Vol 60, No 2, June, 2021
UDK 61
ISSN 0365-4478 (Printed)
ISSN 1821-2794 (Online)
www.medfak.ni.ac.rs/amm

ACTA MEDICA MEDIANAE

Naučni časopis
Medicinskog fakulteta Univerziteta u Nišu i
Podružnice Srpskog lekarskog društva u Nišu

AMM

Scientific Journal of the University of Niš Faculty of Medicine
and the Department of the Serbian Medical Society in Niš

Naučni časopis Medicinskog fakulteta Univerziteta u Nišu i Podružnice Srpskog lekarskog društva u Nišu

Scientific journal of the University of Niš Faculty of Medicine and the Department of the Serbian Medical Society in Niš

Acta Medica Medianae
Vol 60, No 2, June, 2021
UDK 61 ISSN 0365-4478 (Printed version)
ISSN 1821-2794 (Online)
http://www.medfak.ni.ac.rs/amm

Acta Medica Medianae (UDK 61; ISSN 0365-4478 štampana verzija; ISSN 1821-2794 elektronska verzija) je zvanični časopis Medicinskog fakulteta Univerziteta u Nišu i Podružnice Srpskog lekarskog društva u Nišu pod pokroviteljstvom Ministarstva za nauku i tehnološki razvoj Republike Srbije. Časopis izlazi četiri puta godišnje od 1962 godine. Izdavač je Medicinski fakultet Univerziteta u Nišu, Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija. Sadržaj i celokupan tekst časopisa dostupan je na sajtu Medicinskog fakulteta http://www.medfak.ni.ac.rs/amm. Uputstvo autorima se objavljuje u svakom broju, pri čemu je autor dužan da se pridržava navedenih uputstava prilikom predaje rukopisa.
Radovi se prijavljuju putem onlajn sistema e-Ur: http://aseestant.ceon.rs/index.php/amm/login , a u izuzetnim slučajevima se mogu slati u elektronskom formatu na adresu: acta@medfak.ni.ac.rs.

Kontakt adresa: Časopis Acta Medica Medianae, Medicinski fakultet, Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: acta@medfak.ni.ac.rs
Tel+381-18-4533001 lok. 122 fax. +381-18-4534336
Tiraž 200 primeraka. Stampa: "Galaksijanis", Lukovo, Svrljig, Srbija.
Acta Medica Medianae je trenutno indeksirana na *Index Copernicus-u, Srpskom citatnom indeksu, DOAJ i EBSCO*

Acta Medica Medianae (UDK 61; ISSN 0365-4478 printed version; ISSN 1821-2794 online) is the official Journal of the University of Niš Faculty of Medicine and the Department of the Serbian Medical Society in Niš published with the help of the Ministry of Science and Technological Development of the Republic of Serbia. The Journal has been published four times a year since 1962. The publisher is the University of Niš Faculty of Medicine, Institutional address: dr Zoran Đinđić 81, 18000 Niš, Serbia. Table of contents and full texts of articles are available on the Institutional Home Page at http://www.medfak.ni.ac.rs/amm. Prices are subject to change. All subscriptions start with the first issue of the current year. For payment details contact the Secreteriat at acta@medfak.ni.ac.rs. Instructions for authors appear in every issue. Manuscripts accepted for publication are not returned to the author(s). *Acta Medica Medianae* retains the right for further distribution and printing of the articles.
Editorial correspodence: Journal Acta Medica Medianae, Faculty of Medicine, Dr Zoran Đinđić 81, 18000 Niš, Serbia.
Electronic submission of the papers: acta@medfak.ni.ac.rs
Phone: +381-18-4533001 lok. 113 fax. +381-18-4534336
Printed on acid-free paper; 200 issues. Press: "Galaksijanis", Lukovo, Svrljig, Serbia.
Acta Medica Medianae is currently indexed in Index Copernicus, Serbian Citation Index, DOAJ and EBSCO

Naučni časopis Medicinskog fakulteta Univerziteta u Nišu i Podružnice Srpskog lekarskog društva u Nišu

Scientific journal of the University of Niš Faculty of Medicine and the Department of the Serbian Medical Society in Niš

Acta Medica Medianae
Vol 60, No 2, June, 2021
UDK 61 ISSN 0365-4478 (Printed version)
ISSN 1821-2794 (Online)
http://www.medfak.ni.ac.rs/amm

Autor fotografije na prednjoj stranici:
Petar Petrović

ACTA MEDICA MEDIANAE

Vol 60, No 2, June, 2021

www.medfak.ni.ac.rs/amm

ACTA
MEDICA
MEDIANAE

Vol 60, No 2, June, 2021

www.medfak.ni.ac.rs/amm

ACTA MEDICA MEDIANAE

Vol 60, No 2, Jun, 2021

Uredništvo

www.medfak.ni.ac.rs/amm

ACTA
MEDICA
MEDIANAE

Vol 60, No 2, Jun, 2021

www.medfak.ni.ac.rs/amm

Original article

UDC: 616.348-006.6:577.152
doi:10.5633/amm.2021.0201

THE IMPORTANCE OF URATE PATHWAY ENZYMES ACTIVITY AND ITS RELATION WITH OXIDATIVE STRESS IN PROGRESSION AND INVASION OF HUMAN COLORECTAL CANCER

