therapy was necessary for 6.21% of students. Their results showed that the need for orthodontic treatment was reduced, and most of the students were in the category of the little need for therapy [20].

Orthodontic treatment depends on the perception of the therapist but also on the perception of the patient. The perception of the patient and the actual need for orthodontic treatment helps in treatment planning. The patient's assessment for orthodontic treatment need is not always in correlation with the professional assessment. This was determined by Hassan [14], Kolawole et al. [16], Aikinis et al. [17], Hamdan [25], and Ousehal et al. [26].

While conducting research on subjects 12 to 18 years old on the territory of Nigeria, Aikinis et al. [17] noticed a significant difference in the rankings of the attractiveness of occlusion between the patients and the therapist. Based on the perception of the therapist, 17.6% of subjects had a great need for therapy. In patients' perception, 6.5% of subjects had a great need for therapy. Age and gender did not have an impact on assessing the need for orthodontic therapy [17].

Moreover, Soh and Sandham [27] found no correlation between the subjects and the therapist. They studied Asian male army recruits aged 17–22 years. The subjects perceived dental aesthetics differently from the therapist, which is similar to that of the present study. As in our study, men were generally more satisfied with their dental appearance and less likely to perceive the need for orthodontic treatment in order to correct their malocclusion.

This lack of understanding of the nature of malocclusion and its consequences suggests promoting further knowledge and awareness of malocclusion.

A significant correlation in grades for AC of the IOTN of the therapist and subjects was found by Albarakati et al. [15], Siddiqui et al. [28], and Ghijselings et al. [29].

Siddiqui et al. [28] conducted a study on this index on children aged 16 to 25 years and found a significant positive relation between the perceptions of the therapists and patients. Compared with the children in younger age groups, patients with the increase in the average age must be more aware of their aesthetic needs [24].

Another study in which patients from 17 to 24 years were tested showed that patients were less critical in assessing the need for orthodontic treatment compared to therapists. In assessing AC, therapists are significantly associated with the real need for therapy, while the aesthetic assessment of the subjects does not affect so much the real need for therapy as gender and personality traits [30].

Based on the therapist's assessment, Cai et al. [30] have established that the need for therapy is present in 32% of subjects, and only 11% of subjects think that orthodontic therapy is necessary. In that study, as in ours, the opinion of young people about the aesthetic appearance of their teeth differs from the opinion of the therapists. They do not have a realistic view of their appearance and are unable to seriously understand their orthodontic irregularity. Before starting treatment, it is important to explain in detail to patients their condition and why further therapy is needed. This improves communication between the patient and the therapist, better understanding, and better results are achieved in the treatment.

In the research of Cai et al. [30], the influence of gender and personality traits on the subjective perception of the AC was also assessed. Similar to our results, it was concluded that young Chinese women are more critical about dental aesthetics than men, and emotionally introverted people are more critical when their dental aesthetics is concerned [30, 31].

Some research has shown that even younger children have a rational view on the aesthetics of teeth and the need for orthodontic treatment. However, some authors believe that AC should not be used in children with mixed dentition because some orthodontic irregularities are often corrected during the period of growth and development or after breaking bad habits. Just for this reason, high rating values for the need for orthodontic treatment occur if AC is used in children with mixed dentition [18]. Nevertheless, current trends toward earlier initiation of the therapy justify the fact that the IOTN is also used in younger children.

The correct identification of patients who need orthodontic treatment from the early years of life allows interceptive treatments so that the increase in the severity of disorders and the need for more complex and expensive corrective orthodontic treatments is prevented [32].

If the patient's understanding of the need for treatment or the aesthetic classification is not the same as the therapist's one, it can pose a problem in the sense of the constraints of the need for therapy, or it may complicate the therapy itself [28, 33]. In order to ensure patient satisfaction and efficient orthodontic treatment, the perception of the patient, not just the professional assessment of the therapist, must be taken into consideration. A good correlation between self-perception and the real need for therapy indicates that patients are able to understand their clinical condition.

Conclusion

The obtained results showed that subjects did not have quite a rational view about the aesthetics of the teeth. They were not aware of the seriousness of orthodontic irregularities and the need for orthodontic treatment.

Before starting therapy, patients need to explain in detail the real need for orthodontic therapy. A better understanding of the patient has a positive effect on the goals of the treatment, reduces the likelihood of compromised outcomes of the treatment, and guarantees better results.

Using the IOTN, it is possible to estimate the need for orthodontic therapy considering dental aesthetics and AC of orthodontic anomalies. Due to the high prevalence of orthodontic irregularities, it would be important to introduce the use of this index in clinical practice in order to determine the priorities for the treatment and allocate the resources of dental health care correctly.

Acknowledgement

The study was a part of the project MFVMA w(No 1/15-17).

R E F E R E N C E S

1. *Špalj S, Katalinić A, Varga S, Radica N.* Ortodontski priručnik. Rijeka: Medicinski fakultet Sveučilišta u Rijeci; 2012.
2. *Mitchell L.* An introduction to orthodontics. New York: Oxford University Press; 1998.
3. *Proffit RW, Fields WH, Sarver MD.* Contemporary orthodontics. 4th ed. St Louis: Mosby; 2007.
4. *Grzywacz I.* Orthodontic treatment needs and indications assessed with IOTN. Ann Acad Med Stetin 2004; 50(1): 115–22. (Polish)
5. *Brook PH, Shaw WC.* The development of an index of orthodontic treatment priority. Eur J Orthod 1989; 11: 309–20.
6. *Graber MT, Vanarsdall LR, Vig WLK.* Orthodontics: current principles and techniques. 4th ed. St Louis: Mosby; 2005.
7. *Millet D, Welbury R.* Orthodontics and paediatric dentistry. London: Harcourt Publishers Limited; 2000.
8. *Torkan S, Pakshir HR, Fattahi HR, Oshagh M, Momeni Danaei S, Salehi P,* et al. An Analytical Study on an Orthodontic Index: Index of Complexity, Outcome and Need (ICON). J Dent (Shiraz) 2015; 16(3): 149–55.
9. *Vishnoi P, Shyagali TR, Bhayya DP.* Prevalence of Need of Orthodontic Treatment in 7-16-Year-Old School Children in Udaipur City, India. Turk J Orthod 2017; 30(3): 73–7.
10. *Cooper S, Mandall NA, DiBiase D, Shaw WC.* The reliability of the index of orthodontic treatment need over time. J Orthod 2000; 27(1): 47–53.
11. *Younis JW, Vig KW, Rinchuse DJ, Weyant RJ.* A validation study of three indexes of orthodontic treatment need in the United States. Community Dent Oral Epidemiol 1997; 25(5): 358–62.
12. *Borzabadi-Farahani A.* An overview of selected orthodontic treatment need indices In: *Naretto S.* editor. Principles in Contemporary orthodontics. Rijeka: InTech 2011. p. 215–36.
13. *Richmond S, Shaw WC, Stephens CD, Webb WG, Roberts CT, Andrews M.* Orthodontics in the general dental service of England and Wales: A critical assessment of standards. Br Dent J 1993; 174(9): 315–29.
14. *Hassan AH.* Orthodontic treatment needs in the western region of Saudi Arabia: a research report. Head Face Med 2006; 2: 2.
15. *Albarakati SF.* Self-perception of malocclusion of Saudi patients using the aesthetic component of the IOTN index. Park Oral Dent J 2007; 27(1): 45–52.
16. *Kolawole KA, Otuyemi DE, Jeboda SO, Umwehi AA.* Awareness for malocclussion and desire for orthodontic treatment need in 11-14-year-old Nigerian schoolchildren and their parents. Aust Orthod J 2008; 24(1): 21–5.
17. *Aikinis EA, DaCosta OO, Onyeaso CO, Isiekwe MC.* Self-perception of malocclusion among Nigerian adolescentes using the aesthetic component of the IOTN. Open Dent J 2012; 6: 61–6.
18. *Souames M, Bassigny F, Zenati N, Riordan PJ, Boy-Lefevre ML.* Orthodontic treatment need in French schoolchildren: an epidemiological study using the Index of orthodontic treatment need. Eur J Orthod 2006; 28(6): 605–9.
19. *Nobile CG, Pavia M, Fortunato L, Angellio IF.* Prevalence and factors related to maloclusion and orthodontic treatment need in children and adolescentes in Italy. Eur J Public Health 2007; 17(6): 637–41.
20. *Hedayati Z, Fattahi HR, Jahromi SB.* The use of index of orthodontic treatment need in an Iranian population. J Indian Soc Pedod Prev Dent 2007; 25(1): 10–4.
21. *Manzanera D, Moutiel-Company JM, Almerich-Silla JM, Gandia JL.* Orthodontic treatment need in spanish schoolchildren: an epidemiological study using the Index of orthodontic treatment need. Eur J Orthod 2009; 31(2): 180–3.
22. *Janošević P, Stošić M, Janošević M, Radojičić J, Filipović G, Čutović T.* Index of orthodontic treatment need in children from the Niš region. Vojnosanit Pregl 2015; 72(1): 12–5.
23. *Đorđević J, Šćepan I, Glišić B.* Procena saglasnosti i korelacija tri okluzalna indeksa u određivanju potrebe za ortodontskim lečenjem. Vojnosanit Pregl 2011; 68(2): 125–9.
24. *Bellot-Arcis C, Montiel-Company JM, Pinho T, Almerich-Silla JM.* Relationship between perception of malocclusion and the psychological impact of dental aesthetics in university students. J Clin Exp Dent 2015; 7(1): e18–e22.
25. *Hamdan AM.* The relationship between patient, parent and clinician perceived need and normative orthodontic treatment need. Eur J Orthod 2004; 26(3): 265–71.
26. *Ousehal L, Lazrak L, Serrhini I, Elquars F.* Evaluation of facial esthetics by a panel of professionals and a lay panel. Int Orthod 2011; 9(2): 224–34.
27. *Soh J, Sandham A.* Orthodontic treatment need in Asian adult males. Angle Orthod 2004; 74(6): 769–73.
28. *Siddiqui TA, Shaikh A, Fida M.* Agreement between orthodontist and patient perception using Index of orthodontic treatment need. Saudi Dent J 2014; 26(4): 156–65.
29. *Ghijselings I, Brosens V, Willems G, Fieuws S, Clijmans M, Lemieve J.* Normative and self-perceived orthodontic treatment need in 11-to 16-year-old children. Eur J Orthod 2014; 36(2): 179–85.
30. *Cai Y, Du W, Lin F, Ye S, YeY.* Agreement of young adults and orthodontists on dental aesthetics & influencing factors of self-perceived aesthetics. BMC Oral Health. 2018; 18(1): 113.
31. *Yin L, Chen WJ, Yu XZ, Yu J, Fang L, Zhou B,* et al. A survey of perception differences of malocclusion between 16 to 22-year-old young adults and orthodontists. Hua Xi Kou Qiang Yi Xue Za Zhi 2011; 29(2): 153–6, 160. (Chinese)
32. *Cruz Lopez MF, Gutierrez Rojo MF, Gutierrez Rojo JF, Rojas Garcia AR.* Comparison between the ICON index and the aesthetic component of the IOTN to determine the need for orthodontic treatment. Rev Mex Ortodon. 2017; 5(1): e10–3.
33. *Alhummayani FM, Taibah SM.* Orthodontic treatment needs in Saudi young adults and manpower requirements. Saudi Med J 2018; 39(8): 822–8.

Received on March 3, 2019.
Revised on May 21, 2019.
Accepted on June 13, 2019.
Online First April, 2019.

ORIGINAL ARTICLE

UDC: 616.132.2-079-06:616.61-07
https://doi.org/10.2298/VSP190418075P

Diagnostic importance of cystatin C and creatinine for contrast-induced acute kidney injury

Značaj cistatina C i kreatinina u dijagnostici akutnog oštećenja bubrega izazvanog kontrastom

Dejan Pilčević*, Nemanja Rančić†‡, Zoran Jović‡§, Violeta Rabrenović*‡, Svetlana Antić*, Marijana Petrović*‡, Dejan Petrović‖¶, Djoko Maksić*‡

Military Medical Academy, *Clinic for Nephrology, †Centre for Clinical Pharmacology, §Clinic for Cardiology, Belgrade, Serbia; University of Defence, ‡Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; Clinical Centre of Kragujevac, ‖Clinic for Urology, Nephrology and Dialysis, Kragujevac, Serbia; University of Kragujevac, Faculty of Medical Sciences, ¶Department of Internal Medicine, Kragujevac, Serbia

Abstract

Background/Aim. Contrast-induced acute kidney injury (CI-AKI) is a common complication after the percutaneous coronary intervention, associated with a prolonged hospital stay, increased medical costs, and risk of adverse clinical outcomes. The aim of this study was to compare changes in levels of serum creatinine (sCr) and cystatin C (sCyC) 24 h after coronary angiography as an early indicator of CI-AKI. **Methods.** The study included 45 patients with chronic renal failure grade I–III scheduled for coronary angiography. Levels of sCr and sCyC were measured a day before and 24 h after coronary angiography. CI-AKI was defined as a 25% and 10% increase of sCr and sCyC levels from baseline within 24 h from contrast media exposure, in the absence of alternative causes. **Results.** Mean sCr and sCyC concentrations were 86.4 ± 22.6 μmol/L and 1.18 ± 0.52 mg/dL, respectively before contrast administration, and 90.6 ± 24.1 μmol/L and 1.24 ± 0.65 mg/dL, respectively 24 h after contrast media exposure. sCr-based CI-AKI occurred in 4 patients (8.89%) and sCyC-based CI-AKI was detected in 19 patients (42.22%) after the contrast procedure ($p < 0.001$). **Conclusion.** sCyC level measured 24 h after contrast media exposure is a more sensitive indicator of CI-AKI than sCR level.

Key words:
kidney failure, acute; kidney failure, chronic; coronary angiography; creatinine; cystatin c.

Apstrakt

Uvod/Cilj. Kontrastom izazvano akutno oštećenje bubrega (KI-AOB) uobičajena je komplikacija nakon perkutane koronarne intervencije i dovodi do produžene hospitalizacije, povećanih medicinskih troškova i rizika od neželjenih kliničkih ishoda. Cilj rada bio je da se uporede promene u nivou serumskog kreatinina (sKr) i cistatina C (sCiC) 24 sata nakon učinjene koronarne angiografije kao ranih indikatora KI-ABO. **Metode.** Studija je obuhvatila 45 bolesnika sa hroničnom bubrežnom insuficijencijom 1–3. stadijuma kojima je planirana koronarna angiografija. Nivoi sKr i sCiC su mereni dan pre, kao i 24 sata posle koronarne angiografije. KI-ABO je bilo definisano kao povećanje nivoa sKr i sCiC od 25%, odnosno 10% u odnosu na bazalni nivo u roku od 24 sata nakon izlaganja kontrastnom sredstvu, a u odsustvu drugih alternativnih uzroka. **Rezultati.** Srednje vrednosti nivoa sKr i sCiC iznosile su 86,4 ± 22,6 μmoL/L i 1,18 ± 0,52 mg/dL, redom, pre primene kontrasta, odnosno 90,6 ± 24,1 μmoL/L i 1,24 ± 0,65 mg/dL, 24 sata nakon izlaganja kontrastnom sredstvu. S obzirom na nivo sKr, KI-ABO evidentirano je kod 4 bolesnika (8,89%), a s obzirom na nivo sCiC kod 19 bolesnika (42,22%) ($p < 0,001$). **Zaključak.** Nivo sCiC je osetljiviji indikator KI-ABO od sKr 24 sata nakon izlaganja kontrastnim sredstvima.

Ključne reči:
bubreg, akutna insuficijencija; bubreg, hronična insuficijencija; angiografija koronarnih arterija; kreatinin; cistatin c.

Correspondence to: Dejan Pilčević, Military Medical Academy, Clinic for Nephrology, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: dejan.pilcevic@gmail.com

Introduction

Contrast-induced acute kidney injury (CI-AKI) is a common complication after the percutaneous coronary intervention (PCI), associated with a prolonged hospital stay, increased medical costs, and risk of adverse clinical outcomes [1, 2]. This complication has become the third cause of hospital-acquired acute kidney injuries (11.3%) [3]. Since effective treatment measures for preventing CI-AKI have not been completely established, early diagnosis in previously identified high-risk patients for the development of this complication is necessary [4]. CI-AKI is usually defined as an absolute increase ≥ 0.3 mg/dL, or a relative increase > 25% of serum creatinine (sCr) from baseline level within the period of 24–48 h after contrast exposure in the absence of an alternative cause [5–11]. However, sCr concentration is affected by gender, age, muscle mass, and diet. Moreover, its increase could be delayed, which can discredit it as a certain indicator of acute renal failure [12–15]. Cystatin C (CyC), a cationic low molecular weight cysteine protease produced by all nucleated cells at a constant rate but not metabolized in the serum, is freely filtered by the glomeruli [16]. Compared with sCr, cystatin C is less affected by the previously mentioned factors. Its half-life is 3 times shorter, and maximum levels are reached within 24 h after contrast exposure, which recommend it as the marker of early changes in glomerular filtration rate (GFR) [17–19]. However, some studies reported its low predictive value for CI-AKI compared with sCr [20, 21]. According to the fact that the majority of our patients are discharged from the hospital 24 hours after the coronary angiography, in the present study, we compare changes in sCr and sCyC levels in that period with the aim to establish a reliable early diagnostic tool for predicting CI-AKI.

Methods

Design and participants

During 2018, 45 consecutive patients over 18 years of age, with chronic renal failure (CRF) stages I–III, scheduled to undergo coronary angiography at the Military Medical Academy (MMA) in Belgrade, Serbia, were prospectively recruited. The exclusion criteria were pregnancy, lactation, malignancy, GFR > 100 mL/min, GFR < 30 mL/min, age < 18 years, recent exposure to contrast medium (CM) (within the period of 3 months before the procedure), and the use of more than 300 mL of CM.

As CM, we used non-ionic, low-osmolality iodinated CM, either iohexol (Omnipaque®, 350 mg I/mL) or iopromid (Ultravist®, 370 mg I/mL) for all patients. For the purpose of CI-AKI prophylaxis, all patients received a continuous intravenous infusion of 1,000 mL isotonic saline at least 1–6 h after the procedure [with or without N-acetyl-cysteine (NAC) – 2 × 600 mg *per os* – the day before and on the day of procedure]. The study protocol was approved by the Ethics Committee of MMA. Informed consent was obtained from all participants.

Data collection, biomarker measurement, and follow-up

Demographic and clinical data were recorded for each participant. All biochemical indicators – sCr, sCyC, hemoglobin (Hgb), albumins, lipids, C-reactive protein (CRP), brain natriuretic peptide (BNP), urinary beta-2 microglobulin (beta-2 MCG), urinary albumin/creatinine ratio (alb/cr) – were collected in the morning prior to the procedure and 24 h after the coronary angiography. They were measured in the Central Biochemistry Laboratory of MMA. sCyC was quantified with particle-enhanced nephelometric immunoassay (PENIA) method (BN II Dade Behring, Germany).

The Chronic Kidney Disease Epidemiology (CKD EPI) formula was used to calculate the estimated GFR (eGFR) [22]. For the purpose of this study, sCr-based AKI was defined as a relative increase > 25% from baseline level within the 24 h after contrast exposure, and sCyC-based AKI was defined as an increase in the sCyC concentration greater than 10% within the 24 h of contrast media exposure in the absence of an alternative cause [23].

Statistical analyses

The continuous variables were presented as the mean ± standard deviation (SD) or median [with interquartile range (IQR): 25th and 75th percentiles] and categorical variables as percentages. For continuous variables, comparisons between groups were made using the independent samples *t*-test for normally distributed data and the Mann-Whitney test for non-normally distributed data. Categorical data were compared using the chi-squared (χ^2) test. The value of $p < 0.05$ was considered significant throughout the analyses. All analyses were performed using SPSS 19.0 software.

Results

The demographic and biochemical characteristics of our patients are shown in Table 1. The majority of them were male (66.67%), mean age 66.9 ± 8.2 years, mean body mass index (BMI) 26.87 ± 3.94 kg/m². Thirty-four patients (75.6%) had high blood pressure, 16 patients (35.6%) were diabetics, the same number of patients was detected in the population of former or active smokers, and 4 patients (8.9%) had asymptomatic heart failure. Baseline levels of sCr and sCyC were 86.4 ± 22.6 μmol/L and 1.18 ± 0.52 mg/dL, respectively. Mean eGFR calculated by CKD EPI formula was 75.04 ± 16.62 mL/min *per* 1.73 m². Mean values of CRP, BNP, Hgb, albumins, lipids, alb/cr ratio in urine were in the normal range. Seven patients (15.56%) had abnormal baseline values of urinary beta-2 MCG. Twenty-five patients (55.6%) were treated with the prophylactic regime with

Table 1

Demographic and biochemical characteristics of the patients

Variables	Values
Sociodemographic characteristics	
age (years), mean ± SD	66.89 ± 8.22
male, n (%)	30 (66.7)
female, n (%)	15 (33.3)
BMI (kg/m²), mean ± SD	26.87 ± 3.94
Comorbidities	
current or prior smoking; n (%)	16 (35.6)
hypertension, n (%)	34 (75.6)
prior MI or stroke, n (%)	6 (13.3)
diabetes mellitus, n (%)	16 (35.6)
NYHA Grade III–IV, n (%)	4 (8.9)
Renal function	
eGFR (mL/min/1.73 m^2), mean ± SD	75.04 ± 16.62
eGFR (90 to 99.9 mL/min/1.73 m^2), n (%)	15 (33.3)
eGFR (60 to 89.9 mL/min/1.73 m^2), n (%)	15 (33.3)
eGFR (30 to 59.9 mL/min/1.73 m^2), n (%)	15 (33.3)
sCr baseline (μmol/L), mean ± SD	86.44 ± 22.64
sCyC baseline (mg/L), median (IQR)	1.06 (0.87–1.25)
Biochemical characteristics	
CRP baseline (mg/L), median (IQR)	2.95 (0.72–7.25)
BNP baseline (pg/mL), median (IQR)	78.10 (31.40–134.56)
Hgb baseline (g/L), mean ± SD	138.51 ± 11.27
Alb baseline (g/L), mean ± SD	43.49 ± 3.22
Chol baseline (mmol/L), mean ± SD	4.64 ± 1.25
Tg baseline (mmol/L), median (IQR)	1.56 (1.14–2.64)
Alb/Cr urine, median (IQR)	0.016 (0.011–0.035)
Beta-2 MCG > 0,200 mg/L, n(%)	7 (15.56)
Contrast protocol	
volume of CM (mL), median (IQR)	100 (100–100)
prophylaxis without NAC, n (%)	25 (55.6)
prophylaxis with NAC, n (%)	20 (44.4)

CI-AKI – contrast-induced acute kidney injury; CM – contrast media; eGFR – estimated glomerular filtration rate; MI – myocardial infarction; NYHA – New York Heart Associations; BMI – body mass index; sCr – serum creatinine; sCyC – serum cystatin C; Chol – cholesterol; Tg – triglycerides; CRP – C-reactive protein; BNP – brain natriuretic peptide; Hgb – hemoglobin; Alb – albumin; Alb/Cr – albumin/creatinine ratio; MCG –microglobulin; NAC – N-acetyl-cysteine; IQR – interquartile range; SD – standard deviation.

isotonic saline alone and another 20 patients (44.4%) with additional NAC (2 × 600 mg *per os*).

In our study, after contrast media exposure, mean sCr and sCyC concentrations were 90.6 ± 24.1 μmol/L and 1.24 ± 0.65 mg/dL, respectively. sCyC based CI-AKI occurred in 19 patients (19/45, 42.22%) including 4 patients (4/45, 8,89%) with sCr based CI-AKI (χ^2 test; $p < 0.001$).

After this finding, we decided to form 2 groups based on CI-AKI development. In the group with CI-AKI, sCyC levels significantly increased 24 h after coronary angiography ($p < 0.03$), and eGFR values were found to be significantly decreased ($p < 0.012$) (Table 2). We also found significant differences in the percentage of changing sCr and sCyC concentrations ($p < 0.001$). Among the demographic parameters, only age and diabetes mellitus were found to be associated with CI-AKI development ($p < 0.015$ and $p < 0.04$, respectively). However, the medication therapy, CM volume, and other demographic characteristics and biochemical parameters

Table 2

Characteristics of patients after contrast applications according to contrast-induced nephropathy

Variables	No CI-AKI (n = 26)	CI-AKI (n = 19)	*p*-value
Age (years), mean ± SD	64.38 ± 8.08	70.32 ± 7.29	0.015[1]
Gender (male/female), n (%)	19 (73.1)/7 (26.9)	11 (57.9)/8 (42.1)	0.455[2]
BMI (kg/m²), mean ± SD	27.38 ± 4.48	26.18 ± 3.04	0.320[1]
Current or prior smoking, n (%)	9 (56.3)	7 (43.8)	1.000[2]
Hypertension, n (%)	7 (26.9)	4 (21.1)	0.919[2]
Prior MI or stroke, n (%)	4 (15.4)	2 (10.5)	0.976[2]
Diabetes mellitus, n (%)	13 (50.0)	16 (84.2)	0.040[2]
NYHA Grade III–IV, n (%)	2 (7.0)	2 (10.5)	0.741[2]
eGFR (90 to 99.9 mL/min/1.73 m^2), n (%)	10 (38.5)	5 (26.3)	
eGFR (60 to 89.9 mL/min/1.73 m^2), n (%)	9 (34.6)	6 (31.6)	0.529[2]
eGFR (30 to 59.9 mL/min/1.73 m^2), n (%)	7 (26.9)	8 (42.1)	
sCr baseline (μmol/L), mean ± SD	87.12 ± 20.28	85.53 ± 26.07	0.819[1]
sCr 24 hours post CM exposure (μmol/L), mean ± SD	86.69 ± 20.42	96.00 ± 27.97	0.203[1]
sCy baseline (mg/L), median (IQR)	1.10 (0.89–1.24)	0.97 (0.82–1.49)	0.654[3]
sCyC 24 hours post CM exposure (mg/L), median (IQR)	1.02 (0.86–1.24)	1.23 (0.95–1.81)	0.030[3]
eGFR baseline (mL/min/1.73 m^2), mean ± SD	78.25 ± 15.90	70.65 ± 17.00	0.131[1]
eGFR 24 hours post CM exposure (mL/min/1.73 m^2), mean ± SD	77.82 ± 14.54	65.09 ± 17.95	0.012[1]
CRP baseline (mg/L), median (IQR)	2.99 (0.86–7.30)	2.95 (0.65–7.69)	0.597[3]
CRP 24 hours post CM (mg/L), median (IQR)	3.42 (1.26–11.17)	5.58 (2.21–12.09)	0.312[3]
BNP baseline (pg/mL), median (IQR)	70.93 (25.17–122–90)	92.31 (49.53–142.83)	0.290[3]
BNP 24 hours post CM(pg/mL), median (IQR)	52.72 (32.37–116.36)	86.40 (51.64–160.81)	0.198[3]
Hgb baseline (g/L), mean ± SD	140.62 ± 11.32	135.63 ± 10.83	0.145[1]
Hgb 24 hours post CM (g/L), mean ± SD	138.19 ± 11.94	138.63 ± 14.66	0.912[1]
Alb baseline (g/L), mean ± SD	43.65 ± 3.11	43.26 ± 3.43	0.692[1]
Alb 24 hours post CM (g/L), mean ± SD	43.35 ± 3.27	44.05 ± 3.20	0.475[1]
Chol baseline (mmol/L), mean ± SD	4.82 ± 1.33	4.40 ± 1.11	0.268[1]
Chol 24 hours post CM (mmol/L), mean ± SD	4.83 ± 1.41	4.35 ± 1.06	0.223[1]
Percentage of changing sCr, median (IQR)	-0.43 (–6.19–7.16)	10.67 (4.61–21.31)	< 0.001[3]
Percentage of changing sCyC; median (IQR)	-3.22 (–10.86–5.45)	18.56 (14.15–29.24)	< 0.001[3]
Tg baseline (mmol/L), median (IQR)	1.75 (1.18–2.86)	1.50 (1.08–2.11)	0.265[3]
Tg 24 hours post CM (mmol/L), median (IQR)	1.73 (1.19–2.42)	1.36 (1.05–1.84)	0.103[3]
Beta-2 MCG > 0.200 mg/L, baseline, n (%)	2 (7.7)	2 (10.5)	1.000[2]
Beta-2 MCG > 0.200 mg/L, 24 hours post CM, n (%)	4 (15.4)	3 (15.8)	1.000[2]
Alb/Cr urine baseline, median (IQR)	0.018 (0.011–0.044)	0.014 (0.010–0.029)	0.638[3]
Alb/Cr urine 24 hours post CM exposure, median (IQR)	0.021 (0.009–0.045)	0.012 (0.010–0.042)	0.296[3]
Volume of CM (mL), median (IQR)	100 (100-105)	100 (100-100)	0.681[3]
Prophylaxis without NAC, n (%)	17 (65.4)	8 (42.1)	0.212[2]
Prophylaxis with NAC, n (%)	9 (34.6)	11 (57.9)	

For abbreviations see under Table 1.

[1]Independent samples *t*-test; [2]Chi-squared test; [3]Mann-Whitney test.

were similar between the two groups (Table 2). The results of our study showed that sCyC can significantly improve the early prediction of CI-AKI.

Discussion

Although sCr is not a reliable biomarker of glomerular filtration rate (according to the well-known variations related to gender, age, muscle mass, and nutrition), CI-AKI has been traditionally diagnosed based on the dynamic changes in sCr level after contrast exposure [24]. CyC, a cysteine protease freely filtered by the glomeruli (without previous metabolization in the serum), has a shorter half-life, a more rapid rise, and an earlier achievement of a new steady-state compared with sCr which recommends it as the alternative to sCr for evaluating GFR [25, 26]. The reliability of sCyC as a biomarker in detecting acute changes in kidney function has been proven in several previous studies, including CI-AKI patients [27–29]. However, like other available biomarkers, it is not ideal – the level of sCyC could be impacted with atherosclerosis and cardiac structural abnormalities processes. Moreover, a very serious problem demonstrated in the previously reported studies was the lack of consensus for the cut-off value for CyC elevation [30]. Yin et al. [31], in a study including a total of 204 patients undergoing primary angioplasty, found that CyC relative increase ≥ 10% within 72 h had a good predictive value for CI-AKI. Briguori et al. [32], in one of the most apostrophized studies related to CyC, which included patients with chronic kidney disease followed for one consecutive year, concluded that CyC increase ≥ 10%, 24 h after contrast media exposure, was the best increment cut-off value for the early diagnosis of CI-AKI. Zhang et al. [33] confirmed this claim in their study. Contrary to these studies, Liu et al. [34], in another study that encompassed 311 patients with CRF, did not find the superiority of CyC for detecting CI-AKI. Moreover, Ribichini et al. [35], in a study that included 166 patients with the risk of developing CI-AKI, found that variations of the

baseline serum creatinine are more reliable for detecting CI-AKI at an earlier stage than similar variations in CyC.

In our study, 19 CI-AKI cases were detected by sCyC and 4 of them fulfilled criterion for sCr based CI-AKI, too. We did not find any case where sCr was superior to sCyC as a biomarker of CI-AKI. The overall incidence of CI-AKI in our analysis (19 cases or 42.22%) was higher than in most previous reports, but the fact that it was conducted on patients with pre-existing CRF stages I–III provides a reasonable explanation for this and corresponds to the previous results in similar patient populations [36–42].

Furthermore, during these procedures, some patients, who underwent coronary angiography with stent implantation, received a significantly higher dose of CM than usually (more than 100 mL). On the other hand, the incidence of sCr-based CI-AKI (8.89%) was less than in other studies, which can be explained by the fact that we have measured sCr 24 hours after contrast media exposure. We believe this percentage is underestimated due to a short follow-up period.

According to these results, we concluded that an increase of 10% in sCyC can be reliable for early diagnosis of CI-AKI. On the other hand, we consider that 25% of the increase in sCr is a too strict criterion for this early period, and perhaps we should define a new cut-off for this marker at that time interval. This claim is further supported by the fact that we found statistically significant results in the percentage of creatinine and eGFR change.

This study had several limitations. Firstly, this was a single-center study with a small number of patients hence the results of our study should be confirmed by further larger multicenter studies. Secondly, the majority of patients were discharged 24 h after coronary angiography, which may have led to an underestimation of the true incidence of CI-AKI. Thirdly, our study is not designed to evaluate long-term outcomes. Additionally, other prevention measures, such as statin, diuretic, or angiotensin converting enzyme (ACE) inhibitors use, were not standardized in our study, which may have influenced the development of this complication [43, 44]. Our upcoming study will include a larger number of patients with the use of other early biomarkers and prophylactic regimes, which can additionally confirm and improve the results of this study.

Conclusion

Patients with a high risk of developing contrast-induced acute kidney injury (especially with chronic renal failure) should be monitored with serum cystatin C for 24 hours after exposure to contrast media or more than 48 hours if serum creatinine is used. On the other hand, when considering the economic cost-effectiveness of using serum cystatin C apart from the difference in the price of these two markers, the costs of prolonged hospitalization due to acute kidney injury treatment should be considered as well. It is certain that the untimely diagnosis of contrast-induced acute kidney injury represents the worst possible scenario for our patients, both financially and in terms of the quality of their treatment, and the same must be avoided. Therefore, the use of contrast-induced acute kidney injury markers should be considered rationally, but with an individual approach (especially in patients with a higher risk of developing contrast-induced acute kidney injury).

REFERENCES

1. *Aubry P, Brillet G, Catella L, Schmidt A, Bénard S*. Outcomes, risk factors and health burden of contrast-induced acute kidney injury: an observational study of one million hospitalizations with image-guided cardiovascular procedures. BMC Nephrol 2016; 17: 167.
2. *Tsai TT, Patel UD, Chang TI, Kennedy KF, Masoudi FA, Matheny ME,* et al. Contemporary incidence, predictors, and outcomes of acute kidney injury in patients undergoing percutaneous coronary interventions: insights from the NCDR Cath-PCI registry. JACC Cardiovasc Interv 2014; 7(1): 1–9.
3. *Nash K, Hafeez A, Hou S*. Hospital-acquired renal insufficiency. Am J Kidney Dis 2002; 39(5): 930–6.
4. *Stacul F, van der Molen AJ, Reimer P, Webb JA, Thomsen HS, Morcos SK,* et al. Contrast Media Safety Committee of European Society of Urogenital Radiology (ESUR) Contrast induced nephropathy: updated ESUR Contrast Media Safety Committee guidelines. Eur Radiol 2011; 21(12): 2527–41.
5. *Zeng X, McMahon GM, Brunelli SM, Bates DW, Waikar SS*. Incidence, outcomes, and comparisons across definitions of AKI in hospitalized individuals. Clin J Am Soc Nephrol 2014; 9(1): 12–20.
6. *Kellum JA, Lameire, Aspelin P, Barsoum RS, Burdmann EA, Goldstein SL,* et al. Kidney disease: Improving global outcomes (KDIGO) acute kidney injury work group. KDIGO clinical practice guideline for acute kidney injury. Kindey Int Suppl 2012; 2(1): 1–138.
7. *Solomon R, Natarajan MK, Doucet S, Sharma SK, Staniloae CS, Katholi RE,* et al. The CARE (Cardiac Angiography in Renally Impaired Patients) Study: A randomized, double-blind trial of contrast-induced nephropathy in high risk patients. Circulation 2007; 115(25): 3189–96.
8. *Aspelin P, Aubry P, Fransson SG, Strasser R, Willenbrock R, Berg KJ,* et al. Nephrotoxic effects in highrisk patients undergoing angiography. N Engl J Med 2003; 348(6): 491–9.
9. *Morcos SK, Thomsen HS*. European Society of Urogenital Radiology. European Society of Urogenital Radiology guidelines on administering contrast media. Abdom Imaging 2003; 28(2): 187–90.
10. *McCullough PA, Wolyn R, Rocher LL, Levin RN, O'Neill WW*. Acute renal failure after coronary intervention: incidence, risk factors, and relationship to mortality. Am J Med 1997; 103(5): 368–75.
11. *Rudnick MR, Goldfarb S, Wexler L, Ludbrook PA, Murphy MJ, Halpern EF,* et al. Nephrotoxicity of ionic and nonionic contrast media in 1196 patients: a randomized trial: the Iohexol Cooperative Study. Kidney Int 1995; 47(1): 254–61.
12. *Bellomo R, Kellum JA, Ronco C*. Defining acute renal failure: Physiological principles. Intensive Care Med 2004; 30(1): 33–7.
13. *American Society of Nephrology*. American Society of Nephrology Renal Research Report. J Am Soc Nephrol 2005; 16(7): 1886–903.
14. *Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P*. Acute Dialysis Quality Initiative workgroup. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004 Aug; 8(4): R204-12.

15. *Wasung ME, Chawla LS, Madero M.* Biomarkers of renal function, which and when? Clin Chim Acta 2015; 438: 350–7.
16. *Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindström V,* et al. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. Clin Chem 1994; 40: 1921–6.
17. *Onopiuk A, Tokarzewicz A, Gorodkiewicz E.* Cystatin C: a kidney function biomarker. Adv Clin Chem 2015; 68: 57–69.
18. *Briguori C, Visconti G, Rivera NV, Focaccio A, Golia B, Giannone R,* et al. Cystatin C and contrast-induced acute kidney injury. Circulation 2010; 121(19): 2117–22.
19. *Rickli H, Benou K, Ammann P, Fehr T, Brunner-La Rocca HP, Petridis H,* et al. Time course of serial cystatin C levels in comparison with serum creatinine after application of radiocontrast media. Clin Nephrol 2004; 61(2): 98–102.
20. *Ribichini F, Gambaro G, Graziani MS, Pighi M, Pesarini G, Pasoli P,* et al. Comparison of serum creatinine and cystatin C for early diagnosis of contrast-induced nephropathy after coronary angiography and interventions. Clin Chem 2012; 58(2): 458–64.
21. *Xu Q, Wang N, Duan S, Liu N, Lei R, Cheng W, Zhou SK.* Serum cystatin c is not superior to serum creatinine for early diagnosis of contrast-induced nephropathy in patients who underwent angiography. J Clin Lab Anal 2017; 31(5): doi: 10.1002/jcla.22096.
22. *National Kidney Foundation.* K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Am J Kidney Dis 2002; 39(2 Suppl 1): S1–266.
23. *Kim GS, Ko YG, Shin DH, Kim JS, Kim BK, Choi D,* et al. Elevated serum cystatin C level is an independent predictor of contrast-induced nephropathy and adverse outcomes in patients with peripheral artery disease undergoing endovascular therapy. J Vasc Surg 2015; 61(5): 1223–30.
24. *Dalton RN.* Serum creatinine and glomerular filtration rate: Perception and reality. Clin Chem 2010; 56(5): 687–9.
25. *Filler G, Bökenkamp A, Hofmann W, Le Bricon T, Martínez-Brú C, Grubb A.* Cystatin C as a marker of GFR – history, indication and future research. Clin Biochem 2005; 38: 1–8.
26. *Wagener G, Jan M, Kim M, Mori K, Barasch JM, Sladen RN,* et al. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. Anesthesiology 2006; 105(3): 485–91.
27. *Rickli H, Benou K, Ammann P, Fehr T, Brunner-La Rocca HP, Petridis H,* et al. Time course of serial cystatin C levels in comparison with serum creatinine after application of radiocontrast media. Clin Nephrol 2004; 61(2): 98–102.
28. *Bellomo R.* Decade in review – acute kidney injury: acute kidney injury - a decade of progress. Nat Rev Nephrol 2015; 11(11): 636–7.
29. *Kim BJ, Sung KC, Kim BS, Kang JH, Lee KB, Kim H,* et al. Effect of N-acetylcysteine on cystatin C-based renal function after elective coronary angiography (ENABLE Study): a prospective, randomized trial. Int J Cardiol 2010; 138(3): 239–45.
30. *Feng Y, Zhang Y, Li G, Wang L.* Relationship of cystatin-C change and the prevalence of death or dialysis need after acute kidney injury: a meta-analysis. Nephrology (Carlton) 2014; 19(11): 679–84.
31. *Yin L, Li G, Liu T, Yuan R, Zheng X, Xu G, Xu Y et al.* Probucol for the prevention of cystatin C-based contrast-induced acute kidney injury following primary or urgent angioplasty: a randomized, controlled trial. Int J Cardiol 2013; 167(2): 426–9.
32. *Briguori C, Visconti G, Rivera NV, Focaccio A, Golia B, Giannone R,* et al. Cystatin C and contrast-induced acute kidney injury. Circulation 2010; 121(19): 2117–22.
33. *Zhang WF, Zhang T, Ding D, Sun SQ, Wang XL, Chu SC,* et al. Use of Both Serum Cystatin C and Creatinine as Diagnostic Criteria for Contrast-Induced Acute Kidney Injury and Its Clinical Implications. J Am Heart Assoc 2017; 6(1): pii: e004747.
34. *Liu XL, Wang ZJ, Yang Q, Yu M, Shen H, Nie B,* et al. Plasma neutrophil-gelatinase-associated lipocalin and cystatin C could early diagnose contrast-induced acute kidney injury in patients with renal insufficiency undergoing an elective percutaneous coronary intervention. Chin Med J (Engl) 2012; 125(6): 1051–6.
35. *Ribichini F, Gambaro G, Graziani MS, Pighi M, Peasrini G, Pasoli P,* et al. Comparison of serum creatinine and cystatin C for early diagnosis of contrast-induced nephropathy after coronary angiography and interventions. Clin Chem 2012; 58(2): 458–64.
36. *Rihal CS, Textor SC, Grill DE, Berger PB, Ting HH, Best PJ,* et al. Incidence and prognostic importance of acute renal failure after percutaneous coronary intervention. Circulation 2002; 105(19): 2259–64.
37. *Iakovou I, Dangas G, Mehran R, Lansky AJ, Ashby DT, Fahy M,* et al. Impact of gender on the incidence and outcome of contrast-induced nephropathy after percutaneous coronary intervention. J Invasive Cardiol 2003; 15(1): 18–22.
38. *Davidson CJ, Hlatky M, Morris KG, Pieper K, Skelton TN, Schwab SJ,* et al. Cardiovascular and renal toxicity of a nonionic radiographic contrast agent after cardiac catheterization: a prospective trial. Ann Intern Med 1989; 110(2): 119–24.
39. *Hall KA, Wong RW, Hunter GC, Camazine BM, Rappaport WA, Smyth SH,* et al. Contrast-induced nephrotoxicity: the effects of vasodilator therapy. J Surg Res 1992; 53(4): 317–20.
40. *Gruberg L, Mintz GS, Mehran R, Gangas G, Lansky AJ, Kent KM,* et al. The prognostic implications of further renal function deterioration within 48 h of interventional coronary procedures in patients with pre-existent chronic renal insufficiency. J Am Coll Cardiol 2000; 36(5): 1542–8.
41. *Gami AS, Garovic VD.* Contrast nephropathy after coronary angiography. Mayo Clin Proc 2004; 79(2): 211–9.
42. *Zhang T, Shen LH, Hu LH, He B.* Statins for the prevention of contrast-induced nephropathy: a systematic review and meta-analysis. Am J Nephrol 2011; 33(4): 344–51.
43. *Gupta R, Moza A, Cooper CJ.* Intravenous hydration and contrast-induced acute kidney injury: too much of a good thing? J Am Heart Assoc 2016; 5(6). pii: e003777.

Received on April 18, 2019.
Accepted June 19, 2019.
Online First June, 2019.

SHORT COMMUNICATIONS

UDC: 577.1::616.94-053.2-07
https://doi.org/10.2298/VSP1801200057K

Accuracy of serum procalcitonin, C-reactive protein, and soluble CD14 subtype levels in diagnosis of sepsis in children

Tačnost nivoa serumskog prokalcitonina, C-reaktivnog proteina i rastvorljivog CD14 podtipa u dijagnozi sepse kod dece

Sanja Knežević Rangelov*†, Slobodan M. Janković*‡

University of Kragujevac, *Faculty of Medical Sciences, Kragujevac, Serbia; Clinical Center of Kragujevac, †Pediatric Clinic, ‡Department of Clinical Pharmacology, Kragujevac, Serbia

Abstract

Background/Aim. Despite the widespread use of procalcitonin, C-reactive protein (CRP), and soluble CD14 subtype (sCD14-ST), their diagnostic accuracy in children with sepsis is not yet clear. The aim of the study was to establish and compare the diagnostic accuracy of procalcitonin, CRP, and sCD14-ST in children admitted to the hospital under suspicion of having sepsis. **Methods**. The study was designed as a retrospective cross-sectional study on children admitted to the Pediatrics Clinic in Kragujevac, Serbia, under suspicion of sepsis, during a 6-month period. Diagnostic accuracy was tested by the construction of receiver operating characteristic (ROC) curves and their comparison in terms of area under the curve (AUC). **Results**. Procalcitonin had the largest AUC [0.75; 95% confidence interval (CI) 0.63–0.88], followed by CRP (0.68; 95% CI 0.54–0.81) and sCD14-ST (0.65; 95% CI 0.52 – 0.79). Differences between the areas under the ROC curves were not significant (CRP vs. procalcitonin z = 1.054, p = 0.291; CRP vs. sCD14-ST z = 0.238, p = 0.812; procalcitonin vs. sCD14-ST z = 1.089, p = 0.286). **Conclusion**. Our study showed relatively low sensitivity and moderate specificity of procalcitonin, C-reactive protein and sCD14-ST in diagnosing sepsis among children, as well as similar diagnostic accuracy of the three biomarkers.

Key words:
biomarkers; c-reactive protein; child; diagnosis; presepsin protein, human; sensitivity and specificity; sepsis.

Apstrakt

Uvod/Cilj. Uprkos rasprostranjenom merenju nivoa prokalcitonina, C-reaktivnog proteina (CRP) i rastvorljivog CD14 podtipa (sCD14-ST) u serumu, njihova tačnost u dijagnozi sepse kod dece još nije jasna. Cilj studije bio je da se utvrdi i uporedi dijagnostička tačnost prokalcitonina, CRP-a i sCD14-ST-a kod dece primljene u bolnicu zbog sumnje na sepsu. **Metode**. Studija je bila dizajnirana kao retrospektivna studija preseka i sprovedena na deci primljenoj u Pedijatrijsku kliniku Kliničkog centra Kragujevac tokom šestomesečnog perioda pod sumnjom na sepsu. Dijagnostička tačnost je bila testirana konstrukcijom kriva prijemnik-operator (KPO) za svaki od testova i poređenjem površina ispod njih. **Rezultati**. Prokalcitonin je imao najveću površinu ispod krive [0,75; 95% interval poverenja (CI) 0,63–0,88], zatim slede CRP (0,68; 95% CI 0,54–0,81) i sCD14-ST (0,65; 95% CI 0,52–0,79). Razlike između površina ispod KPO krivih nisu bile značajne (CRP vs. prokalcitoninu z = 1,054, p = 0,291; CRP vs. sCD14-ST-u z = 0,238, p = 0,812; prokalcitonin vs. sCD14-STu z = 1,089, p = 0,286). **Zaključak.** Naša studija je ukazala na relativno nisku senzitivnost i umerenu specifičnost prokalcitonina, CRP-a i sCD14-ST-a u dijagnozi sepse kod dece, kao i sličnu dijagnostičku tačnost ta tri biomarkera.

Ključne reči:
biološki pokazatelji; c-reaktivni protein; deca; dijagnoza; presepsin protein, humani; senzitivnost i specifičnost; sepsa.

Introduction

According to the International Consensus Conference on Pediatric Sepsis held in 2005, sepsis could be defined as a joint occurrence of systemic inflammatory response syndrome with either microbiological confirmation of infection or clinical syndrome associated with a high probability of infection [1]. Apart from these clinical and microbiological criteria, several serum markers of inflammation are used for strengthening the diagnosis of sepsis; procalcitonin (PCT),

Correspondence to: Sanja Knežević Rangelov, Clinical Center Kragujevac, Zmaj Jovina Street 30, 34 000 Kragujevac, Serbia.
E-mail: sanjaknez1980@yahoo.com

C-reactive protein (CRP), and soluble CD14 subtype (sCD14-ST) ("presepsin") are among the most frequently used inflammatory markers. In a recent systematic review of diagnostic accuracy studies involving PCT, CRP, and sCD14-ST in a patient with sepsis, it was shown that the usefulness of these biomarkers for diagnosing sepsis remains debatable, as well as the significance of the difference in sensitivity and specificity between the three [2].

Diagnostic accuracy is especially problematic in children with sepsis, as recent meta-analysis reported high sensitivity (85%) but low specificity (54%) of PCT [3], and some other studies reported moderate sensitivity (87.5%) and specificity (70.9%) of CRP [4], and high sensitivity (94%) and specificity (100%) of sCD14-ST [5]. However, not all studies confirmed these figures in pediatric patients, thus the true role of these biomarkers for diagnosing sepsis, especially in newly admitted children, remains to be established [6].

The aim of our study was to establish and compare the diagnostic accuracy of PCT, CRP, and sCD14-ST in children admitted to the hospital under suspicion of having sepsis.

Methods

The study was designed as a retrospective, observational cross-sectional study on children admitted to Pediatric Clinic in Kragujevac, Serbia (part of the Clinical Center of Kragujevac) under suspicion of sepsis during the first 6 months of 2017. The Inclusion criteria were the following: age below 18 years, admission to the hospital, values of PCT, sCD14-ST, and CRP measured upon admission, and suspicion of sepsis regardless of the source of the infection. The exclusion criteria were the following: septic shock, incomplete patient file, and antibiotic treatment during the last 15 days prior to admission. The study sample was not random but consecutive, as all patients admitted to the hospital during the study period, due to suspicion of sepsis, were enrolled if the criteria for inclusion and exclusion were satisfied.

Blood samples were taken from a peripheral vein on admission, and sera were separated by centrifugation and sent to the central laboratory of the Clinical Center of Kragujevac. PCT was measured by electrochemiluminescence method (COBAS, Roche), CRP by immunoturbidimetry (AU680 and AU400, Beckman Coulter Analyzers), and sCD14-ST by chemiluminescence (PATHFAST immunoanalyzer, Mitsubishi Chemical Europe). The laboratory was accredited by the Serbian Interlaboratory Control body. The following variables were collected from the patients' files: serum levels of PCT, sCD14-ST, and CRP on admission, age, gender, serum level of creatinine, white cell count, results of microbiological analysis of blood and tissue samples, data about body temperature on admission, data about chest X-ray if available, and vital parameters (all variables were measured upon admission if not stated otherwise). The existence of sepsis was confirmed based on the criteria set by the International Consensus Conference on Pediatric Sepsis. The study was approved by the Institutional Review Board of Pediatric Clinic in Kragujevac.

The sample size was calculated based on the following assumptions: power of the study at least 80%, probability of type one error 0.05, the difference between the areas under the receiver operating characteristic (ROC) curves (AUC) tested by Student's *t*-test for independent samples, expected difference between the AUCs taken from the study of Julián-Jiménez et al. [7] (0.79 vs. 0.72) and standard deviation of AUCs measurement of 0.15. The calculation was performed using G-power software version 3.1 [8].

Statistics

Distributions of data from the study were tested for normality by Kolmogorov-Smirnov test and then described by measures of central tendency (median) and variability (interquartile range). The differences among the study groups in regard to continuous variables were tested for significance by the Mann-Whitney *U* test, and those in rates by the χ^2 test. AUCs were calculated for PCT, CRP, and sCD14-ST, together with 95% confidence intervals (CI). Optimal cut-off values were determined by the Manhattan method using online calculator created by the Charite–Universitätsmedizin Berlin [9]. The significance of differences between the AUCs was tested by the De Long's method [10] using MedCalc software. All other calculations were performed by the Statistical Software for Social Sciences (SPSS) version 20.0.

Results

The study included 80 children, out of which 36 had sepsis according to the International Pediatric Sepsis Consensus Conference criteria. Characteristics of the groups with and without sepsis are shown in Table 1. The Kolmogorov-Smirnov test showed that, on admission, only white cell count and creatinine serum level in children without sepsis were normally distributed ($p = 0.200$ and $p = 0.210$, respectively), precluding the use of parametric tests for comparison of the study groups.

In the group of children with sepsis, 24 (66.7%) children had a microorganism isolated: *Enterococcus* spp. 2 (8.3%), *Salmonella enteritidis* 1 (4.2%), *Micrococcus luteus* 1 (4.2%), *Streptococcus* beta-*haemolyticus* 1 (4.2%), *Serratia* spp. 1 (4.2%), *Streptococcus pneumoniae* 2 (8.3%), *Klebsiella* spp. 5 (21%), *Staphylococcus* spp. 4 (16.6%), *Escherichia coli* 4 (16.5%), *Pseudomonas* spp. 2 (8.3%), and *Neisseria meningitidis* 1 (4.2%). The isolation sites in this group were as follows: cerebrospinal fluid in 7 (29.2%) cases, blood in 7 (29.2%) cases, urine in 2 (8.3%) cases, tracheal aspirate in 7 (29.2%) cases, and stool in 1 (4.1%) case.