Andrej Veljković[1], Jovan Hadži-Djokić[3], Goran Stanojević[2], Branko Branković[2], Aleksandar Skakić[2], Ivana Damjanović[1], Mladen Stojanović[2], Andrija Šmelcerović[1], Gordana Kocić[1]

Colorectal cancer (CRC) is one of the main reasons for the mortality connected with tumor diseases. There is still a shortage of examination including the influence of urate pathway enzymes in the progressiveness and invasion of CRC, so the present study investigated the role of xanthine oxidase (XO), adenosine deaminase (ADA) and 5'-nucleotidase (5'-NT) activity, concerning TBA-reactive substances (TBARS) as an oxidative stress (OS) marker in progression, also an invasion of human colorectal cancer.

We took tissue specimens from 50 patients with colon cancer, in all four TNM clinical stages of the disease. They were divided into 3 groups: cancer tissue, tissue surrounding the tumor and healthy control tissue group. We made 10% homogenates in which we conducted the study with proper methods.

The activity of ADA and XO in tumor tissue and tissue adjacent to the tumor is statistically higher in comparison to healthy colon tissue. The 5'-NT is not significantly higher in carcinoma tissue. The highest activity of ADA and XO is in T2 and T3 tumor stages. TBARS has the highest concentration in T3 and T4 stages of the tumor.

Presented results suggest that the possible cause of OS in colon carcinoma is high XO and ADA activity. It may include those enzymes in the transformation of the colon tissue, as well as in the progression of CRC. So, the ADA and XO activity might be helpful in determing the margins of colon resection. They can have significance in diagnosis, but in the prognosis of the disease as well.

Acta Medica Medianae 2021;60(2):05-14.

Key words: *colorectal cancer, adenosine deaminase, 5'-nucleotidase, xanthine oxidase, oxidative stress*

[1]University of Niš, Faculty of Medicine, Niš, Serbia
[2]University Clinical Center Niš, Niš, Serbia
[3]Serbian Academy of Sciences and Arts, Belgrade, Serbia

Contact: Andrej Veljković
81 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: veljkovicandrej@yahoo.com

Introduction

Colorectal cancer (CRC) is the third most frequent tumour in the human population. It is one of the main reasons for mortality connected with tumor diseases (1). Despite the fast development of diagnostic and treatment strategies, the five-year survival rate of CRC remains poor, mostly because of recurrence and metastasis. However, the precise mechanisms resulting in the initiation and progression of CRC remain unclear, and it has focused considerable scientific interest on the molecular pathogenesis of CRC. Emerging evidence has shown that enzymes of purine nucleotides are included in the pathogenesis and progression of CRC.

Adenosine deaminase enzyme (ADA) catalyses the conversion of adenosine to inosine which is in the end transformed into uric acid (2). Adenosine is a vital signalling molecule that exerts main anti-inflammatory movements in tumorous conditions including inhibition of tumour infiltration in lymphoid cells (3). Higher ADA activity can also additionally have an impact on scavenging of a vital molecule, adenosine.

Adenine nucleotide catabolism that embodies vital pathways of the intermediary metabolism maintained the regulatory effector (adenosine) and molecular energy compound, adenosine triphosphate (ATP). In a lot of tissues, it gives an excellent

adenine nucleotide pool through a specialised mechanism that correlates with adenosine 5' monophosphate (AMP) metabolism (4). Two essential enzyme sequences typically take a component withinside the catalysis of the original AMP metabolism pathway. The first is AMP deaminase, which catalyses the deamination of AMP to provide inosine monophosphate (IMP). The 2nd is 5'-nucleotidase (5'-NU), which catalyses the dephosphorylation of AMP to provide adenosine. In addition, the catabolism method consists of the conversion of adenosine to inosine through adenosine deaminase (ADA) catalytic activity (5).

Human adenosine deaminase-ADA (E.C. 3.5.4.4.) exists in lots of molecular forms, with specific molecular weights. It performs an important function as a key enzyme involved in the salvage of purine nucleosides and the utilization of purines (6). Many researchers have found out the best activity and significant molecular heterogeneousness of intestinal adenosine deaminase. Besides soluble adenosine deaminase shape, a particulate-membrane bound shape turned into isolated additionally from the normal gut, however, a cancer-unique shape became isolated in some colorectal tumours (7, 8).

The second is 5'-ribonucleotide phosphohydrolase-5'-nucleotidase (5'-NT E.C.3.1.3.5.), which catalyses the dephosphorylation of AMP to produce adenosine. This was first described by Reis in 1934. It is a phosphomonoesterase because it catalyses the hydrolytic degradation of monophosphate nucleotides (AMP, GMP, CMP, UMP, IMP) and their deoxy analogues (9). Although the activity of this enzyme has been shown to be reduced in tissues and neoplasm cells in some studies (10), some researchers have additionally found high 5'-NT activity in cancer tissue relative to surrounding normal tissue (11).

Xanthine oxidase (XO) catalyses the final degradation of purine bases which generate uric acid, that's the final product of purine catabolism (5). It is widely recognized that xanthine oxidase (XO) is an enzyme found in interconvertible forms, dehydrogenase and oxidase. Results of our previous examinations have proven that during most cancers the tissue through oxidation of sulfhydryl groups or restricted proteolysis, dehydrogenase XO activity is transformed to oxidase form that produces hydrogen peroxide and superoxide. (12). Simultaneously with the production of uric acid, XO activity liberates hydrogen peroxide and superoxide anion, which are one of the major ROS and oxidative stress-inducers. DNA damage caused through ROS performs a vital function in the carcinogenic transformation of the cell (13). There are lots of pathological conditions at some stage in which elevated plasma XO exists, like cholecystitis, shock, ischaemia-reperfusion injury, acute virus infection, adult respiration distress syndrome, carcinogenesis (14). It has not been clarified but whether or not the activity of XO will increase or decline in human cancers. Since there is still a shortage of examination including influence of these enzymes in progressiveness and invasion of CRC, the present study investigated the role of urate pathway enzyme activity, such as ADA, 5'-NT and XO, in relation to oxidative stress in progression and invasion of human colorectal cancer.

Materials and methods

Patients and tissue samples

The investigation was conducted in 50 patients with CRC at the Clinical Centre Niš, Serbia. All patients gave their knowledgeable consent for inclusion prior to their participation in the study. The investigation was carried out in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Faculty of Medicine in Niš (Decision No. 01-1591/8).

We took tissues from 50 patients with primary colorectal cancers. Patients were at different stages of the disease according to TNM classification. Tissue specimens used for this study were obtained during the surgery as soon as possible after resection of the carcinoma. In all patients, ph confirmed adenocarcinoma, and we excluded patients with other types of tumours from the study. We also excluded patients with: gravidity, co-malignancies, inoperable tumoirs, preceding chemotherapy or radiotherapy. Tumour stages are shown in Table 1.

Table 1. Tumour Stages (TNM Classification)

Tumor stage	Gender		Age (Mean)
	m	w	
T1	4	2	50.5
T2	8	3	52.5
T3	16	9	63
T4	5	3	61.5
In total	33	17	56.5

Stage I tumours (n = 6) are tumours limited to the bowel wall. Stage II (n = 11) denotes tumours that have penetrated the muscularis propria. Stage III (n = 25), the tumours have spread to involve the regional nodes. Stage IV (8), the tumours which have a faraway metastasis. Also, as a control, we gathered the identical quantity of samples from macroscopically unchanged colon areas farthest from cancer, and tissue immediately surrounding the tumour without a macroscopic or pathological manifestations.

Preparation of tissue samples

We removed tissues quickly during the process of surgery. All samples were placed in iced 0.15 mol/L NaCl solution, perfused with an isotonic solution to get rid of blood cells and other tissue residues. Further on, after removal of fat, connective tissue, and major vessels, the tissue was cut into small pieces and washed with de-mineralized water to remove RBC as much as possible and subsequently with 0.15 M phosphate-buffered (30 HIM) saline (pH 7.5). We homogenized the tissue with a homogenizer with a teflon pestle; made 10% homogenates and centrifuged them at 3,000 x g for 15 min, and the supernatant was frozen at -80 ^{O}C and kept until assayed.

Biochemical assays

The ADA activity

We measured activity of ADA according to the slightly modified method of Pederson and Berry (15, 16). We expressed enzyme activity in U/g protein.

The 5'-NT activity

The activities of the 5'-nucleotidase were determined by Wood and Wiliams method (17). Substrate was AMP at an optimal pH of 7.5 using a barbiturate-HCL buffer. 5'-nucleotidase activity is expressed as IJ/mg protein.

Xanthine oxidase activity

XO activity was evaluated with spectrophotometric method, by using xanthine as a substrate where the uric acid formation was measured. Enzyme activity was expressed in IJ/mg of protein (18).

TBARS concentration

TBA concentration of reactive substances in the homogenate was measured using the slightly modified Nabavi et al. method (19). MDA-reactive lipid peroxidation products were measured at 532 nm. We expressed concentration in nmol/mg of protein.

Protein content

The amount of protein was determined by Lowry et al. method, where by bovine serum albumin was a standard (20).