In the group of children without sepsis, 22 (50.0%) had a microorganism isolated: *Enterococcus* spp. 2 (9.1%), *Salmonella enteritidis* 1 (4.5%), *Streptococcus*

Table 1

Clinico-epidemiologic characteristics of the study groups on admission

Variable	Children with sepsis (n = 36)	Children without sepsis (n = 44)	Significance of difference
Age in months, median (IQR)	15 (1.3–56.0)	9 (1.25–41.0)	Mann-Whitney *U* test = 659.5; 0.200
Gender (m/f), n (%)	13/23 (36.1/63.9)	21/23 (47.7/52.3)	Pearson χ^2 test= 1.093; 0.296
Febrile, n (%)	29 (80.5)	25 (56.8)	Pearson χ^2 test = 5.086; 0.024*
White cells count ($\times 10^9$/L), median (IQR)	15.2 (11.1–18.9)	13.4 (10.8–18.8)	Mann-Whitney *U* test = 752.0; 0.699
Serum creatinine (μmol/L), median (IQR)	43.0 (39.0–48.0)	42.0 (34.0–47.5)	Mann-Whitney *U* test = 651.5; 0.392
CRP (mg/L), median (IQR)	76.6 (9.9–131.0)	17.1 (3.5–67.7)	Mann-Whitney *U* test = 450.5; 0.001*
Procalcitonin (ng/mL), median (IQR)	2.130 (0.144–5.220)	0.261 (0.108–0.615)	Mann-Whitney *U* test = 218.5; 0.001*
sCD14-ST (pg/mL), median (IQR)	259.0 (163.0–535.5)	189.0 (127.0–267.5)	Mann-Whitney *U* test = 498.0; 0.004*
Primary site of bacterial infection and diagnosis at discharge from the hospital, n (%)	Blood, sepsis – 14 (38.9) Cerebrospinal fluid, meningitis – 8 (22.2) Lungs, bacterial bronchopneumonia – 6 (16.7) Urine, pyelonephritis – 2 (5.6) Gut, gastroenterocolitis, bacterial – 6 (16.7)	Viral bronchopneumonia – 10 (22.7) Gastroenterocolitis, viral – 13 (29.6) Omphalitis – 5 (11.4) Cystitis – 4 (9.1) Viral pharyngitis – 6 (13.6) Not found – 6 (13.6)	na

IQR – interquartile range; m – male; f – female; CRP – C-reactive protein; *statistically significant difference; na – not applicable.

pneumoniae 1 (4.5%), *Klebsiella* spp. 1 (4.5%), *Staphylococcus* spp. 5 (22.8%), *Proteus* spp. 2 (9.1%), *Escherichia coli* 6 (27.4%), *Pseudomonas* spp. 2 (9.1%), *Herpes* virus 1 (4.5%) and *Enterobacter* 1 (4.5%). The isolation sites in this group were as follows: umbilical skin in 5 (22.8%) cases, blood in 2 (9.1%) cases, urine in 8 (36.4%) cases, tracheal aspirate in 3 (13.6%) cases, skin in 3 (13.6%) cases, and stool in 1 (4.5%) case.

ROCs for PCT, CRP, and sCD14-ST measured at the admission of the children to the hospital are shown in Figure 1.

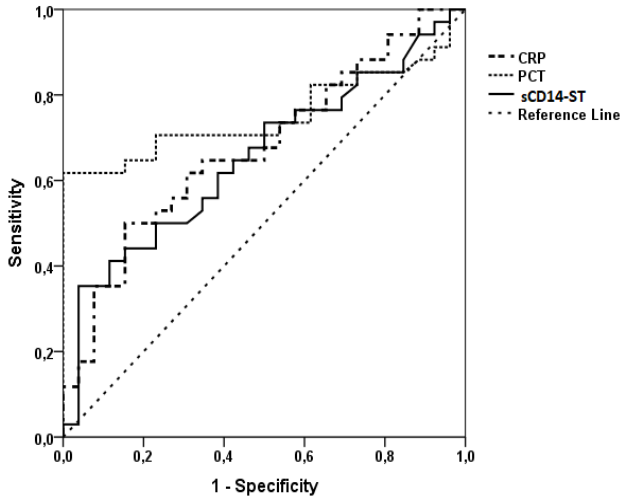


Fig. 1 – Receiver operating characteristic (ROC) curves for procalcitonin (PCT), C-reactive protein (CRP), and soluble CD14 subtype (sCD14-ST) if diagnosing sepsis in children on admission to a hospital.

PCT had the largest AUC (0.753 ± 0.065), followed by CRP (0.716 ± 0.057) and sCD14-ST (0.686 ± 0.061). The sensitivity and specificity of PCT, CRP, and sCD14-ST calculated for cut-off values determined by the Manhattan method are shown in Table 2.

Table 2

Cut-off values, sensitivity and specificity of procalcitonin (PCT), C-reactive protein (CRP), and soluble CD14 subtype (sCD14-ST) for diagnosing sepsis in children on admission to hospital

Parameter	PCT	CRP	SCD14-ST
Cut-off value	1.42 ng/mL	22.1 mg/L	319.5 pg/mL
Sensitivity (%)	61.8	63.9	55.6
Specificity (%)	100	75.0	88.6

Discussion

Our study showed that PCT, CRP, and sCD14-ST had relatively low sensitivity and much higher specificity for diagnosing sepsis in children. Besides, a significant difference in the diagnostic accuracy of these biomarkers was not observed.

When compared with the results of other studies and meta-analyses, values of sensitivity for PCT, CRP, and sCD14-ST in our study were much lower (almost 20%), which could underestimate the diagnostic value of these biomarkers in children with sepsis. However, such results could be explained in one of the following ways: (1) due to the retrospective character of our study, the validity of

diagnosing sepsis, established by the consensus criteria, could not have been checked, and it depended on the performance of the attending physicians; (2) other studies could have overestimated the diagnostic accuracy since many of them included in the control group either healthy children or patients easily differentiated from those who had sepsis [11]. Although several studies confirmed higher diagnostic accuracy of sCD14-ST (comparing area under the ROC curves) than that of CRP and PCT in patients with sepsis [12], our results did not show any significant difference.

Our study has several limitations that could affect the results. First, the age range of our patients was very wide, as we included both newborns and adolescents. Since there are inherent age-related differences in response to infection, cut-off values that we calculated could not have been appropriate completely for both very young and older children.

Conclusion

Our study showed relatively low sensitivity and moderate specificity of PCT, CRP, and sCD14-ST in diagnosing sepsis among children, as well as similar diagnostic accuracy of the three biomarkers. PCT, CRP, and sCD14-ST should not be relied upon completely when assessing the presence of sepsis in children but rather taken into account together with the clinical picture. Further research in this area is necessary, especially on groups of children with a narrower age range (newborns, infants, toddlers, etc.).

Acknowledgement

The authors are grateful to the group of trainees from the educational event held in Sarajevo, November 3rd, 2017, for their active watching while the authors were writing the manuscript.

REFERENCES

1. *Goldstein B, Giroir B, Randolph A.* International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 2005; 6(1): 2–8.
2. *Wu CC, Lan HM, Han ST, Chaou CH, Yeh CF, Liu SH*, et al. Comparison of diagnostic accuracy in sepsis between presepsin, procalcitonin, and C-reactive protein: a systematic review and meta-analysis. Ann Intensive Care 2017; 7(1): 91.
3. *Pontrelli G, De Crescenzo F, Buzzetti R, Jenkner A, Balduzzi S, Calò Carducci F,* et al. Accuracy of serum procalcitonin for the diagnosis of sepsis in neonates and children with systemic inflammatory syndrome: a meta-analysis. BMC Infect Dis 2017; 17(1): 302.
4. *Mkony MF, Mizinduko MM, Massawe A, Matee M.* Management of neonatal sepsis at Muhimbili National Hospital in Dar es Salaam: diagnostic accuracy of C-reactive protein and newborn scale of sepsis and antimicrobial resistance pattern of etiological bacteria. BMC Pediatr 2014; 14: 293.
5. *Poggi C, Bianconi T, Gozzini E, Generoso M, Dani C.* Presepsin for the detection of late-onset sepsis in preterm newborns. Pediatrics 2015; 135(1): 68–75.
6. *Henriquez-Camacho C, Losa J.* Biomarkers for sepsis. Biomed Res Int 2014; 2014: 547818.
7. *Julián-Jiménez A, Gutiérrez-Martín P, Lizcano-Lizcano A, López-Guerrero MA, Barroso-Manso Á, Heredero-Gálvez E.* Usefulness of procalcitonin and C-reactive protein for predicting bacteremia in urinary tract infections in the emergency department. Actas Urol Esp 2015; 39(8): 502–10.
8. *Faul F, Erdfelder E, Lang AG, Buchner A.* G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007; 39(2): 175–91.
9. *Budczies J, Klauschen F, Sinn BV, Győrffy B, Schmitt WD, Darb-Esfahani S,* et al. Cutoff Finder: a comprehensive and straightforward Web application enabling rapid biomarker cutoff optimization. PLoS One 2012; 7(12): e51862.
10. *DeLong ER, DeLong DM, Clarke-Pearson DL.* Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988; 44(3): 837–45.
11. *López Sastre JB, Pérez Solís D, Roqués Serradilla V, Fernández Colomer B, Coto Cotallo GD, Krauel Vidal X,* et al. Grupo de Hospitales Castrillo. Procalcitonin is not sufficiently reliable to be the sole marker of neonatal sepsis of nosocomial origin. BMC Pediatr 2006; 6: 16.
12. *Behnes M, Bertsch T, Lepiorz D, Lang S, Trinkmann F, Brueckmann M,* et al. Diagnostic and prognostic utility of soluble CD 14 subtype (presepsin) for severe sepsis and septic shock during the first week of intensive care treatment. Crit Care 2014; 18(5): 507.

Received on January 20, 2018.
Revised on May 16, 2019.
Accepted May 17, 2019.
Online First May, 2019.

SHORT COMMUNICATION

UDC: 358.4:61]::612.843.1
https://doi.org/10.2298/VSP181113081R

Stereo vision in air force pilots in human centrifuge during +Gz acceleration

Stereo vid kod pilota ratnog vazduhoplovstva na humanoj centrifugi u toku +Gz ubrzanja

Danijela Randjelović*, Tatjana Šarenac Vulović†‡, Nenad Petrović†‡, Sunčica Srećković†‡

***Aero Medical Institute in Zemun, Department of Ophthalmology, Belgrade, Serbia;**
†Clinical Center of Kragujevac, Clinic for Ophthalmology, Kragujevac, Serbia;
‡University of Kragujevac, Faculty of Medical Sciences, Department of Ophthalmology, Kragujevac, Serbia

Abstract

Background/Aim. Stereo vision guarantees good vision and is one of the three main elements of binocular vision, besides simultaneous perception and fusion. It represents the third degree of binocular vision and enables estimation of distance, depth, and space between objects, i.e., allows perception of a three-dimensional image, which is crucial for a pilot. The aim of this study was to investigate the effect of +Gz acceleration on stereo vision in pilots in the air force and student pilots. **Methods.** Two groups of respondents were tested (30 student pilots and 65 air force pilots – a total of 95 respondents). We considered the differences between these two groups as they provide important information about the condition of stereo vision at the beginning of the professional career and after a large number of flight hours over years of flying. We tested variations in stereoscopic vision based on the degree of acceleration of +5Gz by using the Randot Test, which enabled us to determine the degree of three-dimensional vision. **Results.** Temporary changes in stereo vision in student pilots were greater when compared to these changes in air force pilots when exposed to the same acceleration (+5Gz acceleration). The detailed analysis showed that the most sensitive physiological indicators were changes in stereo vision. **Conclusion.** We confirmed that individual physiological pilot training in a human centrifuge, where they are exposed to real G acceleration, improves tolerance to accelerations.

Key words:
aerospace medicine; pilots; acceleration; vision tests; space perception; centrifugation.

Apstrakt

Uvod/Cilj. Stereo vid je garant dobrog vida i jedan je od tri osnovna elementa binokularnog vida pored simultane percepcije i fuzije. Predstavlja treći stepen binokularnog vida i omogućava procenu rastojanja, dubine i razmaka između pojedinih predmeta, tj. omogućava viđenje slike sa tri dimenzije što je od izuzetnog značaja za profesiju pilota. Cilj ove studije bio je da se ispita uticaj +Gz ubrzanja na stereo vid kod pilota borbene avijacije i studenata pilota. **Metode.** Testirane su dve grupe ispitanika (30 studenata pilota i 65 pilota borbene avijacije – ukupno 95 ispitanika). Ispitivali smo razlike između ove dve grupe ispitanika, zbog važnosti informacija o stanju stereo vida na početku profesionalne karijere i nakon višegodišnjih sati letenja. Posmatrali smo dobijene razlike u stereoskopskom vidu na osnovu stepena ubrzanja od +5Gz. U toku našeg istraživanja koristili smo Randot test pomoću koga smo mogli da stepenujemo trodimenzionalnost vida. **Rezultati.** Naši rezultati su pokazali da su prolazne promene stereo vida kod studenata pilota bile veće u odnosu na promene stereo vida kod pilota borbene avijacije, kada su oni bili izloženi ubrzanju istih vrednosti (+5Gz ubrzanju). Na osnovu detaljne analize ustanovljeno je da su najosetljiviji fiziološki pokazatelji bile promene u stereo vidu. **Zaključak.** Potvrdili smo da individualna fiziološka trenaža pilota u humanoj centrifuge, gde su oni izloženi uslovima realnog Gz ubrzanja, poboljšava toleranciju na ubrzanja.

Ključne reči:
medicina, vazduhoplovna; piloti; ubrzanje; vid, ispitivanje; prostor, orijentacija; centrifugovanje.

Correspondence to: Danijela Randjelović, Aero Medical Institute in Zemun, Deparment of Ophthalmology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: danijela_randjelovic@yahoo.com

Introduction

High speeds during take-off, flight, and landing of modern aircraft place an additional strain on the human visual system. Since the very beginnings of the development of aviation, the visual function has been assigned considerable importance. Owing to its considerable practical importance in air combat, the effect of +Gz acceleration on the organ of vision has been a significant variable in research. Such strain leads to changes caused by inertia forces occurring due to changes in acceleration. In aviation, the applied acceleration is usually referred to as G forces [1].

In the course of instrument flight, the pilot almost entirely depends on his/her organ of vision that allows him/her to read the information on the instruments [2, 3]. Having a high level of visual acuity is considered a quality of utmost importance, even today when there are aircraft capable of reaching extraordinarily high speeds and flying at all altitudes [4]. The information gained through our organ of vision is most important in maintaining orientation on the ground and in the air during a flight. In conditions of limited external visibility, spatial orientation may be affected [5]. Central vision is responsible for producing precise information on distance, speed, and depth, and in the course of instrument flying, it allows the pilot to receive information from flight instruments in the cockpit [6]. Stereo vision guarantees good vision and is one of the three main elements of binocular vision, besides simultaneous perception and fusion. It represents the third degree of binocular vision and enables estimation of distance, depth, and space between objects, i.e., allows perception of a three-dimensional image, which is crucial for a pilot [7]. It occurs when objects in front of and behind the fixation point stimulate simultaneously horizontally disparate retinal points. The whole object is perceived as three-dimensional as light falls on slightly different points. In order to achieve this, images of the object being observed must fall on identical spots on the retina, primarily in the foveola. All elements of binocular vision are interdependent and cannot exist separately, except for simultaneous perception. One requirement for the existence and development of binocular vision is appropriate visual acuity. If visual acuity in one eye is the normal 1.0, and if binocular vision is to be developed, visual acuity in the other eye must be minimum of 0.3. Other requirements are that the visual centers of the brain are able to fuse two retina images and that there is precise coordination of movements of both eyes in all directions. Particularly significant is the binocular vision, which is controlled by optomotoric reflexes that are rather complex in nature and develop until the age of five and are solidified until the age of seven (fixation reflex, fusion, movements, accommodation, convergence). The third and highest degree of binocular vision is the stereo vision which represents a person's sense of three-dimensional space. The sense of three-dimensional space is tested by quantitative and qualitative research methods. The simplest qualitative method of testing the sense of three-dimensional space is by synoptophore with pictures for this particular test. Qualitative testing of stereo vision is conducted through a variety of tests: Stereo Fly Test, Randot Stereo Test with polarized specs, Lang Stereo Test Mark 1 and Mark 2, TNO Stereo Test [8–10]. The Randot Test contains a test with polarized circles (stereo-circle test) and is the most differentiated test that enables precise determination of the degree of three-dimensional vision. Ten sections contain four circles each, out of which only one is polarized. The polarized circle is made of two superposition rings, each positioned at a different angle. The greater the angle of polarization, the more visible the third dimension. The greatest angle is the one of 400 seconds (") of arc, and the smallest is of 20" of arc. The Randot Test is in the form of a test booklet [11, 12].

The aim of this study was to investigate the effect of +Gz acceleration on stereo vision in air force pilots and student pilots.

Methods

The research was carried out in the human centrifuge in the Department of Biodynamics at Aero Medical Institute. It was carried out following the instructions given for each test. We tested variations in stereoscopic vision based on the degree of acceleration of +5Gz. Two groups of male respondents were tested, air force pilots and student pilots. We considered the differences between these two groups as they provide important information on the condition of stereo vision at the beginning of the professional career and after a large number of flight hours over years of flying. This would give us more reliable indicators for a better quality selection of candidates, future pilots. In the course of our research, we performed the Randot Test, which enabled us to determine the degree of three-dimensional vision (Figure 1). The test was placed at a distance of 40 cm from the respondents and was carried out binocularly with the respondents wearing polarized viewing glasses. The respondents were asked which of the three circles in the first section seemed to be in front of the other observed circles. The result was read in the special supplement to the test. The test has ten sections, and it is more difficult to notice the difference between circles in each subsequent section, which means that it is also more difficult to notice the third dimension. Stereopsis is quantified in seconds of arc, the test being able to measure

No	SCORING KEY	Seconds of arc at 16 in.
1	L	400
2	R	200
3	L	140
4	M	100
5	R	70
6	M	50
7	L	40
8	R	30
9	M	25
10	R	20

Fig. 1 – Randot test.

stereoscopic sharpness of 20" of arc. Normal stereo sharpness is 60" of arc. If the respondents reach the tenth section without making a single mistake, they score 20", if they make one, they score 25", two 30", and three 40".

Results

Prior to testing, all respondents in both analyzed groups (student pilots and air force pilots) had a normal stereo vision of 20". Upon linear increase in acceleration, a statistically significant difference was noticed in stereo vision between the two observed groups of respondents ($p = 0.000$) (Table 1). In the student group, a statistically higher frequency of respondents with changes in stereo vision was recorded ($p = 0.000$). In the air force pilots group, 92.3% of respondents had unchanged stereo vision, while 7.7% had a stereo vision of 25°. After the test, in the student group, slightly more than half of the respondents, 53.3%, had a normal stereo vision, 23.3% had a stereo vision of 25°, 16.7% of respondents had changed stereo vision of 30°, and 6.7% of 40°. Therefore, statistically significant differences in stereo vision occurred both in the student group ($p = 0.000$), as well as in the group of air force pilots ($p = 0.025$) (Table 1).

Table 1

Stereo vision in student pilots and air force pilots before and after the test of the linear increase acceleration

Stereo vision	Student pilots (n = 30) n (%)	Air force pilots (n = 65) n (%)	Significance*
Before the test			
20"	30 (100)	65 (100)	
After the test			
20"	16 (53.3)	60 (92.3)	
25"	7 (23.3)	5 (7.6)	
30"	5(16.7)	0 (0)	$p = 0.000$
40"	2 (6.7)	0 (0)	
Significance†	$p = 0.000$	$p = 0.025$	

Statistically significant difference: *between student pilots and air force pilots; †before the test vs. after the test within the observed groups of subjects (χ2-test).

Discussion

Average values for stereoscopic vision in student pilots and air force pilots upon exposure to positive G acceleration showed that there is a statistically significant deviation in comparison to stereo vision values prior to being exposed to acceleration force. Interestingly, no changes of this kind have been recorded by other authors. Stereo vision testing has mostly been conducted on motor vehicle drivers [9, 10, 13–15]. Changes in stereo vision observed in our research may affect flight safety and good performance in combat missions.

Good stereo vision, being the highest degree of binocular vision in the pilot population, allows the pilot to see the landscape and all the perceived objects as they are (slope of the terrain, height, depth, flatness of terrain). Therefore, stereo vision is an important visual function that undergoes considerable changes when exposed to positive acceleration. In our research, stereo vision returned to its normal value 30 minutes after being exposed to acceleration.

Conclusion

The obtained results will contribute to the expansion of knowledge necessary for the quality selection of pilots, the most expensive population in any army. It is important to know the limits of tolerance to positive acceleration and find ways to tolerate such acceleration in the best possible way with minimum consequences to the pilot's visual functions while flying modern high-performance fighter aircraft. Temporary changes in stereo vision in student pilots are greater when compared to the changes in the same functions of vision in air force pilots when exposed to the same acceleration (+5Gz acceleration). The detailed analysis showed that the most sensitive physiological indicators were changes in stereo vision. We confirmed that individual physiological pilot training in a human centrifuge, where they are exposed to real G acceleration, improves tolerance to accelerations.

REFERENCES

1. *Pavlović M.* Fundamentals of Aeronautical Medicine. Belgrade: Media centar; 2014; 71–9. (Serbian)
2. *Tsai ML, Horng CT, Liu CC, Shieh P, Hung CL, Lu DW,* et al. Ocular responses and visual performance after emergent acceleration stress. Invest Ophthalmol Vis Sci 2011; 52(12): 8680–5.
3. *Feigl B, Zeie AJ, Stewart IB.* Mild systemic hypoxia and photopic visual filed sensitivity. Ada Ophthalmol 2011; 89(2): 199–200.
4. *Rudnjanin S.* Physiological effects of positive + G acceleration and the ability to increase the tolerance of the organism to the action of acceleration. [graduate thesis]. Belgrade: Military Medical Academy; 1985. (Serbian)
5. *Pavlović M.* Validity of an experimental model of spatial disorientation, moderate hypoxia, and + Gz acceleration in pilot selection. [dissertation]. Belgrade: Military Medical Academy; 2006. (Serbian)

6. *Rudnjanin S, Preboč M, Radojković V*. Flight acceleration effect. Contemporary in ophthalmology. XVIII Ophthalmic Days of the Ophthalmic Section of the Serbian Medical Society; Belgrade; 21–23 April 1986; Belgrade: Srpsko lekarsko društvo; 1986. p. 87. (Serbian)
7. *Čolić J*. Determining visual acuity using different types of optotype. [professional work]. Novi Sad: University of Novi Sad; Faculty of Mathematics, Department of Physics; 2016. (Serbian)
8. Gene review: Red-Green Color Vision Defects. Available from; https:/www.ncbi.nlm.nih.gov/books/NBK1301.
9. *Nedevschi S, Schmidt R, Graf T, Danescu R, Frentiu D, Marita T*, et al. 3D Lane Detection System Based on Stereovision. In: Proceedings IEEE Intelligent Transportation Systems Conference; 2004 October 3–6. Washington, USA: IEEE Intelligent Transportation Systems Conference; 2004; p.161–6.
10. *Nedevschi S, Danescu R, Frentiu D, Marita T, Oniga F, Pocol C,* et al. High Accuracy Stereovision Approach for obstacle Detection on Non-Planar Roads. In: Proceedings off IEEE Intelligent Engineering Systems (INES); Cluj Napoca, Romania; 2004, September 19–21. Cluj Napoca, Romania; IEEE Intelligent Engineering Systems (INES); 2004. p. 211–6.
11. *Savić S*. Ergoophthalmology. Belgrade: Privredno finansijski vodič; 1982. (Serbian)
12. *Čanadanović V*. Motility disorders, sensibility and low vision. In.: *Pajic VP*, editor. Surgery - selected chapters. Novi Sad: Symbol; 2009. p. 3015–8. (Serbian)
13. *Nedevschi S, Schmidt R, Graf T, Danescu R, Frentiu D, Marita T*, et al. High Accuracy Stereo Vision System for Far Distance Obstacic Detection. In: Proceedings of IEEE Intelligent Vehicles Symposium; 2004 June 14–7. Parma, Italy: IEEE Intelligent Vehicles Symposium; 2004; p. 292–7.
14. *Nedevschi S, Danescu R, Frentiu D, Marita T, Oniga F, Pocol C,* et al. Driving Environment Perception Using Stereovision. In: Proceedings of IEEE Intelligent Vehicles Symposium, 2005 June 4. Las Vegas, USA: IEEE Intelligent Vehicles Symposium; p.331–6.
15. *Bertozzi M, Broggi A, Fascioli A., Nichele S*. Stereo Vision-based Vehicle Detection. In: Proceedings IEEE 2000. Intelligent Vehicles Symposium. 2000 October 3–5. Detroit, USA: IEEE Intelligent Vehicles Symposium 2000; p. 39–44.

Received on November 13, 2018.
Revised on June 10, 2019.
Accepted on July 18, 2019.
Online First September, 2019.

CASE REPORTS

UDC: 616-002.7-07:617.731
https://doi.org/10.2298/VSP190223061B

Optic neuritis in a teenage girl with granulomatosis with polyangiitis

Optički neuritis kod tinejdžerke sa granulomatozom sa poliangiitisom

Dejan Bokonjić*, Nada Avram*, Predrag Minić[†‡], Aleksandra Radosavljević[‡§]

University of East Sarajevo, *Faculty of Medicine, Foča, Republic of Srpska, Bosnia and Herzegovina; [†]Mother and Child Health Care Institute of Serbia "Dr. Vukan Čupić", Belgrade, Serbia; University of Belgrade, [‡]Faculty of Medicine, Belgrade, Serbia; Clinical Centre of Serbia, [§]Clinic for Eye Diseases, Belgrade, Serbia

Abstract

Introduction. Granulomatosis with polyangiitis (GPA), formerly known as Wegener's granulomatosis, is characterized by necrotizing granulomatous inflammation in various tissues, including blood vessels, but primarily in the respiratory tract and kidneys. Clinical manifestations can be diverse, including inflammation of the eye and adnexa. Optic neuritis is a very rare ophthalmological manifestation of GPA, not previously described in a teenager. **Case report.** We presented a case of a 16-year-old girl with a rare extrapulmonary manifestation of GPA. The girl had a previous history of GPA and complained of a sudden blurred vision in the left eye. She was promptly referred to an ophthalmologist who noted a decreased visual acuity of 20/400 in the left eye. Colour vision was impaired in the spectrum of red colour. Clinical examination revealed normal anterior segment findings. On ophthalmoscopy, the left optic nerve oedema was noted. Urgent computed tomography of the left orbit showed a soft tissue mass around the optic nerve in the apex of the orbit. Magnetic resonance imaging confirmed the diagnosis of optic perineuritis. After pulse doses of methylprednisolone, the girl achieved complete resolution of vision in the left eye. **Conclusion.** If untreated, inflammation of the optic nerve can lead to a permanent loss of vision. Prompt diagnostic and adequate treatment of patients with GPA is needed in order to prevent vision-threatening complications and control the systemic disease.