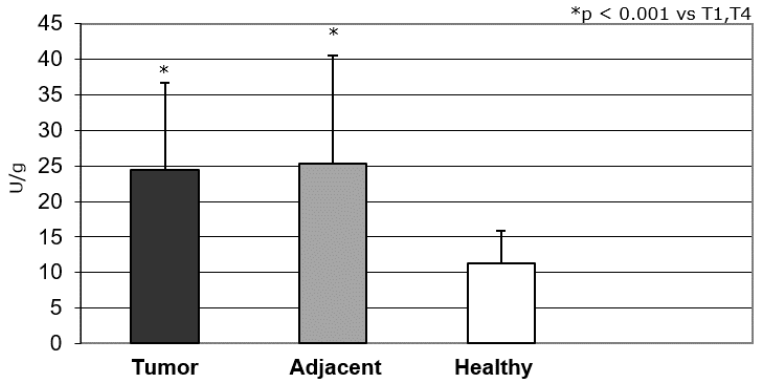
Statistical analysis

The values of all parameters were expressed as X ± SD (mean value ± standard deviation). The examination of received data was assessed via way of means of the t-test evaluating the enzyme activity of mucosa with pathological manifestations or mucosa next to tumour tissue with the activity of corresponding further healthy tissue and with the activity of acquired tissue from patients without pathological manifestations.

The statistical significance of differences between TNM stages of tumour was calculated using the ANOVA test. The limit value of $p < 0.05$ was considered to be statistically significant.

Results

The activity of ADA in tumour tissue was significantly higher in comparison to healthy control tissue ($p < 0.001$). Tissue surrounding the tumour likewise had higher activity of ADA in relation to control tissue ($p < 0.001$) (Graph 1).



Graph 1. The ADA activity in tumour, adjacent and healthy colon tissue

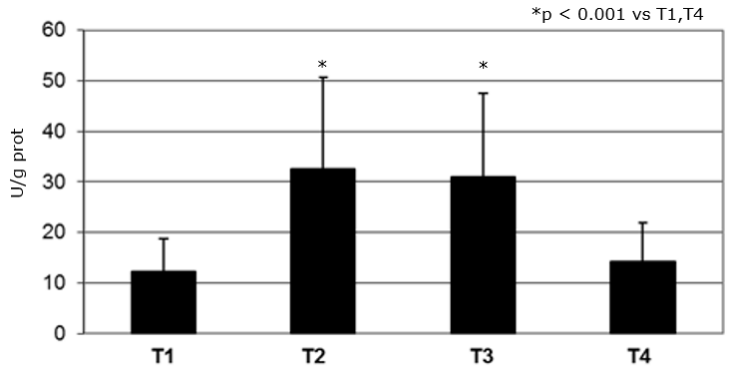
T2 and T3 tumour stages had a significantly higher activity of ADA when compared to T1, and T4 tumour stages ($p < 0.001$). Highest activity was in T2 stage without statistical significance compared to T3 stage (Graph 2).

The activity of 5'-NT was the highest in adjacent tissue, but without statistical significance (Graph 3).

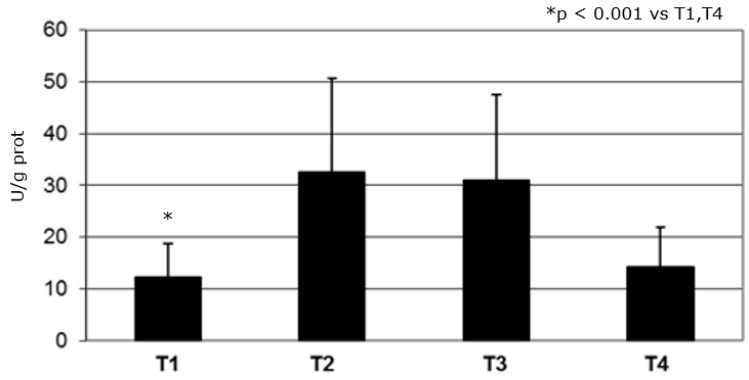
The activity of XO in tumour tissue was notably higher in comparison to control colon tissue ($p < 0.001$). Also, the tissue of tumour had significantly higher XO activity when compared to adjacent tissue ($p < 0.001$) (Graph 4).

T3 and T4 tumour stages had a significantly higher XO activity when compared to T1, T2 tumour stages ($p < 0.001$) (Graph 5).

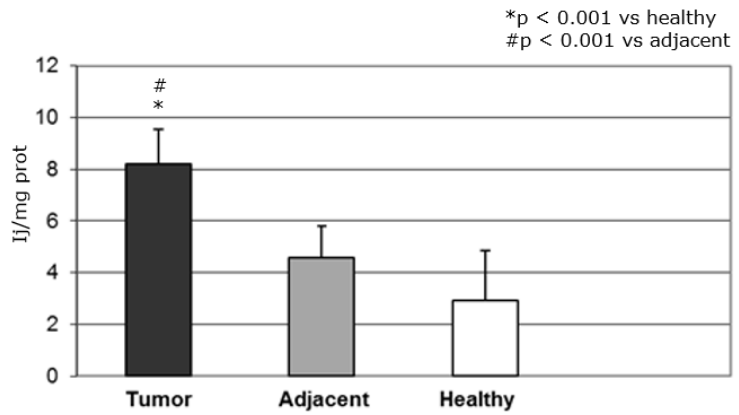
The T4 tumour stage had a significantly higher concentration of TBARS when compared to T1, T2, and T3 tumour stages ($p < 0.001$) (Graph 6).



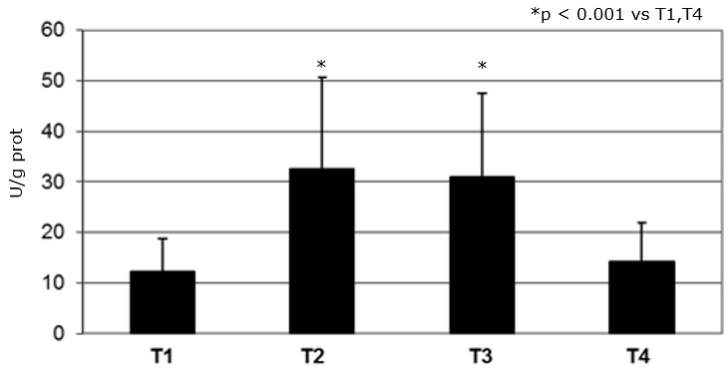
Graph 2. The ADA activity in tumour tissue, patients with T1, T2, T3 and T4 stage



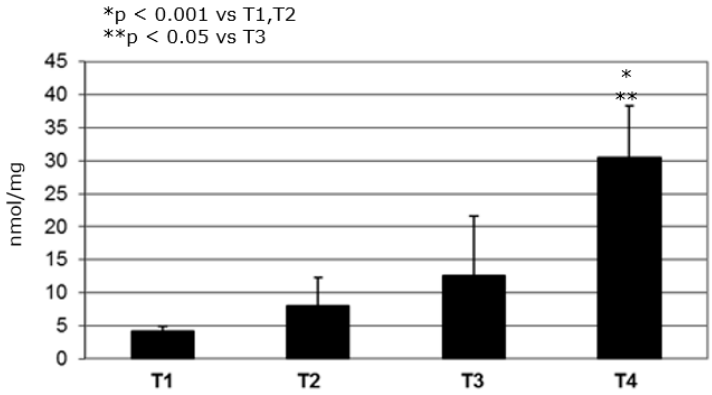
Graph 3. The 5'-NT Activity in tumour, adjacent and healthy colon tissue



Graph 4. The XO activity in tumour, adjacent and healthy colon tissue



Graph 5. The XO activity in tumour tissue of patients with T1, T2, T3, and T4 stage



Graph 6. The concentration of TBARS in tumour tissue of patients with T1, T2, T3, and T4 stage

Discussion

Alterations in the enzymology of the human colorectal tumour absolutely distinguished it from the regular colorectal mucosa. To get a higher knowledge of purine enzymology in colorectal carcinoma, we have paid a great deal interest to investigating the interrelations among the carcinogenic process and the activities of some urate pathway enzymes. CRC is a complex disease, multifactorial, related to accumulated oxidative stress and inflammation followed by fast tissue proliferation. In this study, the role of ADA, 5'-NT, and XO has been investigated concerning oxidative stress in CRC patients.

Adenosine deaminase (ADA) an enzyme of the purine metabolism is widespread in tissues and relatively high levels have been found in the villi intestinal of the large intestine epithelial cells.

Several researches have proven changes of ADA activity withinside the tumour tissue and blood serum in patients with lung, head and neck, breast, and ovarian cancer (21, 22). Additionally, a group of authors founded elevated ADA activity in the cancerous large intestine tissue (23).

ADA is particularly susceptible to stimulation by growth factors and cytokines during rapid tissue proliferation (24). Various researches have documented the rise of ADA in unexpectedly developing malignancies, where it has been documented as a tumour marker, whereas slow-developing well-differentiated tumours do not show prominent enzyme activity (25). Results of our observation additionally display that there may be a higher activity of an enzyme in cancer tissue in comparison to the control healthy tissue of colon in our study. But, the tissue surrounding the cancer has even higher activity of this enzyme. So ADA could be included in tumour proliferation and invasion of large intestine tissue where the tumour is located, directly to adjacent tissue. One of the possible reasons could be lower effect of adenosine.