Key words:
diagnosis; optic nerve; optic neuritis; treatment outcome; wegener granulomatosis.

Apstrakt

Uvod. Granulomatoza sa poliangiitisom (GPA), ranije poznata kao Wegenerova granulomatoza, karakteriše se nekrotizujućom granulomatoznom inflamacijom u različitim tkivima, uključujući krvne sudove, ali primarno unutar respiratornog trakta i bubrega. Kliničke manifestacije mogu biti raznovrsne uključujući i zapaljenje oka i adneksa. Optički neuritis je veoma retka oftalmološka manifestacija GPA, koja do sada nije opisana kod mladih osoba. **Prikaz bolesnika.** Prikazana je 16-godišnja devojčica sa retkom ekstrapulmonalnom manifestacijom GPA. Bolesnica, ranije lečena zbog GPA, požalila se na iznenadni pad vida na levom oku. Hitno je upućena oftalmologu koji je ustanovio smanjenu vidnu oštrinu levog oka 3/60. Kolorni vid je bio oštećen u spektru crvene boje. Kliničkim pregledom utvrđen je normalan nalaz na prednjem segmentu oka. Pregledom očnog dna uočen je edem vidnog živca levo. Kompjuterizovana tomografija leve orbite otkrila je mekotkivnu masu oko optikusa u vrhu orbite. Magnetnom rezonancom mozga potvrđena je dijagnoza optičkog perineuritisa. Posle pulsnih doza metilprednizolona došlo je do potpunog oporavka funkcije vida levog oka. **Zaključak.** Ukoliko se zapaljenje optikusa ne leči, može dovesti do trajnog gubitka vida. Stoga je neophodno hitno sprovesti kompletnu dijagnostiku i adekvatno lečenje obolelih od GPA, kako bi se sprečile komplikacije koje mogu ugroziti vid i kontrolisala sistemska bolest.

Ključne reči:
dijagnoza; optički nerv, neuritis; lečenje; ishod; vegenerova granulomatoza.

Introduction

Granulomatosis with polyangiitis (GPA) is a systemic inflammatory disease of unknown aetiology. Its main clinical features include the formation of granulomas, vasculitis of the upper and lower respiratory tract, glomerulonephritis, and tissue necrosis [1]. Pathophysiology of granulomas is complex. It includes the destruction of normal tissue, a variable degree of obstruction of small and medium-sized blood vessels, and reduction of the amount of blood that

Correspondence to: Aleksandra Radosavljević, Clinical Centre of Serbia, Clinic for Eye Diseases, Pasterova 2, 11 000 Belgrade, Serbia.
E-mail: alexandra.radosavljevic@gmail.com

reaches different tissues and organs [2]. The disease can be manifested at all ages, but most often around 40 years of age. It is rare in the children population [3, 4]. The incidence of GPA is 3 cases per 100,000 people.

One of the first signs of the disease is inflammation of the airways. Lung nodules usually cause symptoms of pneumonia with rapid breathing, cough, and chest pain. Involvement of the kidneys appears only in a small number of patients at the beginning of the disease, but this number increases as the disease progresses. In addition, other systems can be affected, and there are general signs of the disease, such as weight loss, fatigue, fever, night sweats, and joint pain. Occasionally, GPA can start as fulminant form [3]. Inflammation can affect different parts of the eye and manifest with scleritis, ulcerative keratitis, occlusive retinal periarteritis, and uveitis. Regarding the ocular adnexa, nasolacrimal duct obstruction can occur and lead to dacryocystitis, or inflammation can occur in the orbital tissue in the form of orbital pseudotumor [5, 6].

The presence of at least two of the four criteria mentioned above is sufficient for establishing the diagnosis [7]. Antineutrophil cytoplasmic antibodies (cANCAs) are autoantibodies directed against serine elastase 3. They are quite important in the pathophysiology of the disease [1, 5, 8]. On chest radiographs and computed tomography (CT), infiltrates, nodules, and cavities can be usually found. The golden standard for establishing the diagnosis of GPA is the pathohistological confirmation of necrotizing vasculitis, the presence of large areas of necrosis, or granulomatous inflammation in the skin, kidney, or lung biopsy specimens [1].

The treatment of GPA includes a high dose of corticosteroids for prompt reduction of the inflammation and immunosuppressives, or biologics, in order to achieve long-term remission of the disease [9]. The risk of the disease relapse is very high and almost inevitable without maintenance treatment, but also, despite therapy, a 5-year relapse-free rate is as low as 50% [9].

Case report

We presented a case of a 16-year-old girl who has been complaining of dizziness and blurred vision in her left eye that had started a few days before she went to see a doctor (April 2016). She visited an ophthalmologist for a medical check-up. The ophthalmologist noticed impaired vision and also registered oedema of the optic disc.

The patient was a second child from a third controlled pregnancy, delivered on time, via vaginal delivery, birth body weight 3,100 gr, birth body length: 52 cm, immediately started to cry, not reanimated. She was not allergic, fully vaccinated according to age. She denied inheritable diseases in the family. In her past medical history, the girl was treated under the diagnosis of "granulomatous pulmonary disease" since September 2011, when she underwent left thoracotomy due to the presence of a tumorous mass in her left lung. Biopsy showed granulomatous inflammation in the lung, and a diagnosis of GPA was established. cANCAs were positive. Steroid therapy was introduced with 30 mg of prednisone per day, which was very slowly tapered to 2.5 mg every other day, with good clinical response at the beginning. After one month, she had a relapse of the disease manifested with nodules in the lungs, which disappeared after the dosage of prednisone was raised. After tapering the dose of steroids for the second time to 2.5 mg every other day, at the end of April 2016, the eye symptoms appeared.

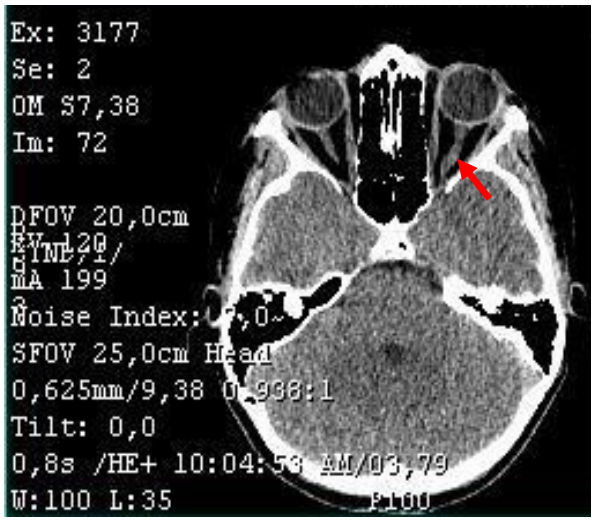
Due to the known underlying disease and the new symptoms reported by the girl, she was admitted to the Pediatric Department for urgent treatment. Upon admission, the 16-year-old girl had body weight 42 kg (50th percentile), body height 146 cm (40th percentile), was conscious, without fever, appeared well hydrated. The pupils symmetrically reacted to light, sclera was white without pathological changes, and conjunctiva was normally vascularised. Her throat was erythematous. During lung auscultation, a normal respiratory sound was noticed, without accompanying whistles and crackles. Cardiac rhythm was normal, tones were clear, murmur was not registered. Respiratory rate was 20 per min, heart rate 88 per min, and blood pressure 100/60 mmHg. The abdomen was neither tender nor distended. The liver and spleen were not palpable. Rough neurological examination showed no abnormalities. Other physical examinations were without pathology. Laboratory findings included: erythrocyte sedimentation rate (ESR), 24/50 mm/h [normal values (nr) 5/15 mm/h]; white blood cells (WBC), 8.9 x 10^9/L (nr: 4.5–11.0 x 10^9); erythrocytes, 4.59 x 10^{12}/L (nr: 4—6.5 x 10^{12}/L); hemoglobin, 12.8 g/dL (nr: 12.0–17.0/g/dL); platelets, 305 x 10^9/L (nr: 150–440 x 10^9/L; serum urea nitrogen, 2.5 mmol/L (nr: 2.8–8.3 mmol/L); serum creatinine (sCr), 46.8 μmol/L (nr: 44–80 μmol/L); asparatate aminotransferase (AST), 34 U/L (nr: 24–49 U/L); alanine aminotransferase (ALT), 32 U/L (nr: 9–20 U/L); alkaline phosphatase (ALP), 91 U/L (nr: 35–105 U/L); blood sugar, 3.2 mmol/L (nr: 4.1–6.1 mmol/L); 24 h diuresis, 2,210 mL; uroproteins, 0.17 g/24 h; clearance of creatinine (ClCr), 153.9 mmol/L/24 h; urine (ClCr = 0.85 x (140 – age in years)/(sCrt) x (body weight/72); C-reactive protein (CRP), 15 mg/L (nr: 0–6 mg/L); cANCAs, 1.6 U/mL (nr: 0–20 U/mL. Urine had a normal appearance.

A complete ophthalmological examination was performed. The best corrected visual acuity (BCVA) was 20/400 in the right and 20/20 in the left eye. In her ophthalmological history, the right eye was amblyopic (previous BCVA was 20/60). The anterior segment of both eyes and the fundus of the right eye had a normal appearance (Figure 1A). Oedema was noted in the left fundus optic disc, with slight elevation, hyperaemia, and unclear boundaries. The funnel of the blood vessels was centrally positioned. Blood vessels had normal calibre. Macula had normal macular reflex (Figure 1B). The initial visual field could not be performed since the patient was treated at the Pediatric Department and was seen by a consultant ophthalmologist.

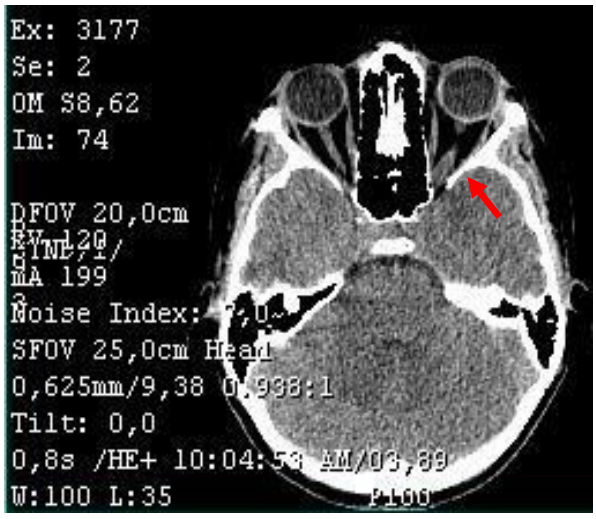
A)

B)

Fig. 1 – Fundus photo of the right (A) and left eye (B) at presentation, showing normal findings in the right and optic nerve oedema in the left eye (arrow).



A)



B)

Fig. 2 – Computed tomography (CT) scan of the brain and orbits shows thickening of the left optic nerve (A) especially in the apex of the orbit (B).

On axial sections and subsequent reconstructions, the CT of the brain and orbits showed that a soft tissue mass was present in the apex of the left orbit, which, according to its size and position, appeared to belong to the thickened and inflamed optic nerve (optic perineuritis). No expansive formations were observed in the right orbit, and the optic nerve was clearly visible. Paranasal sinuses were normally developed and pneumatised, and no abnormal collection or other pathological changes were observed. The nasal septum was centrally positioned. On available sections in the endocranium level, no significant alterations in intensity were observed (Figures 2A and 2B).

Due to unclear delineation of the observed lesion, nuclear magnetic resonance was performed, and previous findings were confirmed.

Ultrasound of the abdomen and radiography of the lungs were also performed to look for the presence of nodules in parenchymal organs. Ultrasound of the abdomen showed that the liver had normal localization, an ordinary shape, a diameter of 108 mm, and a homogeneous echo structure. The gallbladder was without pathological changes. The pancreas had a homogeneous structure and proper size. The right kidney had unchanged localization, normal shape, and size (95 x 43 mm), without urine stoppage and with clear corticomedullary boundary. The left kidney had unchanged localization, normal shape, and size (93 x 47 mm), with a clear corticomedullary boundary. Spleen had normal localization, shape, and size (87 x 33 mm). The free liquid in the abdomen was not noticed. Chest radiography showed no pathological changes in the lung parenchyma.

After the patient was admitted to the hospital, pulse doses of methylprednisolone were introduced along with proton pump inhibitors for protection of the gastric mucosa. Blood pressure and blood glucose levels were regularly monitored, and no major changes were noticed. After three days of pulse therapy, visual acuity started to improve. Therapy with steroids (40 mg/day of oral prednisone) was continued for the next ten days. On medical check-up after 5 (Figures 3A and 3B) and 20 days (Figures 4A and 4B),

A)

B)

Fig. 3 – Fundus photo of the right (A) and left eye (B) after 5 days of treatment, with gradual resolution of optic nerve oedema in the left eye (arrow).

A)

B)

Fig. 4 – Fundus photo of the right (A) and left eye (B) after 20 days of treatment, showing normal findings.

oedema of the left optic nerve gradually and completely resolved, thus steroid therapy was slowly tapered. Finally, when the patient reached the dose of 5 mg of prednisone every other day, she was maintained on this dose in order to prevent further relapses. At the end of the treatment, the vision was completely recovered in the left eye except for minor changes in colour vision. The girl felt well, without any symptoms regarding the respiratory tract or the eyes. Control nuclear magnetic resonance of the brain was performed and confirmed resolution of the lesion (Figure 5). Control visual field showed normal findings in both eyes (Figures 6A and 6B). The patient was followed up for 3 years and had no systemic or ophthalmologic recurrences of GPA.

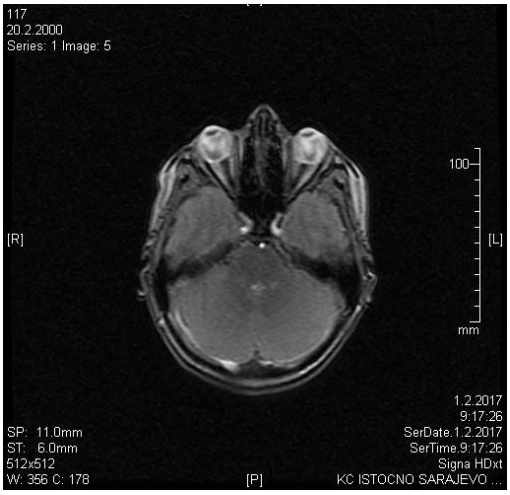
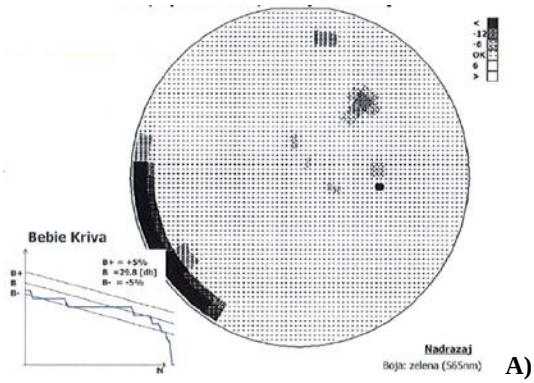
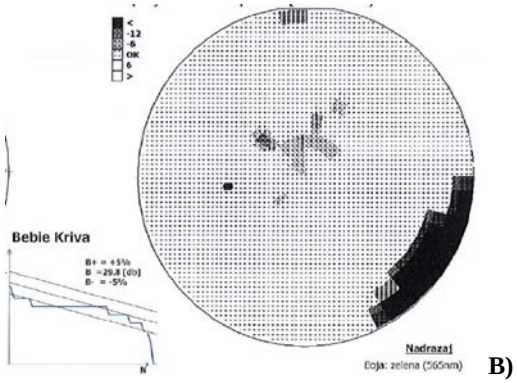


Fig. 5 – Control magnetic resonance imaging of the brain after 18 months of follow-up, shows normal thickness of both optic nerves.



A)



B)

Fig. 6 – Computed visual field (pattern deviation and Bebie curve) of the right (A) and left eye (B) after the treatment, showing normal findings (patient had no fixation errors; 17% false positive errors in the right eye; 5% false positive and 5% false negative errors in the left eye).

Discussion

GPA is one of the ANCA-associated small-vessel vasculitides. It is clinically distinguished from other forms of systemic vasculitides due to the fact that it affects the upper and lower respiratory tracts and kidneys and by the histological presence of granulomatous inflammation. The majority of patients are Caucasian, gender distribution is equal, and the disease usually starts in the fifth decade, but it can occur at any age, including childhood. Differential diagnoses are vast, ranging from infections to other vasculitides, including Henoch-Schönlein purpura, sarcoidosis, and Behcet disease [10]. GPA is characterized by symptoms on the upper and lower respiratory tract and kidneys, as we described. However, unexplained constitutional symptoms like fever and weight loss are very often the initial symptoms of the disease. Ocular manifestations have been reported to occur in 30%–60% of patients with this disease [5, 10]. That is why a complete ophthalmological examination is an important part of the medical check-up in patients suffering from GPA. Any part of the eye may be affected. Keratitis [5, 6], conjunctivitis, scleritis [5, 11], nasolacrimal duct obstruction, uveitis [12], orbital pseudotumor [13], retinal vasculitis [14] and retinal vessel occlusion [5], optic perineuritis (with thickening of the optic nerve in imaging scans) [15–17], or compressive optic neuropathy [18], have all been described. Visual loss has been reported in 8% of patients. CT or nuclear magnetic resonance imaging of the orbit and sinuses may provide important information.

We presented a unique manifestation of GPA-associated optic perineuritis in a teenage patient that was not previously reported at such a young age (all previous reports include only elderly patients in the seventh or eighth decade of life) [15–17]. The patient began therapy with steroids as intravenous pulse therapy and then continued with oral steroid therapy, which was slowly tapered till discontinuance. Our patient responded to treatment with marked improvement in her vision. She tolerated a treatment regimen of 5 mg of prednisone every other day quite well, without new exacerbations, thus there was no need for using immunosuppressive agents.

In our patient, the exacerbations appeared after tapering the dose of steroids below 2.5 mg every other day. It is important to find the minimum dose for keeping the disease under control and prevent exacerbations [14]. Usually, the initial therapy of GPA is daily oral corticosteroid therapy. If that is not enough, pulse dose steroids in combination with immunosuppressives, or even biologic treatment, are needed [9]. It was shown that this treatment has been effective in inducing remission in more than 90% of patients. The mean time to reach remission was 12 months, but in some patients, two years of treatment were necessary before all symptoms have been resolved. Response to treatment is defined as a resolution of the inflammatory manifestations. However, in order to conclude that the patient is a nonresponder to a certain immunosuppressive treatment, at least several months of treatment must pass without any response. Our patient was followed up for three years after treating the optic nerve oedema. The patient is well, without any exacerbations, and she is receiving 5 mg of prednisone every other day.

Conclusion

Visual impairment can occur as a part of underlying systemic disease such as GPA, even in the pediatric population. To our knowledge, this is the first case of optic perineuritis presented in a child. We emphasized the importance of early diagnosis and treatment of the disease, which, if untreated, can lead to permanent loss of vision. A team-work approach and a prompt response are crucial for treating patients suffering from multisystem diseases.

R E F E R E N C E S

1. *Grygiel-Górniak B, Limphaibool N, Perkowska K, Puszczewicz M.* Clinical manifestations of granulomatosis with polyangiitis: key considerations and major features. Postgrad Med 2018; 130(7): 581–96.
2. *Lamprecht P, Kerstein A, Klapa S, Schinke S, Karsten CM, Yu X*, et al. Pathogenetic and clinical aspects of anti-neutrophil cytoplasmic autoantibody-associated vasculitides. Front Immunol 2018; 9: 680.
3. *Jariwala MP, Laxer RM.* Primary vasculitis in childhood: GPA and MPA in childhood. Front Pediatr 2018; 6(8): 226.
4. *Bohm M, Gonzalez Fernandez MI, Ozen S, Pistorio A, Dolezalova P, Brogan P*, et al. Clinical features of childhood granulomatosis with polyangiitis (wegener's granulomatosis). Pediatr Rheumatol Online J 2014; 12: 18.
5. *Tarabishy AB, Schulte M, Papaliodis GN, Hoffman GS*. Wegener's granulomatosis: Clinical manifestations, differential diagnosis, and management of ocular and systemic disease. Surv Ophthalmol 2010; 55(5): 429–44.
6. *Rothschild PR, Pagnoux C, Seror R, Brézin AP, Delair E, Guillevin L.* Ophthalmologic Manifestations of Systemic Necrotizing Vasculitides at Diagnosis: A Retrospective Study of 1286 Patients and Review of the Literature. Semin Arthritis Rheum 2013; 42(5): 507–14.
7. *Leavitt RY, Fauci AS, Bloch DA, Michel BA, Hunder GG, Arend WP,* et al. The American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis. Arthritis Rheum 1990; 33(8): 1101–7.
8. *Thai LH, Charles P, Resche-Rigon M, Desseaux K, Guillevin L.* Are anti-proteinase-3 ANCA a useful marker of granulomatosis with polyangiitis (Wegener's) relapses? Results of a retrospective study on 126 patients. Autoimmun Rev 2014; 13(3): 313–8.
9. *Pagnoux C, Guillevin L.* Treatment of granulomatosis with polyangiitis (Wegener's). Expert Rev Clin Immunol 2015; 11(3): 339–48.
10. *Shafiei K, Luther E, Archie M, Gulick J, Fowler MR.* Wegener granulomatosis: case report and brief literature review. J Am Board Fam Pract 2003; 16(6): 555–9.
11. *Pakrou N, Selva D, Leibovitch I.* Wegener's granulomatosis: ophthalmic manifestations and management. Semin Arthritis Rheum 2006; 35(5): 284–92.

12. *Kubaisi B, Abu Samra K, Foster CS*. Granulomatosis with polyangiitis (Wegener's disease): An updated review of ocular disease manifestations. Intractable Rare Dis Res 2016; 5(2): 61–9.
13. *Ismailova DS, Abramova JV, Novikov PI, Grusha YO*. Clinical features of different orbital manifestations of granulomatosis with polyangiitis. Graefes Arch Clin Exp Ophthalmol 2018; 256(9): 1751–6.
14. *Paović J, Paović P, Vukosavljević M*. Clinical and immunological features of retinal vasculitis in systemic diseases. Vojnosanit Pregl 2009; 66(12): 961–5.
15. *Purvin V, Kawasaki A*. Optic perineuritis secondary to Wegener's granulomatosis. Clin Exp Ophthalmol 2009; 37(7): 712–7.
16. *Takazawa T, Ikeda K, Nagaoka T, Hirayama T, Yamamoto T, Yanagihashi M,* et al. Wegener granulomatosis-associated optic perineuritis. Orbit 2014; 33(1): 13–6.
17. *Shunmugam M, Morley AM, Graham E, D'Cruz D, O'Sullivan E, Malhotra R*. Primary Wegener's granulomatosis of the orbital apex with initial optic nerve infiltration. Orbit 2011; 30(1): 24–6.
18. *Aakalu VK, Ahmad AZ*. Wegener granulomatosis causing compressive optic neuropathy in a child. Ophthalmic Plast Reconstr Surg 2009; 25(4): 327–8.

Received on February 23, 2019.
Revised on May 12, 2019.
Accepted on May 14, 2019.
Online First May, 2019.

CASE REPORT

UDC: 616.61-007.263-089+616.348-007.253-089
https://doi.org/10.2298/VSP181007067P

Secondary renocolic fistula caused by pyonephrosis

Pionefroza kao uzrok sekundarne renokolične fistule

Rade Prelević*†, Boško Milev†‡, Mihajlo Ignjatović†, Mirko Jovanović*, Danilo Prelević‡

Military Medical Academy, *Clinic for Urology, ‡Clinic for General Surgery, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Introduction. Renoalimentary fistulas represent infrequent pathology with 27 literature reports. The oldest report is from the year 1953. Nowadays, they usually arise after the cryoablation of renal tumors. In this case, we reported secondary renocolic fistula as an unusual complication of pyonephrosis, as well as the treatment modality, providing a literature review that favors a conservative or minimally invasive approach in most cases of renocolic fistula. **Case report.** In our case, the patient was a young female with a long course of kidney disease, which eventually led to pyonephrosis with renocolic fistula. Initially, the patient was hospitalized due to life-threatening urosepsis, successfully treated with a conservative approach. Afterward, we decided to proceed with surgical treatment. Regarding the poor right kidney function of the patient and the presence of concurrent sepsis, the right hemicolectomy with primary ileocolic anastomosis and the right nephrectomy were performed. The postoperative course was without complications, and the patient was discharged from the hospital on the 10th day. Follow-up did not reveal any complications. **Conclusion**. Regarding the available literature, a conservative and minimally invasive approach is most frequently employed in such cases. However, in cases of haemorrhage, sepsis, and impaired kidney function, surgery offers the only chance for cure. In patients with concurrent gastrointestinal pathology, surgery is usually the only option. Kidney preservation should be imperative in all cases, except in the case of impaired kidney function. The laparoscopic approach can be utilized in selected cases.

Key words:
fistula; urinary fistula; digestive system fistula; pyonephrosis; sepsis; surgical procedures, operative.

Apstrakt

Uvod. Renoalimentarne fistule predstavljaju retku patologiju sa 27 objavljenih slučajeva u literaturi. Najstariji prikaz datira iz 1953. godine. U današnje vreme najviše slučajeva javlja se nakon krioablacije bubrežnih tumora. Prikazali smo bolesnicu sa sekundarnom renokoličnom fistulom, kao neuobičajenom komplikacijom pionefroze, i tretman izbora u njenom slučaju, uz prikaz literature koja favorizuje neoperativni ili minimalno invazivni pristup lečenja u većini slučajeva. **Prikaz bolesnika**. U našem slučaju, radilo se mlađoj ženskoj osobi sa renokoličnom fistulom koja je imala dugotrajnu primarnu bubrežnu bolest komplikovanu pionefrozom sa razvojem renokolične fistule. Inicijalno, bolesnica je bila hospitalizovana zbog životno ugrožavajuće urosepse, tretirane neoperativno. U daljem toku bolesti odlučili smo se za hiruršku interevenciju. Urađena je desna nefrektomija i desna hemikolektomija sa primarnom ileokoličnom anastomozom. Postoperativni tok protekao je bez komplikacija i bolesnica je nakon 10 dana otpuštena na kućno lečenje. U periodu postoperativnog praćenja nisu uočene komplikacije. **Zaključak.** U dostupnoj literaturi, u tretmanu renoalimentarnih fistula najčešće je korišćen neoperativni i minimalno invazivni modalitet lečenja. U slučajevima krvarenja, sepse, smanjene bubrežne funkcije kao i u slučaju prisustva istovremene gastrointestinalne patologije, hirurgija predstavlja jedinu opciju za izlečenje. Očuvanje bubrega treba da bude imperativ u svim slučajevima, osim u slučajevima sa prisutnom bubrežnom insuficijencijom. U odabranim slučajevima moguć je i laparoskopski pristup.

Ključne reči:
fistula; fistula, urinarna; fistula, digestivni sistem; pionefroza; sepsa; hirurgija, operativne procedure.

Introduction

Renoalimentary fistulas represent pathological communication between parts of the small and large bowel and urinary system, either kidneys or the pyeloureteral tract. They are usually acquired rather than congenital, and they are classified as primary fistulas developed in the case of underlying gastrointestinal and/or urinary disease prone to fistulization (Crohn's dis

Correspondence to: Mihajlo Ignjatović, Military Medical Academy, Clinic for General Surgery, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: mihajloignjatovic@yahoo.com

ease, diverticulosis, ulcer disease, malignancy, and renal tuberculosis) [1–3]. Secondary fistulas arise after invasive diagnostic and therapeutic procedures (colonoscopy, pyeloureterolithotomy, ureterorenoscopy, and percutaneous cryoablation of renal tumors) [1–7]. Signs of renocolic fistula include the following: pneumaturia, fecaluria, haematuria, recurrent acute and chronic urinary tract infections, abscess formation, pyonephrosis, renal atrophy, urinary steal syndrome, and hematochezia [1–7]. A small number of cases are reported in the literature with different approaches to the treatment of this condition. The aim of this case report was to show the clinical course of this rare complication which, in this case, was the consequence of a long-standing primary renal disease. This paper also aimed to show the treatment modality of choice used in this case based on the clinical features that included the presence of sepsis, decreased kidney function, and primary kidney disease.

Methods

We presented a 23-year-old female patient with an ongoing history of kidney diseases, with an onset of the disease in childhood. Initially, the disease presented as vesicoureteral reflux of high grade which led to chronic pyelonephritis and urinary stones formation. Pyeloplasty was performed 17 years ago due to stenosis of pyelon and ureterolithotomy 4 years ago.

The patient was referred to our institution with signs and symptoms of urosepsis, which was successfully treated conservatively. Diagnostic workup included complete blood count (CBC), biochemistry, computed tomography (CT) scan, and upper and lower endoscopy. The upper endoscopy did not reveal any abnormalities.

Colonoscopy revealed oedema of bowel mucosa in the region of hepatic flexure with petechial hemorrhages (Figure 1). The CT scan revealed hypertrophy of the left kidney with atrophy of the right kidney associated with hydronephrosis grade III/IV. Urinoma in the proximity of the kidney was also present with the passage of contrast to the right colon, which was highly suspicious of the presence of a renocolic fistula (Figure 2). Regarding the course of the disease, previous operations, poor right kidney function with concomitant pyonephrosis, *en bloc* resection of the right kidney and the right colon were performed with primary ileocolic anastomosis. Intraoperative exploration revealed the presence of a fistula, which communicated with the renal excretory system and the right colon (Figures 3 and 4).

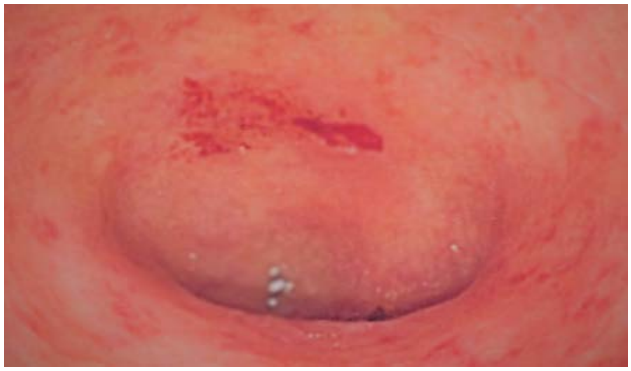


Fig. 1 – Colonoscopy revealed oedema of bowel mucosa in the region of hepatic flexure with petechial hemorrhages.

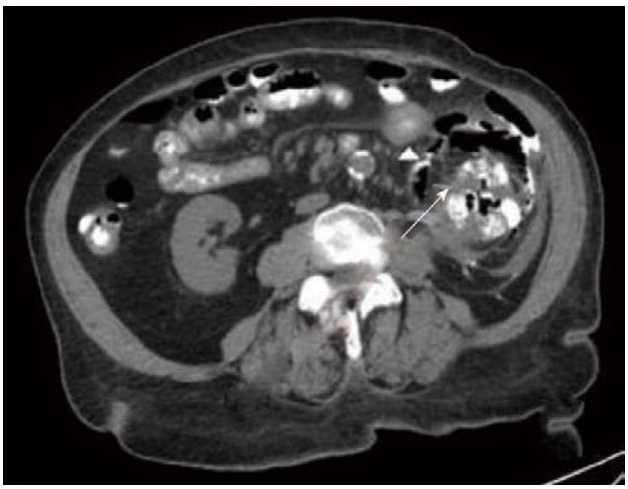


Fig. 2 – Computed tomography scan of the urinoma in the proximity of the right kidney.

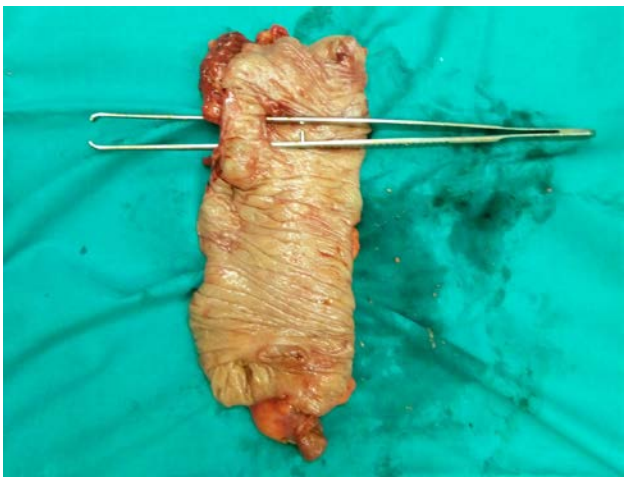


Fig. 3 – Intraoperative exploration – presence of fistula with renal excretory system and right colon.

Fig. 4 – Nephrectomy and resected colon specimen.

The postoperative course was without complications, and the patient was discharged after nine days. Three months of follow-up did not reveal any abnormalities. The histopathological examination revealed renal atrophy, chronic pyelonephritis, perinephritis (Figures 5 and 6), and inflammation of the colonic wall in the area of the fistulous tract.

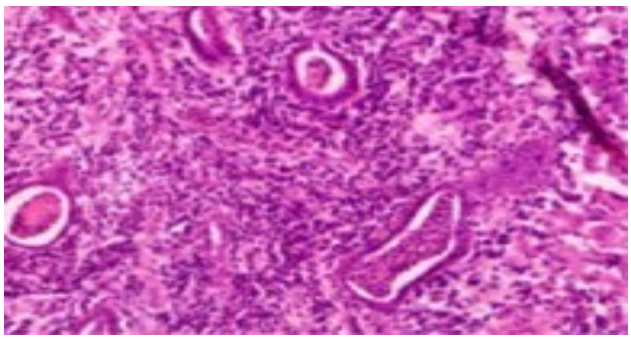


Fig. 5 – Postoperative finding: renal atrophy, chronic pyelonephritis and perinephritis (haematoxylin-eosin, ×10).

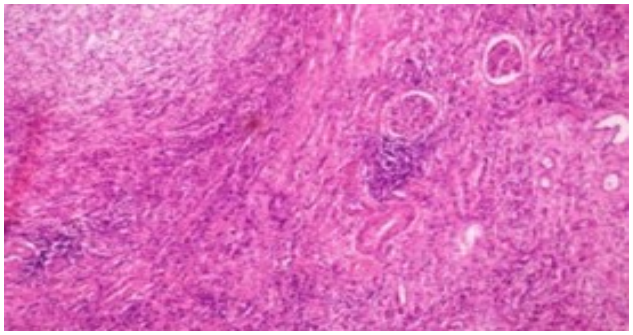


Fig. 6 – Renal atrophy and inflammation of colonic wall around the fistula (haematoxylin-eosin, ×10).

Discussion

There are only 27 papers reported in the Pubmed regarding the problem of renocolic fistulas. The oldest report is from the year 1953.

In our case, the initial presentation was not suggestive of the presence of renocolic fistula regarding the long-standing obstructive renal disease, which led to chronic pyelonephritis and pyonephrosis. Colonoscopy was inconclusive with unspecific inflammatory changes on bowel mucosa. Only contrast CT study revealed urinoma as an indirect sign of communication with the renal excretory tract. Passage of contrast from renal excretory system to the right colon was diagnostic of the presence of renocolic fistula. The surgical procedure was selected based on the following criteria: impaired kidney function, previous episode of urosepsis, and chronically inflamed thickened colonic wall around fistula orifice due to the present perinephritis (as revealed on histopathological examination), which mandated an extensive surgery with nephrectomy and right hemicolectomy. The colonic suture, in this case, was a procedure associated with a high risk of suture dehiscence due to the presence of active inflammation in the inflammatory conglomerate formed between the right kidney and colon.

A similar approach was employed in the case of a 42-year-old female, although a segmental colonic resection with colocolic anastomosis was utilized rather than the right hemicolectomy with ileocolic anastomosis [8]. In the paper of Jallouli et al. [9], severe pyelonephritis with sepsis was initially diagnosed in a 58-year-old female patient. Diagnostic examinations confirmed the existence of a renocolic fistula, thus a nephrectomy with colon suture was performed. The histopathological examination revealed renal tuberculosis, which mandated a long postoperative course of antituberculosis medications. Although these two cases utilized an aggressive surgical approach, two main treatment approaches of reno-alimentary fistulas were described in the literature. The conservative approach includes a nasogastric tube, bowel rest, and total parenteral nutrition with or without ureteral stenting in the case of the fistula with pyeloureteral tract [5, 6]. In the paper of Schmit et al. [10], successful treatment of fistula was performed by CT-guided plugging of the fistulous tract with the clip being placed endoscopically over the fistula orifice. The operative approach requires laparotomy or laparoscopy with resection of affected bowel with or without nephrectomy and gastrointestinal tract reconstruction [11, 12]. In the treatment of renocolic fistulas, four main considerations should be employed in the decision-making algorithm: concurrent gastrointestinal pathology, assessment of kidney function preoperatively, communication of the bowel with the kidney or pyeloureteric tract, and the presence of systemic symptoms. In the case of the fistula with pyeloureteral tract, even in the case of conservative treatment selection, ureteral stenting should be considered in order to close the ureteral fistula orifice, decrease intraluminal pressure, and allow urine outflow in physiologic direction. The presence of impending sepsis, which does not respond to conservative treatment or gastrointestinal bleeding with hemodynamic instability, mandates resection of the affected bowel with or without gastrointestinal reconstruction. In the case of fistulas with duodenum exclusion or resection, procedures should be performed depending on the duodenal segment that is affected. Primary renocolic fistula with concurrent gastrointestinal pathology mandates bowel resection depending on the primary disease behavior (inflammatory bowel disease, diverticulosis, or cancer). Kidney preservation should be imperative in all cases where kidney function is unaffected except in the case where, intraoperatively, the kidney is identified as the source of life-threatening bleeding, in which case partial or total nephrectomy should be considered. Ashfaq et al. [12] performed segmental colectomy laparoscopically with kidney sparring and omentoplasty. If kidney function was severely impaired, concomitant total nephrectomy would be a procedure of choice in all cases.

Conclusion

We reported a case of renocolic fistula that initially presented with urosepsis as a complication of long-standing kidney disease associated with pyonephrosis and severe impairment of renal function, which required an aggressive surgical approach. In our experience, a CT contrast study revealed the presence of a fistula. Regarding the rarity of this complication, a high index of suspicion is necessary for diagnosis since different therapeutic approaches can be utilized. It seems that contrast studies of the gastrointestinal and urinary system (e.g. barium enema and pyelography) are procedures that offer a better chance for definitive preoperative diagnosis.

REFERENCES

1. *Kamani F, Hessami R, Abrishami A.* Benign duodenocolic fistula as a complication of peptic ulcer disease. Gastroenterol Hepatol Bed Bench 2015; 7(1): 72–5.
2. *Kornfield HJ, Hogan RA.* Duodenocolic fistula. Calif Med 1971; 115(5): 58–61.
3. *Le Guillou M, Perron J, Kuss R.* Post-traumatic pyelo-jejunal fistula with urinary steal syndrome. J Urol Nephrol (Paris) 1974; 80(3): 320–22. (French)
4. *Chung SD, Sun HD, Hung SF, Chiu B, Chen Y, Wu JM.* Renal stone-associated squamous cell carcinoma and pyelo-colo-duodenal fistula. Urology 2008; 72(5): 1013.
5. *Vanderbrink BA, Rastinehad A, Caplin D, Ost MC, Lobko I, Lee BR.* Successful conservative management of colorenal fistula after percutaneous cryoablation of renal-cell carcinoma. J Endourol 2007; 21(7): 726–9.
6. *Morgan AI, Doble A, Davies RJ.* Successful conservative management of a colorenal fistula complicating percutaneous cryoablation of renal tumors: a case report. J Med Case Rep 2012; 6: 365.
7. *Bissada NK, Cole AT, Fried FA.* Reno-alimentary fistula: an unusual urological problem. J Urol 1973; 110(3): 273–6
8. *Parasher R, Sasidharan K.* Spontaneous reno-colic fistula. Indian J Urol 2000; 17: 64–5.
9. *Jallouli W, Sellami A, Chaker K, Zehani A, Essid MA, Ben Chehida MA*, et al Reno-colic fistula in a tuberculous kidney: About a case report. Urol Case Rep 2018; 20: 5–6.
10. *Schmit GD, Thompson RH, Buttar NS.* Colorenal fistula repair using a combined percutaneous CT-guided and endoscopic approach. J Vasc Interv Radiol 2016; 27(6): 896–7.
11. *Campobasso D, Granelli P, Maestroni U, Cerasi D, Ferretti S, Cortellini P.* Are Nephroenteric Fistulas Only a Surgical Trouble? Indian J Surg 2015; 77(3): 222–5.
12. *Ashfaq A, Ferrigni R, Mishra N.* Laparoscopic approach to colorenal fistula with renal preservation and omentoplasty: A case report. Int J Surg Case Rep 2017; 35: 53–6.

Received on November 7, 2018.
Revised on May 5, 2019.
Accepted May 5, 2019.
Online First June, 2019.

CASE REPORT

UDC: 616.24-022:578.834]:616.25-003.219
https://doi.org/10.2298/VSP200604142N

Pneumothorax in a patient with pneumonia caused by SARS-CoV-2: A case report

Pneumotoraks kod bolesnice sa pneumonijom izazvanom SARS-CoV-2

Ljiljana Novković*†, Ivan Čekerevac*†

University of Kragujevac, Faculty of Medical Sciences, *Department of Internal Medicine, Kragujevac, Serbia; Clinical Center of Kragujevac, †Clinic for Pulmonology, Kragujevac, Serbia

Abstract

Introduction. The coronavirus disease 2019 (COVID-19) is an acute infectious multisystem disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), manifested by acute respiratory symptoms. The novel coronavirus pneumonia (NCP) is the most common serious clinical manifestation of SARS-CoV-2 infection. In the severe NCP, the systemic manifestations of the disease were also demonstrated, and one of the rare complications, first described in Wuhan (China), is pneumothorax. **Case report.** A 65-year-old female was admitted to the Clinic for Pulmonology with a high fever, shortness of breath, sore throat, and general weakness that started five days before. Laboratory findings revealed lymphopenia, elevated values of inflammatory markers, and liver lesion. A chest X-ray (CXR) demonstrated diffusely accentuated interstitial pattern and reduced parenchymal transparency, left perihilar. Positive SARS-CoV-2 in a nasopharyngeal swab sample was detected in the real-time reverse transcription-polymerase chain reaction (RT-PCR), confirming the diagnosis of NCP. Immediately, nasal oxygen therapy with a flow rate of 8 L/min, with chloroquine phosphate, antibiotics, and symptomatic treatment, was initiated. On the 8th day, her condition suddenly deteriorated, and she developed severe hypoxemia. A repeated CXR showed complete left-sided pneumothorax. Thoracic drainage was successfully performed with complete reexpansion of the lungs the very next day. The patient was released from the hospital in good general condition with normal arterial blood gases. **Conclusion.** Pneumothorax may develop as a complication in patients with pneumonia caused by SARS-CoV-2, without previous pulmonary comorbidities, due to alveolar damage. Acute deterioration with rapid oxygen desaturation in these patients should raise the suspicion of pneumothorax. Early diagnosis and prompt treatment are necessary to reduce mortality.

Key words:
covid-19; pneumonia; pneumothorax; polymerase chain reaction; radiography.

Apstrakt

Uvod. Koronavirusna bolest 2019 (COVID-19) je akutna, infektivna multisistemska bolest koja se najčešće manifestuje akutnim respiratornim simptomima. Izaziva je *severe acute respiratory syndrome coronavirus* 2 (SARS-CoV-2). Nova koronavirusna pneumonija (NCP) je najčešća ozbiljna klinička manifestacija SARS-CoV-2 infekcije. U teškoj NCP ispoljene su i sistemske manifestacije bolesti, a jedna od retkih komplikacija, prvi put opisana u Vuhanu (Kina), je pneumotoraks. **Prikaz bolesnika.** Bolesnica stara 65 godina primljena je u Kliniku za pulmologiju zbog febrilnosti, otežanog disanja, gušobolje i opšte malaksalosti koje je imala prethodnih 5 dana. Laboratorijskim ispitivanjem otkriveni su limfopenija, povišene vrednosti parametara zapaljenja i lezija jetre. Radiografijom (RDG) grudnog koša utvrđeno je difuzno naglašen intersticijum i smanjena transparencija parenhima levo perihilarno. Prisustvo SARS-CoV-2 u uzorku nazofaringealnog brisa otkriveno je lančanom reakcijom polimeraze (PCR), čime je potvrđena dijagnoza NCP. Odmah je započeta terapija kiseonikom preko nazalne kanile protoka 8 L/min, uz hlorokin fosfat, antibiotike i simptomatsku terapiju. Osmog dana, stanje bolesnice se naglo pogoršalo i razvila je tešku hipoksemiju. Ponovljenom RDG grudnog koša potvrđen je kompletan pneumotoraks levo. Torakalna drenaža je uspešno izvedena uz potpunu reekspanziju pluća već sledećeg dana. Bolesnica je otpuštena iz bolnice u dobrom opštem stanju, sa normalnim gasovima arterijske krvi. **Zaključak.** Usled oštećenja alveola, pneumotoraks kao komplikacija pneumonije izazvane SARS-CoV-2, može nastati bez prethodnih plućnih oboljenja. Akutno pogoršanje sa naglom desaturacijom kiseonikom kod tih bolesnika trebalo bi da pobudi sumnju na pneumotoraks. Rana dijagnoza i brzo lečenje su neophodni za smanjenje smrtnosti.

Ključne reči:
covid-19; pneumonija; pneumotoraks; polimeraza, reakcija stvaranja lanaca; radiografija.

Correspondence to: Ljiljana Novković, University of Kragujevac, Faculty of Medical Sciences, Department of Internal Medicine, Svetozara Markovica 69, 34 000 Kragujevac, Serbia. E-mail: ljiljanan1@hotmail.com

Introduction

The coronavirus disease 2019 (COVID-19) is an acute infectious multisystem disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), primarily affecting the respiratory tract. The disease was first detected in December 2019 in Wuhan, China. It spread rapidly throughout the world, and hence the World Health Organization (WHO) declared a pandemic on the 11th of March 2020 [1].

Clinical presentations of SARS-CoV-2 infection have a broad spectrum that can range from asymptomatic forms to critical manifestations of the disease. Asymptomatic persons seem to account for approximately 40% to 45% of the SARS-CoV-2 infection [2]. Even though the majority of cases result in mild symptoms of a typical viral infection, up to 5% of the cases can develop critical illness and multiorgan failure [3]. Pneumonia is the most common serious clinical manifestation of the SARS-CoV-2 infection. It has been identified as novel coronavirus pneumonia (NCP).

As the COVID-19 pandemic progresses, over the past few months, awareness and knowledge of unusual disease presentations, such as pneumothorax, have increased. Pneumothorax is a known and well-described complication of mechanical ventilation (MV) when it supports the COVID-19 treatment and is attributed to barotrauma [4]. Additionally, patients with COVID-19 are often treated with noninvasive ventilation (NIV) or oxygen *via* high-flow nasal cannula (HFNC) for respiratory support. The applied positive pressure can facilitate the development of pneumothorax.

However, recent reports suggest that pneumothorax can be present in the context of COVID-19, even in the absence of MV-related and NIV-related barotrauma [5–10].

We presented a case of a patient with pneumonia caused by SARS-CoV-2 who developed spontaneous pneumothorax as a rare complication.

Case report

A 65-year-old female with a past medical history of hypertension and regulated hyperthyroidism was admitted to the Clinic for Pulmonology, Clinical Center of Kragujevac, Kragujevac, Serbia with a high fever, shortness of breath, sore throat, and general weakness that started five days before. The patient had never smoked, and she denied previous pulmonary diseases. Upon admission, her general condition was poor - she was dyspnoic, adynamic, and dehydrated. Her vital signs showed tachypnea (31 breaths/min), with high temperature (38.5ºC), increased heart rate (123 beats/min), and arterial blood pressure reading of 110/70 mmHg. The initial oxygen saturation (SpO2) was 89%, normal values > 95% on room air and 95% with a binasal cannula, 8 lit/min of O_2. Chest examination revealed basal crackles on the left side. Other systemic examinations were orderly.

Laboratory analysis showed white blood cell (WBC) count 13.63 × 10^9/L [normal range (nr) 3.7 × 10^9/L]. The WBC differential count showed 82.84% neutrophils (nr 44%–72%) and lymphopenia of 8.43% (nr 20%–46%). Initial laboratory tests were significant for elevated C-reactive protein (CRP) of 57.6 mg/L (nr 0–5 mg/L), aspartate aminotransferase (AST) of 62 IU/L (nr 0–40 U/L), alanine aminotransferase (ALT) of 51 IU/L (nr 0–40 U/L), lactate dehydrogenase (LDH) of 855 U/L (nr 220–450 U/L), D-dimer of 1.84 μg/mL (nr < 0.50 μg/mL), and ferritin of 609 μg/L (nr 20–300 μg/mL). A chest X-ray (CXR) on admission demonstrated accentuated interstitial pattern bilaterally, linear-banded perihilar shadows, and reduced left perihilar transparency (Figure 1A).

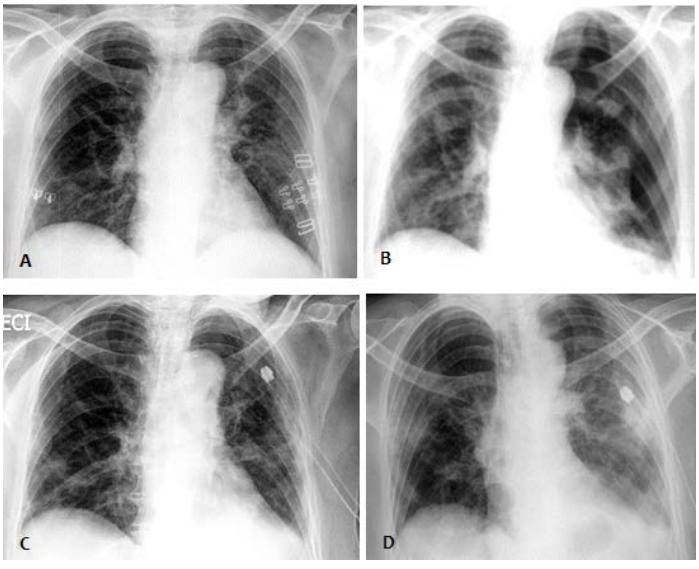


Fig. 1 – Chest X-ray: A) Chest X-ray on admission showing a diffusely accentuated interstitial pattern, linear-banded shadows perihilar and reduced parenchymal transparency left perihilar; B) Chest X-ray on the 8th day of hospitalization showing complete left-sided pneumothorax; C) Chest X-ray showing complete reexpansion of the lung parenchyma on the left side; D) Chest X-ray showing diffusely reduced parenchymal transparency left and consolidation right infraclavicular.

The nasopharynx swab, real-time reverse transcription-polymerase chain reaction (RT-PCR) test for SARS-CoV-2, was positive two days after admission.

The patient was labeled as moderate NCP. She started with the treatment for the SARS-CoV-2 caused pneumonia, guided by the valid local protocol in our country at the moment: chloroquine phosphate, parenteral antibiotics (ceftriaxone and azithromycin), supplemental oxygen with a nasal cannula, vitamins and symptomatic therapy, with a prophylactic dose of low molecular weight heparins (LMWH) in order to prevent venous thromboembolism.

The patient felt subjectively better and hemodynamically stable. For a week, she remained on the 4 L/min oxygen *via* nasal cannula, maintaining an oxygen saturation of 96%. No significant changes in AST and ALT values were observed in control laboratory tests (AST: 55 IU/L, ALT: 68 IU/L). In the electrocardiographic finding, sinus rhythm persisted without extrasystoles and changes in the final oscillation. The value of the QTC interval was 423 ms, normal values < 470 ms.

On day 8, her condition suddenly deteriorated. The patient complained of intense shortness of breath, accompanied by an irritating dry cough and developed O_2 desaturation of 78%. Gas analysis showed recorded severe hypoxemia [arterial pressure oxygen – pO_2 = 6.0 kPa, normal values > 10.6 kPa] and mild hypocapnia [pCO_2 = 4.4 kPa]. The patient required 15 L/min of oxygen *via* a face mask and was transferred to the Iintensive Care Unit. A repeated CXR showed complete left-sided pneumothorax (Figure 1B). The emergency intervention by a thoracic surgeon was undertaken, a chest drain was inserted, and the patient's oxygen saturation improved. The next day, control CXR showed complete reexpansion of the lung parenchyma on the left side (Figure 1C). At no point during her stay, did she require the use of NIV or oxygen *via* HFNC. Her oxygen requirements decreased over the next 2 days, and she was transferred to the medical ward with a binasal cannula, 3 L/min of O_2.