Indeed, improved ADA activity in patients suggests decreased availability of adenosine, which may be a defensive molecule just in case of the tumorous condition. Adenosine is an endogenous purine nucleoside generated from ATP (26). This is an important signalling molecule that exerts important anti-inflammatory action. Tumours have an excessive concentration of adenosine that could inhibit the characteristic of tumour-infiltrating lymphoid cells (2). We may take this as a compensative mechanism against the tumorous conditions. ADA enzyme scavenges adenosine through degrading it into inosine, which eventually gets regenerated into uric acid (3). There are numerous proofs that adenosine acts as a crucial regulative autocrine and paracrine component accumulating in the cellular micro-environment (27). The concentration of adenosine, which is typically low in physiological conditions, will grow in reaction to certain conditions, including

inflammation, hypoxia, ischaemia, or trauma (28). The fast accumulation of extracellular adenosine has a protecting effect, since it prevents immoderate inflammatory reaction of the cells in order to help the tissues return to their physiological state (29). However, in the tumour environment, low adenosine concentration may be associated with immunosuppression leading to neoplasia (30), which is related to our results, which show the highest ADA activity in the tissue surrounding the tumour.

Hypoxia is one of the reasons malignant tumours no longer perform the functions necessary for cellular homeostases, such as the proper production of ATP, resulting in the decomposition of nucleotides and the discharge of adenosine. Some authors, therefore, proposed that adenosine is the main component in promoting tumour increase (31). The direct consequences of adenosine on molecular growth *in vitro* are controversial (32, 33). There is a significant evidence that low levels of extracellular adenosine, both in a paracrine or autocrine manner, can promote tumour increase in numerous ways (31). Next, the available data strongly support the low adenosine concentration as a stimulator of angiogenesis (34). Third, adenosine has a role in lowering the inflammatory and cellular immunity responses and contributes to the formation of specific, tumour immune barriers (35). It can also take part in signal transduction through specific adenosine receptors, which result in the alterations in the adenyl cyclase system and PLC activity (36).

A crucial aspect of most cancers cells is their spreading from the primary tumour that consequently produces diverse clinical symptoms. There are generally four clinical levels of CRC which are highlighted in the literature.

Results of our investigation show the highest activity of ADA in the T2 and T3 stages of the disease (Graph 2). Those are the stages where the tissue invasion and proliferation are the highest. So, ADA might be included in tumour progression.

Other authors additionally found that inflammation progresses with the development of the disorder in patients with clinical degree four compared to the ones having stages 1, 2, or 3 of breast cancer. Mahajan (37) confirmed most activity of ADA at stage three of breast cancer, but at some point of their observation probably they did not come across any affected person with stage 4 breast cancer. High levels of ADA activity can additionally be interpreted as a compensatory mechanism for tumours towards highly toxic adenosine, deoxyadenosine and its derivatives, ADP, and dATP, which are effective inhibitors of ribonucleotide reduction, a restricting enzyme in nucleic acid biosynthesis (38), which can be higher in tumour than in normal colon tissue (39).

The proof of excessive ADA activity in fast and stimulated normal tissue increase is crucial for the existence of useful purine metabolism because of the viable inactivation of adenosine and 2'-deoxyadenosine, toxic metabolites for the cells increase (40). The enzyme is mainly sensitive to stimulation by growth factors and cytokines in the course of rapid tissue proliferation (24). Therefore, a few data show that ADA isn't always directly involved in carcinogenesis, however has a metabolic function in assisting a fast increase state of relevant tissues, through the re-utilisation of nucleosides, related as RNA and associated precursors. When CRC cells are treated with deoxycoformicine, an ADA inhibitor, the cell growth is inhibited (41). The highest ADA activity in T2 and T3 tumour stages may support its influence in the progression and invasiveness of the colon carcinoma.

Some authors additionally related accumulated ADA activity to lower or deficient ADA complexing macromolecule (ADBP), a specific glycoprotein localized in the healthy colon membranes. The monoclonal antibodies against tumour represent also ADBP (42).

We have also investigated the activity of 5'-NT to test the process of enzymes resolving mononucleotide to nucleosides. Although, Sanfilippo et al. (43) have shown that there was no important difference in different activity in tumour and healthy large intestine tissue, within the study by Eroglu et al. (23) activity of 5'-NT in tumour tissue was above in tumour-free tissue. They even found that its level was related to the development of the carcinoma.

Our results do not show a statistically significant higher value of 5'-NT activity in the tumour compared to the control colon tissue and tissue adjacent to the tumour. Low activity of 5'-NT, also the accumulated ADA activity results in reduced levels of adenosine. These conditions result in enhanced OS and generate some complications as a result of the amount of remaining adenosine does not perform its physiological functions. Additionally, adenosine is a generally anti-inflammatory agent, that suppresses neoplasm necrosis factor-alpha (TNF-α) production in monocytes and macrophages, inhibiting the liberation of arachidonic acid and leukotriene production in neutrophils (44). Adenosine can also be an activator in antioxidant enzyme signalling pathways. In our preceding report, we suggested that colon cancer tissue had an extensively higher concentration of oxidative stress parameters which included TBARS and advanced oxidation protein products as compared to healthy colon tissue that represented control. The tissue adjacent to the tumour additionally had a higher concentration of those oxidative products as compared to the control, and it could consist of oxidative stress in the process of tumour development and nearby invasion. Also there may be a decreased activity of anti-oxidative enzymes (45).