However, her condition continued to deteriorate. The patient became febrile and dyspnoic with O_2 desaturation. Laboratory results showed an increased value of D-dimer (3.47 μg/mL) and raised inflammatory markers, CRP: 185 mg/L, procalcitonin: 2.35 ng/mL (normal values < 0.05 ng/mL) and ferritin: 998 μg/L, whereas CXR registered diffusely reduced left parenchymal transparency and consolidation right infraclavicular (Figure 1D).

There was clinical suspicion of bacterial superinfection but also a dilemma about the possible severe SARS-CoV-2 pneumonia. At that time, chest computed tomography (CT) was not available. The patient responded well to parenteral antibiotics (meropenem and vancomycin), a therapeutic dose of LMWH, glucocorticoid (methylprendisolone 40 mg *iv*) with O_2 supplementation for seven days. The drain was removed a week after administration. The patient was discharged from the hospital in a good general condition on day 21. CXR showed marked radiological regression of the described changes (Figure 2). Arterial blood gas analysis without oxygen therapy showed normal values at discharge.

The patient felt well in the following period. She was monitored by a thoracic surgeon, who described a normal CXR. Four months later, when the epidemiological situation allowed, a chest CT scan was performed, which described ground-glass opacity bilaterally in the lower lobes with elements of interstitial fibrosis and thickening of the parietal pleura in the left upper lobe (Figure 3).

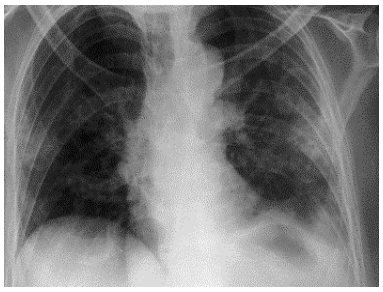


Fig. 2 – Chest X-ray on discharge from the hospital showing radiological regression of the previous described changes (in Figure 1).

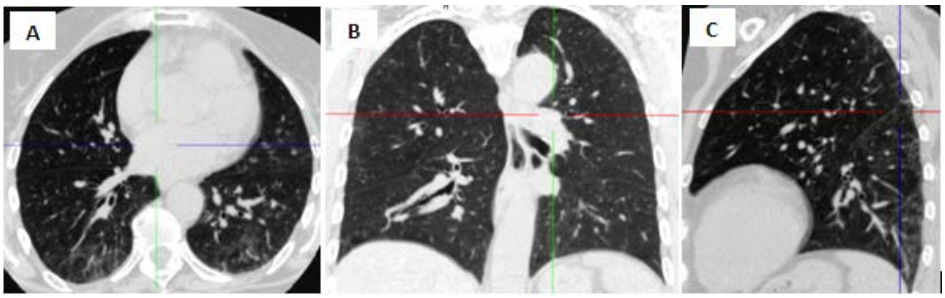


Fig. 3 – Chest computed tomography (CT) scans: A) axial, B) coronal, and C) sagital plane showing ground-glass opacity with elements of interstitial fibrosis bilaterally in the lower lobes and thickening of the parietal pleura in the left upper lobe.

For our patient, pulmonary function testing (spirometry, diffusion capacity for CO), control chest CT, and further monitoring are planned. There are currently no recommendations for the use of glucocorticoids in such patients.

Discussion

The severity of COVID-19 is variable, from mild to critical disease. The most common symptoms of SARS-CoV-2 infection, widely characterized in large-scale studies, include fever, cough, and shortness of breath. NCP is the most common serious clinical manifestation of SARS-CoV-2 infection [11]. Patients with severe NCP usually present with dyspnea (respiratory rate > 30 breaths/min) and/or hypoxemia (SpO_2 < 90% on room air) with bilateral infiltrates present on chest imaging. In very severe cases, the disease can progress rapidly and become complicated by acute respiratory distress syndrome (ARDS) and coagulopathies [11]. To date, it is recommended that the definitive diagnosis of SARS-CoV-2 infection be confirmed by a positive RT-PCR test or genetic sequencing [12].

Pneumothorax is an uncommon and rare finding in patients with NCP, with a frequency of 1% according to the current literature [13].

Pneumothorax is a clinical entity defined as the presence of air in the pleural space [14]. It can occur spontaneously or following a trauma. Spontaneous pneumothorax, being the most common type, can be primary or secondary, depending on the absence or presence of an underlying lung disease [14].

The well-known risk factors for the development of spontaneous pneumothorax include the following: male gender, tobacco use, tall stature, age-group from 10–30 years, and strenuous exercise. Additionally, the most frequent underlying disorders responsible for secondary spontaneous pneumothorax include chronic obstructive pulmonary disease (COPD) with emphysema, interstitial lung disease, tuberculosis, and lung cancer or *Pneumocystis carinii* pneumonia [14].

Pneumothorax is a potential complication usually associated with cystic lung formation due to rupture of the lung tissue.

Liu et al. [15] reported that COVID-19 may independently result in pulmonary cyst formations and the development of pneumothorax. SARS-CoV-2 infected alveolar units tend to be peripheral and subpleural, which is confirmed by radiological findings of COVID-19 in the peripheral lung parenchyma. This tropism of SARS-CoV-2 may increase the risk of peripheral cystic formation facilitating its rupture into the pleural cavity and the development of pneumothorax.

The pathophysiology mechanism of pneumothorax formation in patients with NCP is not completely understood. However, differences between the early and late stages of the disease are indicated.

It is supposed that the complication of pneumothorax occurs secondary due to diffuse alveolar damage from the inflammation caused by a viral infection. The histology, an early phase of NCP, mainly shows the migration of neutrophils, monocytes and macrophages, vascular congestion, mucus-like exudation in the alveoli, edema in the alveolar septum, and microthrombosis. Due to the destruction of the alveolar septa and a sudden increase of alveolar pressure, the alveoli may be prone to rupturing and forming pulmonary cystic lesions [15].

At this stage, the direct cytopathogenic effect of SARS-CoV-2 on type II cells also suggests a possible pathogenetic mechanism. SARS-CoV-2 propagates within type II pneumocytes, a large number of viral particles are released, the cells undergo apoptosis and die [16].

The late stages of NCP determine ischemic parenchymal damage, activation of fibroblasts, lung fibrosis, low lung compliance, and inflammatory fibromyxoid exudates into alveoli and airway. Pulmonary cystic lesions may be formed in response to fibromyxoid exudates, which form a valve in the bronchus. Moreover, due to pulmonary fibrous processes, bronchioles are narrow and distorted, and the valve mechanism could cause pulmonary cystic formation [15].

Pneumothorax, associated with subcutaneous and mediastinal emphysema, is a well-described complication of mechanical ventilation in patients with critical SARS-CoV-2 pneumonia [17]. However, pneumothorax may also develop as a complication of NIV. The use of NIV, or the application of oxygen *via* HFNC, in conditions of continuous and excessive positive airways pressure delivery can lead to an increase of intra-alveolar pressure, rupture of the alveoli, and formation of cyst lesions [15].

In addition, applied positive pressure may facilitate rupture of subpleural cysts and the development of pneumothorax.

Our patient had no predisposing risk factors, no history of previous pulmonary diseases, was a nonsmoker, and of normal body weight. Initial CXR showed no abnormalities in terms of emphysema or bullae. She did not receive NIV nor oxygenation via HFNC for respiratory support. She developed pneumothorax on the eighth day of hospitalization, in an early phase of NCP.

The literature describes patients who developed pneumothorax at different stages of the disease course. Al-Shokri et al. [18] reported three cases of SARS-CoV-2 infection complicated by pneumothorax. The first, second, and third patient developed pneumothorax on days 2, 7, and 15, respectively. Aydin et al. [5] and Chen et al. [13] reported pneumothorax as an initial manifestation in a patient with NCP.

Our case supports the opinion that pneumothorax may develop in pneumonia caused by SARS-CoV-2 due to advanced alveolar damage, rupture of the alveoli, and the formation of pulmonary cystic lesions. The increase in intrapulmonary pressure during a severe cough attack associated with viral infections can lead to cyst rupture and secondary pneumothorax.

Our case is consistent with the one in the recently published article by Sun et al. [9]. As detailed by the authors, pneumothorax could be a consequence of a sudden increase of the alveolar pressure into the pneumonic consolidations.

A recent review of the study by Alhakeem et al. [19] showed 18 case reports describing COVID-19 patients with spontaneous pneumothorax. Only three cases included fe-

males. In addition, only four cases were smokers, and three had underlying lung disease. Ten of these patients underwent chest tube insertion. Three cases were on invasive mechanical ventilation. Twelve patients had a favorable clinical course. The mortality rate was 33%.

In the literature, the diagnostic value of CXR is relatively low, 30%–60% in NCP. Despite its potential limits, some of the complications of NCP can be diagnosed with repeated CXR, as seen in the example of our patient.

Conclusion

Pneumothorax may develop as a complication in patients with SARS-CoV-2 pneumonia, without previous pulmonary comorbidities and ventilator (MV and NIV) respiratory support, due to alveolar damage. Acute deterioration with rapid oxygen desaturation in these patients should raise the suspicion of pneumothorax. Early diagnosis and prompt treatment are necessary to reduce mortality.

R E F E R E N C E S

1. *World Health Organization* WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. Geneva: World Health Organization, 2020. Available from: (https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19--11-march-2020). [accessed 2020 March 29].
2. *Oran DP, Topol EJ.* Prevalence of Asymptomatic SARS-CoV-2 Infection: A Narrative Review. Ann Intern Med 2020; 173(5): 362–7.
3. *Wu Z, McGoogan JM.* Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA 2020; 323(13): 1239–42.
4. *Yao W, Wang T, Jiang B, Gao F, Wang L, Zheng H,* et al. Emergency tracheal intubation in 202 patients with COVID-19 in Wuhan, China: lessons learnt and international expert recommendations. Br J Anaesth 2020; 125(1): e28–e37.
5. *Aydin S, Öz G, Dumanli A, Balci A, Gencer A.* A Case of Spontaneous Pneumothorax in Covid-19 Pneumonia. J Surg Res 2020; 3(2): 96–101.
6. *Mallick T, Dinesh A, Engdahl R, Sabado M.* COVID-19 Complicated by Spontaneous Pneumothorax. Cureus 2020; 12(7): e9104.
7. *Ucpinar BA, Sahin C, Yanc U.* Spontaneous pneumothorax and subcutaneous emphysema in COVID-19 patient: Case report. J Infect Public Health 2020; 13(6): 887–9.
8. *González-Pacheco H, Gopar-Nieto R, Jiménez-Rodríguez GM, Manzur-Sandoval D, Sandoval J, Arias-Mendoza A.* Bilateral spontaneous pneumothorax in SARS-CoV-2 infection: A very rare, life-threatening complication. Am J Emerg Med 2021; 39: 258.e1–258.e3.
9. *Sun R, Liu H, Wang X.* Mediastinal emphysema, giant bulla, and pneumothorax developed during the course of COVID-19 pneumonia. Korean J Radiol 2020; 21(5): 541–4.
10. *Rohailla S, Ahmed N, Gough K.* SARS-CoV-2 infection associated with spontaneous pneumothorax. CMAJ 2020; 192(19): E510.
11. *Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J,* et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA 2020; 323(11): 1061–9.
12. *Barreto HG, de Pádua Milagres FA, de Araújo GC, Daúde MM, Benedito VA.* Diagnosing the novel SARS-CoV-2 by quantitative RT-PCR: variations and opportunities. J Mol Med (Berl) 2020; 98(12): 1727–36.
13. *Chen N, Zhou M, Dong X, Qu, J, Gong F, Han Y,* et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395(10223): 507–13.
14. *Noppen M.* Spontaneous pneumothorax: epidemiology, pathophysiology and cause. Eur Respir Rev 2010; 19(117): 217–9.
15. *Liu K, Zeng Y, Xie P, Ye X, Xu G, Liu J,* et al. COVID-19 with cystic features on computed tomography: A case report. Medicine (Baltimore) 2020; 99(18): e20175.
16. *Zhu N, Wang W, Liu Z, Liang C, Wang W, Ye F,* et al. Morphogenesis and cytopathic effect of SARSCoV-2 infection in human airway epithelial cells. Nat Commun 2020; 11(1): 3910.
17. *Xiang C, Wu G.* SARS-CoV-2 pneumonia with subcutaneous emphysema, mediastinal emphysema, and pneumothorax: A case report. Medicine (Baltimore) 2020; 99(20): e20208.
18. *Al-Shokri SD, Ahmed AOE, Saleh AO, AbouKamar M, Ahmed K, Mohamed MFH.* Case Report: COVID-19-Related Pneumothorax-Case Series Highlighting a Significant Complication. Am J Trop Med Hyg 2020; 103(3): 1166–9.
19. *Alhakeem A, Khan MM, Al Soub H, Yousaf Z.* Case Report: COVID-19-Associated Bilateral Spontaneous Pneumothorax-A Literature Review. Am J Trop Med Hyg 2020; 103(3): 1162–5.

Received on June 4, 2020.
Revised on November 12, 2020.
Accepted December 22, 2020.
Online First December, 2020.

CASE REPORT

UDC: 616-006.325::616.748]:617.58
https://doi.org/10.2298/VSP200125050T

Intramuscular myxoma of a thigh: A case report

Intramuskularni miksom natkolenice

Zoran Terzić*†, Batrić Vukčević†, Marinko Paunović*, Boban Djordjević‡§, Stojan Terzić†

Clinical Center of Montenegro, *Center for Plastic and Reconstructive Surgery, Podgorica, Montenegro; University of Montenegro, †Faculty of Medicine, Podgorica, Montenegro; Military Medical Academy, ‡Clinic for Plastic and Reconstructive Surgery and Burns, Belgrade, Serbia, University of Defence, §Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Introduction. Myxoid lesions may present as benign, locally invasive, or malignant tumors. The incidence of intramuscular myxoma is nearly 1 case in 1,000,000 inhabitants. **Case report.** A 73-year-old man presented to our clinic with a painless, subcutaneous tumor of the adductor region of the left thigh. Computed tomography and magnetic resonance imaging showed a cystic tumor with thin septae located in the adductor muscles. The tumor was extirpated *in toto*, with the histopathological confirmation of an intramuscular myxoma. **Conclusion.** This example may serve to increase the awareness of a successful intramuscular myxoma treatment among surgeons and radiologists in small countries.

Key words:
diagnosis; magnetic resonance imaging; myxoma; surgical procedures, operative; thigh; tomography, x-ray computed.

Apstrakt

Uvod. Miksoidni tumori mogu biti benigni, lokalno invazivni ili maligni. Incidenca intramuskularnog miksoma je oko jedan slučaj na 1 000 000 stanovnika. **Prikaz bolesnika.** Prezentovan je slučaj 73-godišnjeg muškarca sa bezbolnim, potkožnim tumorom aduktorne regije leve natkolenice. Kompjuterizovanom tomografijom i magnetnom rezonancom otkriven je cistični tumor sa tankim septama, lociran u aduktornoj muskulaturi. Tumor je ekstirpiran u celosti, a patohistološki nalaz je pokazao da se radi o intramuskularnom miksomu. **Zaključak.** Prikazani slučaj može pomoći hirurzima i radiolozima u malim zemljama da sagledaju mogućnost uspešnog lečenja intramuskularnog miksoma.

Ključne reči:
dijagnoza; magnetska rezonanca, snimanje; miksom; hirurgija, operativne procedure; natkolenica; tomografija, kompjuterizovana, rendgenska.

Introduction

Myxoid soft tissue tumors represent a group of neoplasms consisting of a rich extracellular gelatinous mucopolysaccharide matrix actively secreted by tumor cells [1]. They usually affect the extremities and can be benign (including the locally invasive tumors) or malignant [2, 3]. Among benign and locally aggressive myxomas, intramuscular myxoma is the most frequent type, while aggressive angiomyxoma, superficial angiomyxoma, myxolipoma, and dermal myxoma are less common [2]. The incidence of intramuscular myxoma is around 1 case in 1,000,000 inhabitants [4]. Due to the hypocellularity of the lesion, excisional biopsy is indicated (instead of fine needle aspiration cytology), while complete excision is almost always curative [5]. We presented a patient with an asymptomatic intramuscular myxoma diagnosed and treated at a tertiary care center in Podgorica, Montenegro.

Case report

A 73-year-old man presented to our clinic with a tumor in the left thigh. He stated that the mass had been growing slowly for several years. He felt no pain and had no other symptoms. His previous medical history included: arterial hypertension, transurethral resection of the prostate for benign prostatic hyperplasia, laparoscopic cholecystectomy for chronic calculous cholecystitis, right-sided inguinal hernia repair, and extirpation of the right great saphenous vein due to venous varices. The laboratory results were unremarkable.

Correspondence to: Batrić Vukčević, University of Montenegro, Faculty of Medicine, 81 000 Podgorica, Montenegro.
E-mail: batricvukcevic@gmail.com

The physical examination revealed a subcutaneous tumor on the medial aspect of the superior third of the left thigh. The tumor was solid, irregularly ball-shaped, and around 6–7 cm in its widest diameter. It was not painful on palpation, and there was no neurovascular deficit on the affected leg. Ultrasonography of the left thigh showed a tumor located among the adductor muscles, heterogeneous in echo sonographic appearance. Color Doppler imaging did not show any tumor blood vessels, and there were no pathological findings on the arterial or venous vessels of the left leg. Computed tomography (CT) showed a 95 x 90 mm tumor in the adductor region of the left thigh adjacent to the inferior *ramus* of the left pubic bone resembling a cystic lesion (Figure 1). Magnetic resonance imaging (MRI) also suggested the cystic nature of the tumor, with thin septae (Figures 2 and 3). No bone or vascular lesions were seen on CT or MRI.

Fig. 1 – Axial computed tomography scan (white arrowhead indicating the tumor).

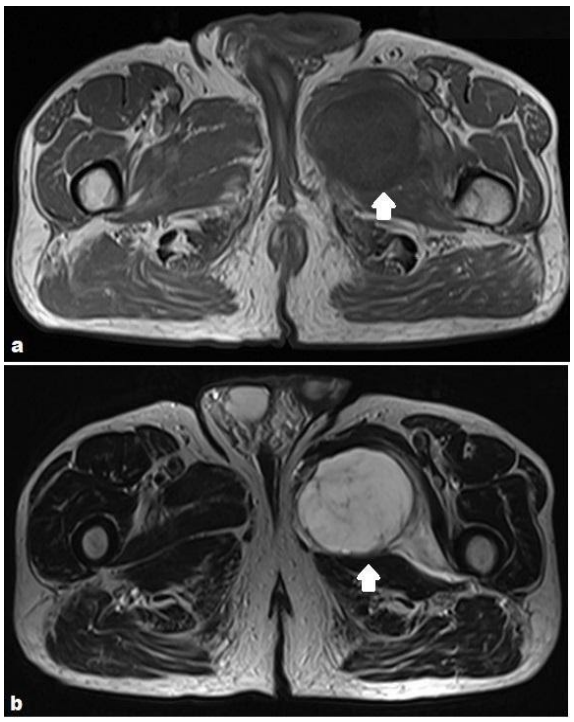


Fig. 2 – Axial magnetic resonance image (white arrowhead indicating the tumor): a) T1 sequence; b) T2 sequence.

Fig. 3 – Coronal magnetic resonance image (white arrowhead indicating the tumor): a) hypointense lesion – T1 sequence; b) hyperintense lesion – T2 sequence.

Surgery revealed an encapsulated tumor measuring 9 × 7 × 6.5 cm in size, located in the adductor muscles of the thigh, arising from the medial plane of the femoral sheath (adjacent to the adventitial layer of the femoral vein) (Figure 4). The tumor was extirpated *in toto* and sent to

Fig. 4 – Intraoperative view of the tumor.

histopathological examination. The patient's recovery was uneventful, and there was no recurrence of the tumor in the next 6 months after surgery. Histopathology showed an overall regular histological and cytological appearance – a tumor consisting of myxomatous stroma, oval and spindle cells without mitoses. Immunohistochemistry was negative for CK, S100, CD34, and actin, while it was positive for vimentin (Figure 5). Therefore, the tumor was diagnosed as a benign myxoma.

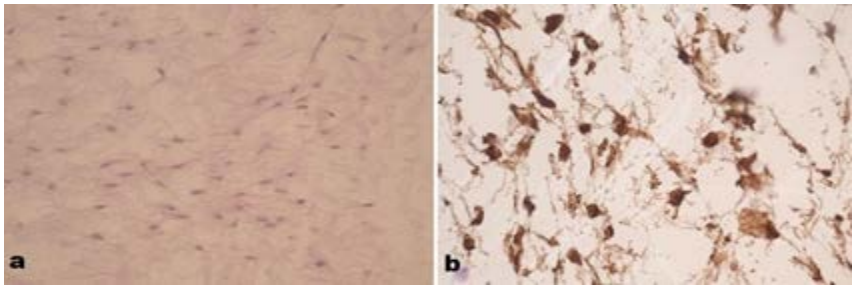


Fig. 5 – a) Uniform tumor cells without mitoses (hematoxylin and eosin, ×200); b) Vimentin positivity on immunohistochemistry (×400).

Discussion

Intramuscular myxoma usually occurs in patients 50–60 years of age, somewhat more often in women [5], most commonly affecting the muscles of the thigh. The tumor is rarely located in the intermuscular planes and more often in the muscle tissue itself [2]. CT image is nonspecific, showing a well-defined hypodense lesion in the intramuscular space. MRI shows homogeneous (81–100%), hypointense lesions on the T1 sequence and hyperintense lesions on the T2 sequence owing to the liquid contents of the tumor, as well as the perilesional rind of fat or edema [6, 7].

Aggressive angiomyxomas usually occur in women, affecting the pelvis or perineum. They exhibit a swirling pattern of infiltration without visceral involvement [2]. Myxofibrosarcoma is a malignant lesion affecting the extremities, with equal sex predilection and common local recurrence due to incomplete resection. It exhibits an infiltrative border with centrifugal spreading along fascial and vascular planes. The tumor is heterogeneous on both T1 and T2 sequences, with a T2-hyperintense curvilinear "tail sign" projection from the primary lesion into the adjacent tissue. The "tail sign" has moderate sensitivity (64–77%) and specificity (79–90%) for this diagnosis [8], and it should be differentiated from perifocal edema by the presence of contrast enhancement [9]. Due to its heterogeneity, myxofibrosarcoma is most difficult to distinguish from myxoid liposarcoma (intralesional hemorrhage might mimic fat tissue, but fat-suppressed T1 images are of great value in these cases) [4].

In order to help determine malignant from benign cyst-like lesions on MRI, Harish et al. [10] proposed several factors: heterogeneity on T1 sequence, average tumor size ≥ 7 cm, with the largest tumor size ≥ 10 cm. Peterson et al. [11] suggested that benign myxoid lesions exhibit the following characteristics: uniform low signal intensity on T1 sequence and increased signal intensity on T2 sequence, homogeneous enhancement, sufficient circumscription, and intramuscular localization. In a 2016 study on 95 myxoid tumors (26 benign and 69 malignant), Crombe et al. [3] identified several MRI characteristics of malignant lesions: ill-defined margin, hemorrhagic component, fibrosis, "tail sign", and intratumoral fat. In their study, malignant lesions were misdiagnosed due to the concomitant absence of all the aforementioned characteristics [3]. The radiographic and histopathologic descriptions of the tumor presented herein implied that it is a benign intramuscular myxoma. The absence of distant metastases, as well as the lack of local recurrence after resection, confirmed the nature of the tumor.

Montenegro has roughly 600,000 inhabitants, and the aforementioned incidence of intramuscular myxoma makes it a unique case in this country. While there is a sufficient number of case reports and research articles published on benign and malignant myxoid tumors worldwide, there are not many case reports on this subject originating from the Balkan countries.

Conclusion

The awareness of intramuscular myxomas and a possibility of their successful treatment among the surgeons and the radiologists from that region should be increased.

Conflict of interest

None declared.

REFERENCES

1. *Henderson – Jackson EB, Bui MM.* Molecular Pathology of Soft-Tissue Neoplasms and Its Role in Clinical Practice. Cancer Control 2015; 22(2): 186–92.
2. *Baheti AD, Tirumani SH, Rosenthal MH, Howard SA, Shinagare AB, Ramaiya NH,* et al. Myxoid Soft-Tissue Neoplasms: Comprehensive Update of the Taxonomy and MRI Features. AJR Am J Roentgenol 2015; 204(2): 374–85.
3. *Crombe A, Alberti N, Stoeckle E, Brouste V, Buy X, Coindre JM,* et al. Soft tissue masses with myxoid stroma: Can conventional magnetic resonance imaging differentiate benign from malignant

tumors? Eur J Radiol 2016; 85:1875–82.
4. *Petscavage-Thomas JM, Walker EA, Logie CI, Clarke LE, Duryea DM, Murphey MD*. Soft-tissue myxomatous lesions: review of salient imaging features with pathologic comparison. Radiographics 2014; 34(4): 964–80.
5. *Erwteman AS, Balach T*. Clinical evaluation and management of benign soft tissue tumors of the extremities. Cancer Treat Res 2014; 162: 171–202.
6. *Bancroft LW, Kransdorf MJ, Menke DM, O'Connor MI, Foster WC*. Intramuscular myxoma: characteristic MR imaging features. AJR Am J Roentgenol 2002; 178(5): 1255–9.
7. *Sung J, Kim JY*. Fatty rind of intramuscular soft-tissue tumors of the extremity: is it different from the split fat sign? Skeletal Radiol 2017; 46(5): 665–73.
8. *Lefkowitz RA, Landa J, Hwang S, Zabor EC, Moskowitz CS, Agaram NP,* et al. Myxofbrosarcoma: prevalence and diagnostic value of the "tail sign" on magnetic resonance imaging. Skeletal Radiol 2013; 42(6): 809–18.
9. *Kaya M, Wada T, Nagoya S, Sasaki M, Matsumura T, Yamaguchi T,* et al. MRI and histological evaluation of the infltrative growth pattern of myxofbrosarcoma. Skeletal Radiol 2008; 37(12): 1085–90.
10. *Harish S, Lee JC, Ahmad M, Saifuddin A*. Soft tissue masses with "cyst-like" appearance on MR imaging: Distinction of benign and malignant lesions. Eur Radiol 2006; 16(12): 2652–60.
11. Peterson KK, Renfrew DL, Feddersen RM, Buckwalter JA, el-Khoury GY. Magnetic resonance imaging of myxoid containing tumors, Skeletal Radiol. 1991; 20(4): 245–50.

Received on January 25, 2020.
Accepted on May 6, 2020.
Online First May, 2020.

HISTORY OF MEDICINE

UDC: 616.34
https://doi.org/10.2298/VSP180727065S

Historical development of the understanding of coeliac disease

Istorijski razvoj saznanja o celijačnoj bolesti

Biljana Stojanović*, Sveta Janković†‡, Nela Djonović‡§, Vladimir Radlović‖¶, Stevan Jovanović*, Biljana Vuletić†‡

***High Health School of Professional Studies, Belgrade, Serbia; University of Kragujevac, †Faculty of Medical Sciences, Kragujevac, Serbia; Clinical Center of Kragujevac, ‡Pediatric Clinic Kragujevac, Serbia; Institute of Public Health Kragujevac, §Department of Hygiene and Ecology, Kragujevac, Serbia; University of Belgrade, ‖Faculty of Medicine, Belgrade Serbia; ¶University Children's Hospital, Belgrade, Serbia**

Key words:
biopsy; celiac disease; diagnosis; history of medicine; therapeutics.

Ključne reči:
biopsija; celijakija; dijagnoza; istorija medicine; lečenje.

Introduction

Celiac disease (CD), also known as the malabsorption syndrome or gluten-sensitive enteropathy, is an immune-mediated disorder that occurs in individuals with a genetic predisposition as a result of gluten consumption. Gluten is found in wheat, rye, barley, and oats [1]. CD occurs in about 1% of the total population. The prevalence of CD varies from country to country (0.3% in Germany, 0.7% in Italy, 0.8% in Sweden, 2.4% in Finland, and 0.7%–0.8% in the USA). It is a lifelong disease that is associated with reduced quality of life and high-risk comorbidity and death [2, 3]. Differences in the incidence of the disease depend not only on genetic factors and diet but also on the availability of modern diagnostic technology. The disease occurs in children and adults, but its typical form is more frequent in early life, between the 7th and 24th month [4]. The diagnosis is based on small intestinal biopsy, tissue transglutaminase (tTG) antigen test, and human leukocyte antigen markers (HLA DQ2 and HLA DQ8) [5]. The mainstay of treatment is a gluten-free diet (GFD) [5–7]. Appropriately diagnosed and treated patients have a reasonable chance of living a normal life. However, about 85% of people with CD are asymptomatic, although serological parameters and histopathological findings in the small bowel mucosa might reveal increased intraepithelial lymphocyte infiltration [8, 9].

Earliest descriptions

CD has been known since the ancient times. For years, it was exclusively considered a disease of the Old Continent because it was found that it primarily occurred in the white population, especially in certain groups, while it occurred less frequently in people of other races [10]. Today, it is known that CD is present in different groups of people, and it is widespread throughout the world [11]. A long time ago, humans lived in hunter-gatherer groups, and their diet consisted of fruits, drupes, roots, and occasional meat [10]. In the New Stone Age (Neolithic), humans first started to domesticate animals, cultivate the land, and grow crops for human consumption. The way of life and their former diet had been replaced during the period of the agricultural revolution. Hunting and gathering fruits were replaced by growing crops and animals, which challenged the human gastrointestinal tract to adapt to a new diet and a new, previously unknown, antigenic stimulation [10, 12, 13].

The ancient Greek physician Aretaeus of Cappadocia gave the first known description of the disease in the first century, anno domini (AD). Aretaeus worked as a physician during the reign of Nero. He most probably studied in Alexandria and lived and worked in Alexandria and Rome [13]. He described a disease encompassing the disturbance of “pepsis” and “anadosis”, which could be loosely translated into modern terms as digestion and absorption [14]. He suggested that it was a chronic disease in adults, manifested

Correspondence to: Sveta Janković, Clinical Center Kragujevac, Pediatric Clinic; Zmaj Jovina 30, 34 000 Kragujevac, Serbia.
Email: svetajankovic.201128@yahoo.com

by general debility, dehydration, generalized wasting, the passage of undigested food, and malodorous white clay-like stools. The disease was not transmitted and was prone to recur. Aretaeus believed that the problem was a lack of heat in the stomach that was essential for digestion. He also believed that this was a disease of older people, more common in women, and that it never occurred in children. He believed that the disease was not chronic and even thought that the "consumption of large amounts of cold water after a strong thirst" might be a possible cause. He also emphasized the importance of a modified diet but did not give any details of the diet composition [14]. In his work, Aretaeus also described a single patient who was pale, thin, weak, incapable of working, and had abdominal pain. Diarrheal stools were whitish, malodorous, and followed by flatulence.

CD in the XIX century

After Aretaeus, no tangible progress had been made in understanding CD until the modern era. Here, Francis Adams ought to be credited for keeping the scientific society aware of Aretaeus's work in his lecture given at the Sydenham Society in London in 1856 [14]. The first detailed description of CD dates back to 1887 and is associated with the English paediatrician Samuel Gee.

Samuel Gee [15] (1839–1911) gained a sufficient reading skill in ancient Greek. He gave a modern description of the condition in a lecture at St. Bartholomew's Hospital and Hospital for Sick Children, Great Ormond Street, London. A year later, the lecture was published in the reports of his hospital. It represents the first modern clinical description of CD, along with the theory that highlights the importance of the diet in patients with CD. This work is considered the first comprehensive description of the condition and is usually referred to in all subsequent publications [16]. Gee [15] further investigated the disease in his research and acknowledged the previously existing term coined after the Greek word *coeliacus*, loosely translated as the abdominal cavity. Thus, it was emphasized that a large stomach, along with very thin arms and legs, dominated the condition, and disorder in digestion was established as a basic problem. Gee [15] described patients' stools as heavy, greasy, and extremely malodorous, i.e. severe steatorrhea and cachexia were present due to poor appetite in persons of all ages. Contrary to Aretaeus, Gee included children, mainly those aged 1 to 5 years. Unfortunately, most of these children died soon due to severe cachexia. After their death, Gee examined their intestines, but, as the wall of the small intestine rapidly decays after death, he failed to find the cause of CD [10].

CD in the first half of the XX century

In the early 20th century, the diagnosis of CD was based on clinical features, distinctive appearance of stools, and typical age at which the disease occurred [16–18]. It was not until the beginning of the XX century that it became clear that the cause of CD was a disorder of absorption in the small intestine [11]. Gee [15] believed that children suffering from the disease could be cured by a dietary regime, so he recommended avoiding starch-rich foods. He forbade the intake of milk, rice, fruit, and vegetables. He particularly recommended the intake of shellfish, but almost no child could bear this type of diet for a longer period of time [11].

Christian Archibald Herter, an American physician, introduced a new name for this disease in 1908 – intestinal infantilism – considering that an intestinal disorder was the cause of the disease [16]. In 1908, Herter [17] wrote a book on children with CD titled "Intestinal Infantilism". The author noted that the growth of these children was slow and they had better fat tolerance compared with carbohydrates, while the disease was described as severe insufficiency of digestion. In 1924, Haas and Haas [18] promoted the positive effect of a banana diet for treating CD. During their career, they treated over 600 patients with CD. In 1951, their son, Dr. Merrill P. Haas, joined them and published the medical textbook – "The Management of Coeliac Disease". Until 1940, the phosphorylation of fats and insufficient secretion of digestive juices and enzymes (particularly pancreatic) were thought to be the possible causes of CD disturbances. On the other hand, it was also thought that the disease might be a result of a variety of conditions, therefore, celiac syndrome was mentioned [19]. During this period, the disease was treated by trying various diets. In England, Leonard Parsons [20] advised the exclusion of fats from the diet, while carbohydrates were excluded on the recommendation of John Howland [21] in the USA.

CD in the second half of the XX century

In his dissertation published in 1950, the Dutch paediatrician Dr. Willem Dicke [22] observed the exclusion of wheat from children's diet. He concluded that it led to dramatic improvement, while the disease was getting worse once the wheat was included again. This observation was the result of a natural experiment conducted during wartime when wheat was scarce. This was later confirmed under laboratory conditions by a paediatrician Charlotte Anderson who discovered that wheat gluten caused severe symptoms. The medical team from Birmingham, Anderson et al. [23], concluded in 1952 that gluten was a necessary factor for the development of damage to the mucous membrane of the small intestine in patients with CD.

During the 1950s, the diagnosis was based on the characteristics of malabsorption and clinical observations. In the mid-50s, Shiner [24], in England, and Royer [25], in Argentina, independently of one another, constructed the instruments for peroral small intestine mucosal biopsy. The application of these devices allowed Margot Shiner [26] in 1957 to discover that children with CD had villous atrophy in the small intestine. Intestinal biopsy has become the gold standard for CD ever since.

It was not until the 50s that the individual works of Wim Dicke [26] and those made in collaboration – Dicke et al. [27] – announced the discovery of gluten and led to major progress in the knowledge and treatment of the disease. Their

work, however, did not win much understanding and acceptance by the general medical community of the time and was published with a delay of several years.

The implementation of peroral aspiration biopsy of the small intestinal mucosa using a capsule developed by Crosby and Kugler [28] enabled subsequent progress in the histopathological examinations since it made the procedure easier and more comfortable for the patients. In their statement published in 1990, The European Society for Paediatric Gastroenterology and Nutrition (ESPGHAN) working group recommended using the biopsy capsule rather than the endoscopic biopsy in order to ensure diagnostically adequate specimens [5]. This procedure has become more and more popular and is still being further developed [29, 30].

+Paulley [31] provided the description of typical morphological changes in the small intestinal mucosa in adults in 1954, while Sakula and Shiner [32] proved these changes in children in 1957. Throughout the 1960s, other characteristics of CD were being described, while the importance of the hereditary factor in the emergence of this disease was established in 1965 [33].

Numerous methods of laboratory tests of metabolism and absorption of nutrients were developed simultaneously. The European Society for Paediatric Gastroenterology (and Nutrition – as added later – ESPGHAN) was founded in 1968 in Paris with 14 members, with Dolf Weijers as the first president because of the better cooperation, more precise classification and definition of malabsorption, and diagnosis and treatment of CD. According to the first ESPGHAN diagnostic criteria adopted in Interlaken (Switzerland) in 1969, besides the initial intestinal biopsy, it was necessary to obtain at least two additional biopsy specimens, one after 2–4 years of GFD and the other one during the 3–6 months period of reintroduction of gluten [34]. An important contribution to diagnosis was the use of a stereomicroscope which allows three-dimensional visualization and ideal preparation of the sample drawn from the small bowel mucosa for histopathological analysis. Due to the experience gained and further advances in the use of stereomicroscope, as well as the introduction of serological indicators specific to CD, the 1970 criteria were substantially supplemented and corrected at the ESPGHAN meeting in Budapest in 1989 [5].

In 1975, it was established that gluten peptides lead to a cell-mediated immune response in the small intestine [35]. HLA class II molecules present epitopes in their binding groove to CD4+ T-helper cells and activate the immune system against the gluten, resulting in a characteristic enteropathy with intraepithelial lymphocytosis, hyperplasia of the crypts, and villous destruction [36]. Later on, it was discovered that gluten-specific CD4+ T-cells could be isolated from the small intestine of CD patients but not in controls [37, 38]. Along with the cellular response, a strong B-cell response was also discovered in the form of auto-antibodies, defined as antireticulin, and then anti-endomysium to indicate a poorly defined reaction to an extracellular matrix component of the intestine [39]. In the late 1990s, it was discovered that enzyme tTG triggered these antibodies [40]. Subsequently, tTG was implicated in the deamidation of gliadin [41, 42]. In this reaction, the glutamine in gliadin is transformed into glutamic acid, thus making gluten antigen fit perfectly in the binding groove of HLA-DQ2.5 and HLA-DQ8 molecules, which results in a stronger immune response [43–45].

During this long period, CD was a common but often unrecognized disease. This is partly due to its variable clinical presentation and symptoms that range from malabsorption followed by chronic diarrhea, growth retardation in children, abdominal distention, and weight loss to nonspecific signs and symptoms such as fatigue, osteoporosis, iron deficiency, or anaemia. Serological indicators of the disease, although highly sensitive and specific, had no absolute diagnostic value. Serological tests have been generally recommended as the first step when CD is suspected in order to identify patients who should undergo intestinal biopsy [5].

CD nowadays

The diagnostic criteria for CD were proposed by ESPGHAN and published in 1990. The criteria have not been renewed for more than 20 years. During this time, the perception of CD has changed from a rather uncommon enteropathy to a common multiorgan disease with a strong genetic predisposition associated mainly with human leukocyte antigen HLA-DQ2 and HLA-DQ8. The studies of monozygotic twins found a multitude of genetic factors responsible for CD susceptibility [46]. Recently, genome-wide association studies have identified 39 non-HLA loci that also predispose CD [47]. The diagnosis of CD has also changed as a result of the availability of CD-specific antibody tests, based mainly on tTG type 2 antibodies [48]. Environmental factors have been found to play a role in the emergence of the disease at least to some extent. Infection with rotavirus has been investigated, and the results demonstrate an increased risk of CD autoimmunity in children [49]. Early feeding habits, such as the milk feeding type and breastfeeding duration, can influence the intestinal microenvironment [50], which is characterized by an increased number of intestinal Gram-negative bacteria and a lower level of Bifidobacteria in CD patients [51].

CD is now considered to be a systemic immune-mediated disorder [52–54]. Activated CD4+ T-lymphocytes produce high levels of either a T-helper 1 or a T-helper 2 pattern of pro-inflammatory cytokines, which causes a clonal expansion of plasma-cells secreting anti-gliadin and anti-tTG antibodies [55]. An increased density of CD8+ intraepithelial cells is considered a hallmark of CD [56], and tTG also enhances the gliadin-specific T-cell responses [57].

ESPGHAN summarized the scientific progress to publish the latest guidelines for the diagnosis of CD in 2012 [58]. The guidelines underline the gluten-dependent symptoms, CD-specific antibody levels, HLA markers, and specific small intestinal biopsy findings as a ground for diagnosing CD. It was also suggested that if a high antibody level is present, then performing the biopsy is not necessary. Moreover, the decline of antibody levels can be used to

confirm the diagnosis and follow the response to GFD. However, the 2012 guidelines reserve the small intestinal biopsy and gluten challenge for all uncertain cases [58]. These current guidelines are due to be comprehensively scrutinized and reevaluated.

Currently, adherence to a strict lifelong gluten-free diet is the only available treatment for CD [58]. Research performed since the beginning of the 21st century aims to explore the possibilities for developing effective therapies that could reduce the burden of GFD. Such are dietary modulation with enzyme-treated coeliac-safe wheat [59], wheat gene modulation, and bacterial fermentation [61]. Oral exogenous enzyme intake has been considered in order to reduce gluten toxicity by decreasing the immunogenicity of peptide sequences before ingestion or in the gut [62–65]. Modulation of intestinal permeability for gluten has also been investigated [66, 67]. Experimental therapies attempting to reduce immunogenicity or suppress inflammation include the restoration of oral tolerance by administering gluten peptides pretreated with enzymes secreted by Lactococcus [68], immunomodulation by helminths [69], tTG inhibitors [70, 71], HLA-DQ groove antagonists [72], and inhibitors of adhesion molecules [69]. Clinical trials have been conducted to evaluate the efficacy of a vaccine based on a set of gluten peptides [73]. However, the potential risks of immune system activation, clinical effectiveness, safety, and affordability require further investigations of the vaccine.

Conclusions and future directions

The understanding of CD has greatly improved since the first description in 1887. Intensive studying has changed many attitudes about the disease, opened a number of questions, and, thus, imposed the necessity of additional research and decision-making. Unfortunately, CD is increasingly becoming a public health problem. CD is now more widely discussed, and symptomatic patients are more easily recognized. It is very important that the environment in which the patient lives is aware of the problem and alleviates their suffering.

R E F E R E N C E S

1. *Abadie V, Sollid LM, Barreiro LB, Jabri B.* Integration of genetic and immunological insights into a model of celiac disease pathogenesis. Annu Rev Immunol 2011; 29: 493–525.
2. *Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S*, et al. The prevalence of celiac disease in Europe: Results of a centralized, international mass screening project. Ann Med 2010; 42(8): 587–95.
3. *Ludvigsson JF, Card TR, Kaukinen K, Bai J, Zingone F, Sanders DS*, et al. Screening for celiac disease in the general population and in high-risk groups. United European Gastroenterol J 2015; 3(2): 106–20.
4. *Radlović N.* Celiac disease. Srp Arh Celok Lek 2013; 141(1–2): 122–6.
5. Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. Arch Dis Child 1990; 65(8): 909–11.
6. *Troncone R, Auricchio S.* Celiac disease. In: *Wyllie R, Hymas JS,* editors. Pediatric Gastrointestinal and Liver Disease. Philadelphia: Saunders Elsevier Inc; 2006. p. 517–27.
7. *Mäki M.* Celiac disease. In: *Kleinman RE, Sanderson IR, Goulet O, Sherman PM, Mieli-Vergani G, Shneider BL,* editors. Walker's Pediatric Gastrointestinal Disease. Hamilton: BC Decker Inc; 2008. p. 319–27.
8. *Marsh M.* Mucosal pathology in gluten sensitivity. In: *Marsh M,* editor. Coeliac disease. Oxford: Blackwell Sci Publ; 1992. p. 136–91.
9. *Arranz E, Bode J, Kingstone K, Ferguson A.* Intestinal antibody pattern of coeliac disease: association with gamma/delta T cell receptor expression by intraepithelial lymphocytes, and other indices of potential coeliac disease. Gut 1994; 35(4): 476–82.
10. *Farrell RJ, Kelly CP.* Celiac sprue. N Engl J Med 2002; 346(3): 180–8.
11. *Catassi C, Fabiani E, Iacono G, D'Agate C, Francavilla R, Biagi F*, et al. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. Am J Clin Nutr 2007; 85(1): 160–6.
12. *Tjon JM, van Bergen J, Koning F.* Celiac disease: how complicated can it get? Immunogenetics 2010; 62(10): 641–51.
13. *Freeman HJ.* The Neolithic Revolution and Subsequent Emergence of the Celiac Affection. Int J Celiac Dis 2013; 1(1): 19–22.
14. *Adams F,* translator. On the Coeliac Affection. The Extant Works of Aretaeus, the Cappadocian. London: Sydenham Society 1856; 350–1.
15. *Gee SJ.* On the coeliac affection. St. Bartholomew's Hospital. Report 1888; 24: 17–20.
16. *Abel EK.* The Rise and Fall of Celiac Disease in the United States. J Hist Med Allied Sci 2010; 65(1): 81–105.
17. *Herter CA.* On intestinal infantilism from chronic intestinal infection. New York: Macmillan, Co; 1908.
18. *Haas SV, Haas MP.* The treatment of celiac disease with the specific carbohydrate diet; report on 191 additional cases. Am J Gastroenterol 1955; 23(4): 344–60.
19. *Brown A.* Some etiological factors in the coeliac syndrome. Arch Dis Child 1949; 24(118): 99–106.
20. *Parsons LG.* Coeliac disease. Am J Dis Child 1932; 43: 1293–346.
21. *Howland J.* Prolonged intolerance to carbohydrates. Trans Amer Pediat Soc (N.Y.) 1921; 38: 393–6.
22. *Dicke WK.* Coeliakie: een onderzoek naar de nadelige invloed van sommige graansoorten op de lijder aan coeliakie [thesis]. Netherlands: University of Utrecht; 1950. (Dutch)
23. *Anderson CM, French JM, Sammons HG, Frazer AC, Gerrard JW, Smellie JM.* Coeliac disease; gastrointestinal studies and the effect of dietary wheat flour. Lancet 1952; 1(6713): 836–42.
24. *Shiner M.* Jejunal-biopsy tube. Lancet 1956; 270(6907): 85.
25. *Royer M, Croxatto O, Biempica L, Balcazar Morrison AJ.* Duodenal biopsy by aspiration under radioscopic control. Prensa Med Argent 1955; 42(33): 2515–9.
26. *Shiner M.* Small intestinal biopsies by the oral route; histopathologic changes in the malabsorption syndrome. J Mt Sinai Hosp N Y 1957; 24(3): 273–85.
27. *Dicke WK, Van De Kamer JH, Weijers HA.* Celiac disease. Adv Pediatr 1957; 9: 277–318.
28. *Crosby WH, Kugler HW.* Intraluminal biopsy of the small intestine; the intestinal biopsy capsule. Am J Dig Dis 1957; 2(5): 236–41.

29. *Koprowski R.* Overview of technical solutions and assessment of clinical usefulness of capsule endoscopy. Biomed Eng Online 2015; 14: 111.
30. *Ciaccio EJ, Lewis SK, Bhagat G, Green PH.* Coeliac disease and the videocapsule: what have we learned till now. Ann Transl Med 2017; 5(9): 197.
31. *Paulley JW.* Observation on the aetiology of idiopathic steatorrhoea; jejunal and lymph-node biopsies. Br Med J 1954; 2(4900): 1318–21.
32. *Sakula J, Shiner M.* Coeliac disease with atrophy of the small-intestine mucosa. Lancet 1957; 273(7001): 876–7.
33. *MacDonald WC, Dobbins WO 3rd, Rubin CE.* Studies of the familial nature of celiac sprue using biopsy of the small intestine. N Engl J Med 1965; 272: 448–56.
34. *Meeuwisse GW.* Diagnostic criteria in coeliac disease. Acta Paediatr Scand 1970; 59: 461–3.
35. *Ferguson A, MacDonald TT, McClure JP, Holden RJ.* Cell-mediated immunity to gliadin within the small-intestinal mucosa in coeliac disease. Lancet 1975; 1(7912): 895–7.
36. *Marsh MN.* Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). Gastroenterology 1992; 102(1): 330–54.
37. *Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O,* et al. Gliadin-specific, HLA-DQ(a1*0501,b1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. J Exp Med 1993; 178(1): 187–96.
38. *Lundin KA, Scott H, Fausa O, Thorsby E, Sollid LM.* T cells from the small intestinal mucosa of a DR4, DQ7/DR4, DQ8 celiac disease patient preferentially recognize gliadin when presented by DQ8. Hum Immunol 1994; 41(4): 285–91.
39. *Mäki M, Hällström O, Marttinen A.* Reaction of human noncollagenous polypeptides with coeliac disease autoantibodies. Lancet 1991; 338(8769): 724–5.
40. *Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO,* et al. Identification of tissue transgluta-Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997; 3(7): 797–801.
41. *van de Wal Y, Kooy Y, van Veelen P, Peña S, Mearin L, Papadopoulos G,* et al. Selective deamidation by tissue transglutaminase strongly en-hances gliadin-specific T cell reactivity. J Immunol 1998; 161(4): 1585–8.
42. *Molberg O, Mcadam SN, Körner R, Quarsten H, Kristiansen C, Madsen L* et al. Tissue transglutaminase selectively modifies gliadin peptides that are recog nized by gut-derived T cells in celiac disease. Nat Med 1998; 4(6): 713–7.
43. *Osman AA, Günnel T, Dietl A, Uhlig HH, Amin M, Fleckenstein B,* et al. B-cell epitopes of gliadin. Clin Exp Immunol 2000; 121(2): 248–54.
44. *Aleanzi M, Demonte AM, Esper C, Garcilazo S, Waggener M.* Celiac disease: antibody recognition against native and selectively deamidated gliadin peptides. Clin Chem 2001; 47(11): 2023-8.
45. *Arentz-Hansen H, Körner R, Molberg O, Quarsten H, Vader W, Kooy YM,* et al. The intestinal T cell re-The intestinal T cell response to alpha-gliadin in adult celiac disease is fo-cused on a single deamidated glutamine targeted by tissue transglutaminase. J Exp Med 2000; 191(4): 603–12.
46. *Kuja-Halkola R, Lebwohl B, Halfvarson J, Wijmenga C, Magnusson PK, Ludvigsson JF.* Heritability of non-HLA genetics in coeliac disease: a population-based study in 107 000 twins. Gut 2016; 65(11): 1793–8.
47. *Dubois PC, Trynka G, Franke L, Hunt KA, Romanos J, Curtotti A,* et al. Multiple common variants for celiac disease influencing immune gene expression. Nat Genet 2010; 42(4): 295–302.
48. *Volta U, Villanacci V.* Celiac disease: diagnostic criteria in progress. Cell Mol Immunol 2011; 8(2): 96–102.
49. *Stene LC, Honeyman MC, Hoffenberg EJ, Haas JE, Sokol RJ, Emery L,* et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am J Gastroenterol 2006; 101(10): 2333–40.
50. *Silano M, Agostoni C, Guandalini S.* Effect of the timing of gluten introduction on the development of celiac disease. World J Gastroenterol 2010; 16(16): 1939–42.
51. *Sanz Y, De Pama G, Laparra M.* Unraveling the ties between celiac disease and intestinal microbiota. Int Rev Immunol 2011; 30(4): 207–18.
52. *du Pré MF, Sollid LM.* T-cell and B-cell immunity in celiac disease. Best Pract Res Clin Gastroenterol 2015; 29(3): 413–23.
53. *Kim SM, Mayassi T, Jabri B.* Innate immunity: actuating the gears of celiac disease pathogenesis. Best Pract Res Clin Gastroenterol 2015; 29: 425–35.
54. *Mazzarella G.* Effector and suppressor T cells in celiac disease. World J Gastroenterol 2015; 21(24): 7349–56.
55. *Björck S, Lindehammer SR, Fex M, Agardh D.* Serum cytokine pattern in young children with screening detected coeliac disease. Clin Exp Immunol 2015; 179(2): 230–5.
56. *Troncone R, Jabri B.* Coeliac disease and gluten sensitivity. J Intern Med 2011; 269(6): 582–90.
57. *Matthias T, Neidhöfer S, Pfeiffer S, Prager K, Reuter S, Gershwin ME.* Novel trends in celiac disease. Cell Mol Immunol 2011; 8(2): 121–5.
58. *Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R,* et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012; 54(1): 136–60.
59. *Gianfrani C, Siciliano RA, Facchiano AM, Camarca A, Mazzeo MF, Costantini S,* et al. Transamidation of wheat flour inhibits the response to gliadin of intestinal T cells in celiac disease. Gastroenterology 2007; 133(3): 780–9.
60. *Van den Broeck HC, Van Herpen TW, Schuit C, Salentijn EM, Dekking L, Bosch D,* et al. Removing celiac disease-related gluten proteins from bread wheat while retaining technological properties: a study with Chinese Spring deletion lines. BMC Plant Biol 2009; 9: 41–53.
61. *De Angelis M, Rizzello CG, Fasano A, Clemente MG, De Simone C, Silano M,* et al. VSL#3 probiotic preparation has the capacity to hydrolyze gliadin polypeptides responsible for celiac sprue. Biochim Biophys Acta 2006; 1762(1): 80–93.
62. *Gass J, Bethune MT, Siegel M, Spencer A, Khosla C.* Combination enzyme therapy for gastric digestion of dietary gluten in patients with celiac sprue. Gastroenterology 2007; 133(2): 472–80.
63. *Tye-Din JA, Anderson RP, Ffrench RA, Brown GJ, Hodsman P, Siegel M,* et al. The effects of ALV003 pre-digestion of gluten on immune response and symptoms in celiac disease in vivo. Clin Immunol 2010; 134: 289–95.
64. *Fuhrmann G, Leroux JC.* In vivo fluorescence imaging of exogenous enzyme activity in the gastrointestinal tract. Proc Natl Acad Sci US A 2011; 108(22): 9032–7.
65. *Dhal PK, Polomoscanik SC, Avila LZ, Holmes-Farley SR, Miller RJ.* Functional polymers as therapeutic agents: concept to market place. Adv Drug Deliv Rev 2009; 61(13): 1121–30.
66. *Leffler DA, Kelly CP, Green PH, Fedorak RN, DiMariano A, Perrow W,* et al. Larazotide Acetate for Persistent Symptoms of Celiac Disease Despite a Gluten-Free Diet: A Randomized Controlled Trial. Gastroenterology 2015; 148(7): 1311–9.e6.
67. *Paterson BM, Lammers KM, Arrieta MC, Fasano A, Meddings JB.* The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a

proof of concept study. Aliment Pharmacol Ther 2007; 26(5): 757–66.