Erkiliç et al. (46) reported that ADA would increase the production of ROS, like H2O2, O2-, NO, and 1O2. The high concentration of ROS causes OS, which leads to inflammation by enhancing lipid peroxidation next to the membrane.

The lower 5'-NT activity is considered to be a consequence of destruction of the membrane and its structure because of the high levels of ROS (47). Extracellular AMP is hydrolysed by the action of 5'-NT on free phosphate and adenosine. The most commonly suggested reason for depletion of activity is precisely the oxidative modification of the 5'-NT sulfhydryl (-SH) groups and the interaction with LPO also, the final product of oxidative stress.

This conclusion is derived from a previous study which shown the inhibition of 5'-NT activity by impaired sulfhydryl groups compared with many different enzymes (48).

But our results show the highest concentration of TBARS in T3 and especially T4 stadium of the disease (Graph 6). The highest ADA activity is in T2 and T3 stages, so ADA is not likely the source of ROS.

Further, the enhanced ADA activity which raises the xanthine concentration might lead to higher XO activity. Therefore, the high activity of XO may be linked to the high xanthine levels present in the cancer tissue, because it is the substrate of XO. Therefore, higher levels of xanthine raise XO activity, which might be the source of high oxidative stress. It is the main enzyme that links the metabolism of purines and free radicals along with oxidative stress.

The results of this study showed a high rise in XO activity in cancer tissues when compared with the healthy tissue and also the adjacent tissue. Tissue surrounding the tumour had lower activity when compared to tumour tissue, so XO is not the main reason of tumour proliferation.

Most of the previous studies reported lower XO activity in cancers, and it is suggested that the lower purine catabolism and higher activity of salvage pathway enzymes could favour tumour cell growth (49). Some of the previous researches have refuted the thesis that XO activity is the source of the oxidative stress (50). The lower XOR activity in more aggressive cancer cells has unexplained effects on tumour development and leads to cancer growth and metastasis.

We could relate increased XO activity to the change of the dehydrogenase form of XO into the oxidase one, by process of the oxidation of thiol groups or by proteolytic degradation caused by higher level of peroxynitrite. Furthermore, higher activity of ADA that raises the xanthine pool could lead to increased XO activity. Therefore, the high XO activity could be explained by the higher levels of xanthine.

Our results showed the highest XO activity in T3 and T4 stage (Graph 5) and it is in correlation with the TBARS concentration in the higher stage of the disease.

ROS induced by the activity of XO can influence the higher hypoxia-inducible factor 1a expression and activate NF-kB, and thus contribute to cancer-associated inflammatory signalling and to tumour progression (51, 52). One recent article has shown that xanthine oxidase inhibition could suppress migration of the cell and metastastatic potential of breast cancer (53).

High level of OS in the last cancer stage in our investigation shows the advancement of the tumour since the higher OS gives rise to inflammatory processes. ROS can also act as a secondary messenger by activating intracellular signalling pathways, particularly NF-kB, one of the major modulators of carcinogenesis. Oxidative activation, stimulates the expression of many pro-inflammatory cytokines in the epithelium of the gut, such as TNF-α, IL-8, and COX-2, and leads to inflammation and tumorigenesis (54).

Increased XO activity in the tissue of patients with cancer in our study suggests that OS may be increased in cancerous changes and processes, and could affect the course of the disease. We may relate higher XO activity in our study to increased levels of TBARS in tumour tissue representing markers of oxidative damage.

Conclusion

We can conclude that colon cancerogenesis could require a higher concentration of many metabolic changes responsible for tumour development in a co-operative fashion. Presented results could suggest that the possible cause of OS in colon carcinoma is high XO and ADA activity. It may include those enzymes in the transformation of the colon tissue, as well as in the progression of CRC. So, the ADA and XO activity might be helpful in determining the margins of colon resection. They can have significance in diagnosis, but in the prognosis of the disease as well.

The simplicity of measuring activity asserts the usefulness of these enzymes in some patients where cytopathological findings cannot lead to a clear conclusion, this simple test, together with all clinical findings can have significance in diagnosis, but in the prognosis of the disease as well.

Acknowledgments

This research was supported by the Internal Scientific project of the Faculty of Medicine in Niš (Project number 45), and the projects of the Ministry of Science and Technological development of the Republic of Serbia; and Academy of Sciences and Arts, a branch in Niš (Projects number O-06-17 and O-07-17).