68. *Huibregtse IL, Marietta EV, Rashtak S, Koning F, Rottiers P, David CS,* et al. Induction of antigenspecific tolerance by oral administration of Lactococcus lactis delivered immunodominant DQ8-restricted gliadin peptide in sensitized nonobese diabetic Ab° Dq8 transgenic mice. J Immunol 2009; 183: 2390–6.
69. *Schuppan D, Junker Y, Barisani D.* Celiac disease: from pathogenesis to novel therapies. Gastroenterology 2009; 137: 1912–33.
70. *Pardin C, Roy I, Lubell WD, Keillor JW.* Reversible and competitive cinnamoyl triazole inhibitors of tissue transglutaminase. Chem Biol Drug Des 2008; 72(3): 189–96.
71. *Ozaki S, Ebisui E, Hamada K, Goto J, Suzuki AZ, Terauchi A,* et al. Potent transglutaminase inhibitors, aryl beta-aminoethyl ketones. Bioorg Med Chem Lett 2010; 20(3): 1141–4.
72. *Siegel M, Xia J, Khosla C.* Structure-based design of alpha-amido aldehyde containing gluten peptide analogues as modulators of HLA-DQ2 and transglutaminase 2. Bioorg Med Chem 2007; 15(18): 6253–61.
73. *Sollid LM, Khosla C.* Novel therapies for coeliac disease. J Intern Med 2011; 269(6): 604–13.

Received on July 27, 2018.
Revised on May 10, 2019.
Accepted on May 27, 2019.
Online First June, 2019.

LETTERS TO THE EDITOR
(RESEARCH LETTERS)

UDC: 616.8-07/-08::616-036.21
https://doi.org/10.2298/VSP210205018R

Treatment of neurology patients during the COVID-19 pandemic in Serbia

Lečenje neuroloških bolesnika tokom pandemije COVID-19 u Srbiji

To the Editor:

In the period of the COVID-19-precipitated state of emergency in Serbia, up until the 30th September 2020, patients with neurological disorders, who required tertiary level of medical care, have been treated at the following centers: for Belgrade - Neurology Clinic of the Military Medical Academy and its outpatient clinic, Special Hospital for Cerebrovascular Diseases "Saint Sava", and outpatient clinics of the Accident and Emergency Department of the Clinical Center of Serbia; for Vojvodina – Neurology Clinic within the Clinical Center of Vojvodina; and for central and Southern Serbia – Neurology Clinics within the Clinical Centers of Kragujevac and Niš. The Neurology Clinic within the Clinical Center of Niš has been relocated to the Military Hospital Niš for the majority of this period.

Regarding the outpatient appointments for neurology patients in the first five months of the pandemic (Mart to July), only the Outpatient Clinic of the Military Medical Center Novi Beograd had been open to all categories of population (civilian and military). Since the end of August, the Consultant Neurology Clinic within the Military Medical Academy has also admitted patients with neurological disorders. On the other hand, the Outpatient Clinic of the Clinical Center of Serbia has commenced only a reduced level of appointment bookings, starting from the end of May.

In the observed period, the above-mentioned tertiary centers have hospitalized and treated over 3,000 patients with severe neurological conditions outside the COVID system. The staff of these clinics exhibited heroic efforts in order to provide timely and accurate diagnostic assessment and, in particular, differentiate neurological conditions *per se* from neurological complications of COVID-19. Considering that neurological complications of COVID-19 are common, and outcomes of COVID-19 in patients with existing neurological problems are often severe, such level of dedication and effort was critical in order to avoid spreading of COVID-19 within the neurology wards, which would, in turn, dramatically increase morbidity and mortality rates. An additional strain for neurology units, particularly in Belgrade, came from the fact that neurology wards within other tertiary institutions, such as Clinical Centers "Zemun", "Dragiša Mišović", and City Hospital "Zvezdara", have not participated in the treatment of neurological patients, since they have been transformed into a part of the COVID system. Moreover, the Neurology Clinic within the Clinical Center of Serbia, the institution with the greatest capacity for neurological hospitalizations in the country, redirected patients to the Department of Emergency Neurology of the Clinical Center of Serbia. Together with the strict observation of the intrahospital epidemiological measures, such as increased distancing within patient rooms, the above-mentioned issues further reduced the capacity of the active wards by over 60% in comparison with the period before the pandemic. Despite this, the Specialised Hospital for Cerebrovascular Diseases "Saint Sava" treated the highest number of patients with cerebrovascular stroke and performed the greatest number of hospitalizations amongst all neurology clinics in Serbia.

In order to provide robust and reliable statistical data, a Working Group of the Society of Serbian Neurologists prepared a database of all neurology patients, including the COVID-19 positive ones, treated at outpatient or hospital settings during the COVID-19 pandemic in Serbia. Thereby, data relating to the admittance, severity, treatment, and outcomes of neurology patients at outpatient and hospital institutions are presented in tabular form, illustrating difficulties, complexities, risks, demands, and dangers of medical care for these patients during the COVID-19 pandemic. It has already become clear that apart from the treatment, which needs to be provided for neurological conditions *per se*, determining the COVID-19 status for these patients and the causal relationship between COVID-19 and neurological symptomatology and outcomes has been critical. Furthermore, including the measures for avoiding the infection from spreading to other patients and medical staff has become crucial while maintaining, at the same time, high standards of treatment for COVID-19 patients in neurology wards.

Correspondence to: Ranko Raičević, Military Medical Academy, Clinic for Neurology, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: ranko.raicevic1@gmail.com

Table 1

Number of outpatient admittances in polyclinics, outpatient accident and emergency centers, and number of hospitalizations in neurological institutions of tertiary level in Serbia, including the COVID-19 positive patients who have been hospitalized or treated as outpatients

Institution	Outpatient examinations in polyclinic services	Examinations in emergency centers	Hospitalized patients	Number of COVID-19 positive patients O: outpatients H: hospitalized
Special Hospital "Saint Sava", Belgrade		3,500	1,200	O: 0 H: 42
Clinic for Neurology, MMA, Belgrade	Two outpatient clinics of the Military Medical Center in Belgrade and two outpatient clinics at the Special Polyclinic of the MMA (about 5,000 examinations)	8,294	589	O: 21 H: 17
Clinic for Neurology, CC Niš, Niš	Reduced scheduling due to dislocation	6,159	412	O: 55 H: 7
Clinic for Neurology, CCV, Novi Sad	Reduced scheduling due to dislocation	4,500	400	O: 0 H: 11
Clinic for Neurology CC Kragujevac, Kragujevac	Reduced scheduling due to dislocation	3,000	200	O: 0 H: 0

MMA – Military Medical Academy; CC – Clinical Center; CCV – Clinical Center of Vojvodina.

In Table 1, the number of outpatient-treated and hospitalized neurology patients during the COVID-19 pandemic in Serbia (period March-September, 2020) is given, while in Table 2, the frequency and characteristics of neurological disorders in COVID-19 patients are presented.

Neurological manifestations of COVID-19 are not uncommon, and in the studies published so far, they count in one-third to as many as half of the treated patients [1–9]. Symptoms and signs may involve the nervous system at all levels, from the brain to muscles. For now, studies most often confirm previous results given in the Chinese population, in which about 30% of patients have neurological manifestations of COVID-19. We presented neurological manifestations that we have observed in 153 COVID-19 positive patients treated at neurological clinics. Like in the other presented study, encephalopathy was the most frequent central nervous system manifestation reported [1–6]. Most cases of altered consciousness were secondary to severe hypoxemia and closely related to the severity of the disease [5–9]. We must

Table 2

Frequency and characteristics of neurological disorders in COVID-19 positive patients

Institution*	Stroke	Neuroinfection of the periphery and central nervous system	Epileptic seizures	Loss of smell and taste	Encephalopathy and psychiatric symptoms	Fatigue and muscle pain
Speicail Hospital "Saint Sava", Belgrade	42/42 (100%)	0	0	6/42 (14.3%)	10/42 (23.8%)	4/42 (9.5%)
Clinic for Neurology, MMA, Belgrade	11/38 (29%)	3/38 (7.9%)	7/38 (18.4%)	5/38 (13.2%)	11/38 (29%)	21/38 (55.3%)
Clinic for Neurology CC Niš	9 /62 (14.5%)	7/62 (11.3%)	5/62 (8.1%)	15/62 (24.2%)	12/62 (19.4%)	20/62 (32.3%)
Clinic for Neurology CCV, Novi Sad	5/11 (45.5%)		2/11 (18.2%)	4/11 (36.4%)	4/11 (36.4%)	4/11 (36.4%)

***For abbreviations see under Table 1.**
Certain patients exhibited more than one neurological manifestation.
All values are presented as numbers (percentages).

emphasize the fact that more and more studies report a higher percentage of cerebrovascular events, which were the initial results reported in the study by Mao et al. [1]. A possible explanation could be the fact that in one number of patients, the acute cerebrovascular disease was the first sign of COVID-19. All this indicates that the neurological manifestations, their development, and unpredictable course in COVID-19 have not been fully studied. Literature data highlight the problem of muscle damage and the consequent feeling of weakness and the appearance of myalgias. Although our data indicate that these symptoms occur in more than thirty percent of neurological manifestations, there are not enough data yet to speculate about the pathogenesis of muscular involvement [1–9].

Ranko Raičević*†, Željko Živanović‡§, Marijana Vukićević‖, Miroslava Živković¶, Viktor Pasovski*, Mirjana Stojković*, Marija Grunauer*, Tija Apostolović‖, Aleksandra Ilić‡§

Military Medical Academy, *Clinic for Neurology, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; Clinical Center of Vojvodina, ‡Clinic for Neurology, Novi Sad, Serbia; University of Novi Sad, §Faculty of Medicine, Novi Sad, Serbia; ‖Special Hospital for Cerebrovascular Diseases "Saint Sava", Belgrade, Serbia; Clinical Center of Niš, ¶Department of Neurology, Niš, Serbia

REFERENCES

1. *Mao L, Jin H, Wang M, Hu Y, Chen S, HeQ,* et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020; 77(6): 683–90.
2. *Correia AO, Feitosa PWG, Moreira JLS, Nogueira SAR, Fonseca RB, Nobre MAP.* Neurological manifestations of COVID-19 and other coronaviruses: a systematic review. Neurol Psychiatry Brain Res 2020; 37: 27–32.
3. *Baj J, Karakuła-Juchnowicz H, Teresiński G, Buszewicz G, Ciesielka M, Sitarz E,* et al. COVID-19: Specific and Non-Specific Clinical Manifestations and Symptoms: The Current State of Knowledge. J Clin Med 2020; 9(6): 1753.
4. *Oxley TJ, Mocco J, Majidi S, Kellner CP, Shirah H, Singh IP,* et al. Large-vessel stroke as a presenting feature of COVID-19 in the young. N Engl J Med 2020; 382(20): e60.
5. *Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW,* et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. JAMA 2020; 323(20): 2052–9.
6. *Baig AM.* Neurological manifestations in COVID-19 caused by SARS-CoV-2. CNS Neurosci Ther 2020; 26: 499–501.
7. *Helms J, Kremer S, Merdji H, Clere-Jehl* R, *Schenk M, Kummerlen C,* et al. Neurologic features in severe SARS-CoV-2 infection. N Engl J Med 2020; 382(23): 2268–70.
8. *Zhao H, Shen D, Zhou H, Liu J, Chen S.* Guillain– Barré syndrome associated with SARS-CoV-2 infection: causality or coincidence? Lancet Neurol 2020; 19(: 383–4.
9. *Ye M, Ren Y, Lv T.* Encephalitis as a clinical manifestation of COVID-19. Brain Behav Immun 2020; 88: 945–6.

Received on February 5, 2021.
Accepted on February 10, 2021.
Online First February, 2021.

LETTER TO THE EDITOR
(RESEARH LETTER)

UDC: 612.017:612.371]:[616.98:578.834
https://doi.org/10.2298/VSP200220019B

Long-term antibody-response monitoring following primary exposure to SARS-COV-2 and afterward mRNA COVID-19 vaccination: A case report

Dugoročno praćenje odgovora posredovanog antitelima posle primarne ekspozicije SARS-COV-2 i posle mRNA COVID-19 vakcinacije: Prikaz slučaja

Key words:
antibodies; covid-19; covid-19 serotherapy; infections; vaccination.

Ključne reči:
antitela; covid-19; covid-19 seroterapija; infekcije; vakcinacija.

To the Editor:

The majority of individuals infected by the novel coronavirus, or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), typically have a mild/moderate form of the resulting "coronavirus disease 2019" (COVID-19). However, the current pandemic counts of COVID-19 cases are higher than 111 million infected individuals worldwide, with approximately 2.5 million deaths. The most important preconditions for COVID-19 "expansion" are: 1) high potential of "human-to-human" virus transmission and 2) existence of an "immunologically-naive" background, i.e., population [1–4].

Rapid and almost limitless spreading of the disease has inspired the emergence of intense fundamental and preclinical studies, as well as initial clinical trials. The aim of these investigations was: 1) to determine multiple immune-mediated and other morphological, functional, and molecular "damaging-events" in targeted tissues; 2) to improve diagnostic tools in order to verify or exclude SARS-CoV-2 infection; 3) to "update" available therapy and improve newly-developed treatment options in order to reduce global healthcare-system crisis and decrease morbidity/mortality rate [3–5]. However, multiple prospective studies are needed to determine treatment directions, dosing, and side-effects of these medications.

Antibody-response to the receptor-binding domain (RBD) of the spike (S) protein of SARS-CoV-2 after infection remains incompletely evaluated. Dynamics/kinetics intensity and duration of antibody production, as well as anti-SARS-CoV-2 cross-reactivity with other coronaviruses and antibody-mediated protection after infection, are still undetermined [6,7]. Potential treatments incorporate medicaments, such as antiviral drugs, anti-interleukin-6 receptor monoclonal antibodies (mAbs), and allogeneic convalescent plasma with neutralizing anti-SARS-CoV-2 antibodies, which have been used for some earlier indications and innovative therapeutic approaches/strategies [4–9]. Finally, numerous safe, well-tolerated, and immunogenic COVID-19 vaccines have been developed. Thus, to this day, different vaccines have been already certified or are still progressing through phase-3-trials [10, 11]. Although researchers are not absolutely sure whether the infection itself or the use of vaccines generate a more powerful antibody-response, one fact is undoubtedly evident – the use of vaccines is much safer [7, 10, 11].

The purpose of this letter is to present our results of a 10-month continuous anti-SARS-CoV-2 antibody level monitoring in serum/plasma by enzyme-linked immunosorbent assay (ELISA), after the "initial/natural" exposure to SARS-CoV-2 (infection), followed by the application of the mRNA COVID-19 vaccine. Moreover, some "diagnostic-steps" and data concerning convalescent plasma collection by apheresis – designed for upcoming basic studies and/or potential therapeutic use – will be summarized.

On April 6, 2020, a 67-year-old male was diagnosed with COVID-19, owing to positive molecular testing, using the quantitative Polymerase Chain Reaction (qPCR) technique. SARS-CoV-2 RNA, isolated from nasopharyngeal/oropharyngeal swabs, was reversely transcribed to cDNA

Correspondence to: Bela Balint, Serbian Academy of Sciences and Arts, Kneza Mihaila 35, 11 000 Belgrade, Serbia.
E-mail: balintbela52@yahoo.com

and subsequently amplified using QuantStudio-5 Real-Time PCR-System (Thermo-Fisher Scientific; USA). The results of relevant laboratory testing were the following: white blood cells (WBC) = 7.4 × 10^9/L, lymphocytes = 2.9 × 10^9/L, C-reactive protein (CRP) = 6.9 mg/L (normal ≤ 5.0 mg/L), D-dimer = 0.35 μg/mL (normal ≤ 0.5 μg/mL), and chest radiography was without signs of a pathological process. The patient was self-isolated at home according to the regulations at that time (28-days quarantine) with mild symptoms of COVID-19, such as rare subfebrility (up to 37.3°C), dry cough, and throat scratching. On April 20 and May 5, 2020, the results of the PCR-testing were negative. After that, the patient's quarantine was canceled.

On June 9, 2020, the results of rapid-antibody-testing were negative for IgM and positive for IgG, using Vazyme 2019-nCoV IgG/IgM Detection-Kit (Vazyme Biotech Co. Ltd.; China).

For detecting IgG antibodies to SARS-CoV-2, sera samples were firstly inactivated at 56 ºC for 30 minutes. These assays detected IgG antibodies, targeting the spike (S1) and nucleocapsid (N) proteins. Positive, negative, and cut-off controls were run with each test run. The cut-offs were calculated according to the manufacturer, as well as the antibody index, which was estimated as the ratio of sample and mean cut-off optical densities. Sera displaying antibody indices < 4 are considered as negative, those from 4–6 as equivocal, and those > 6 were presented as positive.

Simultaneously with the rapid-antibody-testing (June 9, 2020), the results of the first IgG anti-SARS-CoV-2 investigations were: 47.5 (positive > 6) by ELISA, using VirClia device (Vircell, Spain), and 7.4 (positive > 1.4) by two-step chemiluminescent microparticle immunoassay (CMIA) using Architect i2000SR system (Abbott, Germany), respectively. After that, levels of IgG antibodies were monitored only by ELISA. Kinetics of antibody levels was nearly constant in the first 6 months after the onset of the symptoms – on August 5, 2020, antibody index was 47.9, and on October 15, 2020, it was 33.3, respectively. These results were comparable with the data from literature [6, 7]. Afterward, progressive decreases in IgG levels were shown on November 30, 2020, and January 11, 2021 – indexes were 18.8 and 17.3, respectively (Figure 1).

On June 15, 2020, convalescent plasma was collected from the investigated person by apheresis. Plasmapheresis was performed by Spectra Optia device (Terumo BCT; USA). The total volume of collected plasma was 960 mL: 360 mL for studies and 750 mL for potential therapeutic use. The patients was non-reactive for hepatitis B and C viruses (HBV and HCV, respectively), human immunodeficiency virus (HIV), and syphilis (lues) markers. Plasma samples (10 mL per tubes; 6 samples) and units (150 mL per bag; 6 units) were cryopreserved with uncontrolled-rate technique ("dump-freezing"; cooling rate: 1–2°C/min) by simple placing of tubes/units into a mechanical freezer ULT C75 (Nordic Lab; Denmark). They will be stored at –90 ± 5°C thawing and investigation (or potential therapeutic use).

On January 13, 2021, the investigated person received the first dose of the mRNA COVID-19 vaccine (Pfizer-BioNTech; USA). The first vaccination was well tolerated, without adverse events or complications. Afterward, the IgG antibody level rapidly increased – the IgG index was 67.87 on January 27, 2021. Finally, the second dose of the same vaccine was applied on February 03, 2021. Following the second dose of vaccine, transient chills manifested 6–8 hours after application. On February 18, 2021, the IgG index (70.3) was also high, as shown in Figure 1. The presented data generally agree with those reported in the literature on both the antibody levels and the duration of their presence in human plasma [6–10, 12].

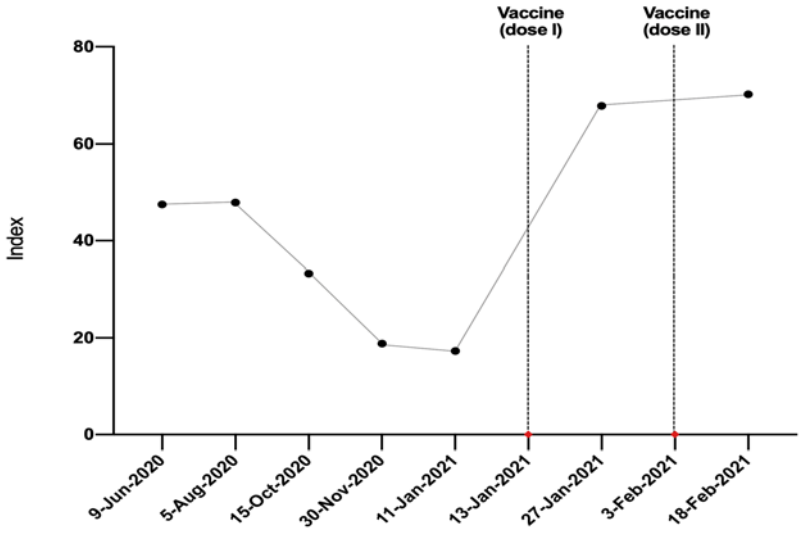


Fig. 1 – Antibody plasma levels in the investigated person before and after application of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine.

As recently verified, immune-response mediated by specific antibodies to RBD epitopes of the SARS-CoV-2 S protein positively and closely correlates with their neutralizing-capacity because RBD is responsible for binding to angiotensin-converting enzyme 2 (ACE2). Thus, the synthesis and elevated plasma level of these antibodies could make an effective platform for SARS-CoV-2 elimination and corre-

late with a milder course of the disease, as well as superior clinical recovery [2, 7]. Antibody-response correlates clearly with SARS-CoV-2 neutralizing activity (virus deactivation/elimination) [6, 7]. Furthermore, as presented, antibody titers remain relatively stable for several (6–8) months after the primary exposure to SARS-CoV-2 [6, 12]. Although the titer of antibodies may significantly decline with time in some persons, the specific T and B memory cells remain [12].

The SARS-Cov-2 infection could be treated by allogeneic plasma collected from recovered COVID-19 patients, typically simultaneously with antiviral agents, steroids, and other medication [4, 8, 9]. Although polyclonal antibodies (existing in collected plasma) are already in routine therapeutic use, further controlled clinical trials are needed to confirm the concept of COVID-19 treatment by convalescent plasma infusion [8, 9]. There are also data concerning the production of mAbs for treating COVID-19. A major disadvantage of this therapeutic approach is an insufficient mAb quantity for expected oversize requests in healthcare systems and their high cost [5]. Finally, since the RBD-region is a potent immunogenic epitope, it is most likely an ideal "antigen-candidate" for vaccine design [7].

In conclusion, the anti-SARS-CoV-2 antibodies detected in this pilot study, particularly their increased plasma level after vaccination, could be protective enough against a possible new COVID-19. We speculate that they could provide a more effective virus elimination following a (re)infection. Besides, in presenting this case, we point out that vaccination (particularly the use of the first dose) to date has demonstrated neither critical side-effects nor inferiority in antibody-response when compared to the infection itself. The results presented require further basic studies and prospective clinical studies.

Conflict of interests

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

Bela Balint*†, Milena Todorović Balint‡§, Zorana Andrić‖, Milica Jovičić‖, Glorija Blagojević‖, Miodrag Čolić*

Serbian Academy of Sciences and Arts, *Department of Medical Sciences, Belgrade, Serbia; Institute of Cardiovascular Diseases "Dedinje", †Department of Transfusion Medicine, Belgrade, Serbia; University Clinical Center of Serbia, ‡Clinic for Hematology, Belgrade, Serbia; University of Belgrade, §Faculty of Medicine, Belgrade, Serbia; ‖Blood Transfusion Institute of Serbia, Belgrade, Serbia

REFERENCES

1. *Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F,* et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med 2020; 383(2): 120–8.
2. *Garvin MR, Alvarez C, Miller JI, Prates ET, Walker AM, Amos BK,* et al. A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm. Elife 2020; 9: e59177.
3. *Khalaf K, Papp N, Chou JT, Hana D, Mackiewicz A, Kaczmarek M.* SARS-CoV-2: Pathogenesis, and advancements in diagnostics and treatment. Front Immunol 2020; 11: 570927.
4. *Ali MJ, Hanif M, Haider MA, Ahmed MU, Sundas F, Hirani A*, et al. Treatment options for COVID-19: A review. Front Med (Lausanne) 2020; 7: 480.
5. *Tuccori M, Ferraro S, Convertino I, Cappello E, Valdiserra G, Blandizzi C*, et al. Anti-SARS-CoV-2 neutralizing monoclonal antibodies: clinical pipeline. MAbs 2020; 12(1): 1854149.
6. *Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M*, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science 2020; 370(6521): 1227–30.
7. *Yin S, Tong X, Huang A, Shen H, Li Y, Liu Y,* et al. Longitudinal anti-SARS-CoV-2 antibody profile and neutralization activity of a COVID-19 patient. J Infect 2020; 81(3): e31–e32.
8. *Xu TM, Lin B, Chen C, Liu LG, Xue Y.* Non-optimal effectiveness of convalescent plasma transfusion and hydroxychloroquine in treating COVID-19: a case report. Virol J 2020; 17(1): 80.
9. *Zeng H, Wang D, Nie J, Liang H, Gu J, Zhao A,* et al. The efficacy assessment of convalescent plasma therapy for COVID-19 patients: a multi-center case series. Signal Transduct Target Ther 2020; 5(1): 219.
10. *Izda V, Jeffries MA, Sawalha AH.* COVID-19: A review of therapeutic strategies and vaccine candidates. Clin Immunol 2021; 222: 108634.
11. *Awadasseid A, Wu Y, Tanaka Y, Zhang W.* Current advances in the development of SARS-CoV-2 vaccines. Int J Biol Sci 2021; 17(1): 8–19.
12. *Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE,* et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021; 371(6529): eabf4063.

Received on February 20, 2021.
Accepted February 24, 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 right 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 desnom 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