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca A Cancer J Clin 2018; 68:394-424. [CrossRef] [PubMed]
2. Lokshin A, Tatiana R, Huang X, Zacharia LC, Jackson ED, Gorelik E. Adenosine-mediated inhibition of the cytotoxic activity and production by activated natural killer cells. Cancer Res 2006;66:7758-65. [CrossRef] [PubMed]
3. Eltzschig HK, Weissmuller T, Mager A, Eckle T. Nucleotide metabolism and cell cell interactions. Methods Mol Biol 2006;341:73-87. [CrossRef] [PubMed]
4. Banihani SA. Role of uric acid in semen. Biomolecules 2018;8(3):E65. [CrossRef] [PubMed]
5. Kimoloi S. Modulation of the de novo purine nucleotide pathway as a therapeutic strategy in mitochondrial myopathy. Pharmacol Res 2018;138:37-42. [CrossRef] [PubMed]
6. Daddona PE, Kelley WN. Analysis of normal and mutant forms of human adenosine deaminase - a review. Mol Cell Biochem 1980;29(2):91-101. [CrossRef] [PubMed]
7. Piggot CO, Brady TG. Purification of multiple forms of adenosine deaminase from rabbit inetstine. Bioch Biophys Acta 1976;429:600-7. [CrossRef] [PubMed]
8. Pedley AM, Benkovic SJ. A new view into the regulation of purine metabolism: The purinosome. Trends Biochem Sci 2017;42:141-54. [CrossRef] [PubMed]
9. Kate J, Wijnen JT, Herbschleb-Voogt E, Griffionen G, Bosman FT, Khan PM. Adenosine deaminase (ADA, E.C. no 3.5.4.4.) In colorectal adenocarcinoma in man. Adv Exp Biol Med 1984;165:299-303. [CrossRef] [PubMed]
10. Durak I, Isik AU, Canbolat O, Akyol B, Kavutcu M. Adenosine deaminase, 5'-nucleotidase, xanthine oxidase, superoxide dismutase and catalase activities in cancerous and noncancerous human laryngeal tissues. Free Radic Biol Med 1993;15:681-4. [CrossRef] [PubMed]
11. Canbolat O, Durak I, Cetin R, Kavutcu M, Demirci S, Oztürk S. Activities of adenosine deaminase, 5'-nucleotidase, guanase, and cytidine deaminase enzymes in cancerous and non-cancerous human breast tissues. Breast Cancer Res Treat 1996;37:189-93. [CrossRef] [PubMed]
12. Veljković A, Hadži-Dokić J, Sokolović D, Bašić D, Veličković-Janković L, Stojanović M, et al. Xanthine Oxidase/Dehydrogenase Activity as a Source of Oxidative Stress in Prostate Cancer Tissue. Diagnostics 2020;10(9):668. [CrossRef] [PubMed]
13. Solomon H, Brosh R, Buganim Y, Rotter V. Inactivation of the p53 tumor suppressor gene and activation of the Ras oncogene: cooperative events in tumorigenesis. Discovery Medicine 2010;9(48):448-54. [PubMed]
14. Nath K, Fischerder M, Hostetter HT. The role of oxidants in progressive renal injury. Kidney Int 1994;45: S-111—S-115. [PubMed]
15. Pederson RC, Berry AJ. Sensitive, optimised assay for serum AMP deaminase. Clin Chem 1997;23:1726-33. [CrossRef] [PubMed]
16. Lauber K. Photometric determination of nitrogen: Wet incineration followed by formation of indophenol blue with salicylate/hypochlorite. Clin Chem 1976;67:107-10. [CrossRef] [PubMed]
17. Wood RJ, Williams DG. Colorimetric determination of serum 5'-nucleotidase without deproteinization. Clin Chem 1981;27(3):464-5. [CrossRef] [PubMed]
18. Smelcerovic Z, Veljkovic A, Kocic G, Yancheva D, Petronijevic Z, Anderluh M, et al. Xanthine oxidase inhibitory properties and anti-inflammatory activity of 2-amino-5-alkylidene-thiazol-4-ones. Chemico-Biological Interactions 2015;229:73-81. [CrossRef] [PubMed]
19. Nabavi SM, Nabavi SF, Eslami S, Moghaddam AH. In vivo protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. Food Chem 2012;132:931-5. [CrossRef]
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent, The Journal of Biological Chemistry 1951;193(1):265-75. [CrossRef] [PubMed]
21. Tayyar Kalcioglu M, Kizilay A, Ramazan Yilmaz H, Uz E, Gulec M, Ozturan O, et al. Adenosine deaminase, xanthine oxidase, superoxide dismutase, glutathione peroxidase activities and malondialdehyde levels in the sera of patients with head and neck carcinoma. Kulak Burun Bogaz Ihtis Derg 2004;12(1-2):16-22. [PubMed]
22. Aghaei M, Karami-Tehrani F, Salami S, Atri M. Adenosine deaminase activity in the serum and malignant tumors of breast cancer; the assessment of isoenzyme ADAI and ADA2 activities. Clin Biochem 2005; 38(10):887-91. [CrossRef] [PubMed]
23. Eroglu A, Canbolat O, Demirci S, Kocaoglu H, Eryavuz Y, Akgul H. Activities of adenosine deaminase and 5'-nucleotidase in cancerous and noncancerous human colorectal tissues. Med Oncol 2000;17(4):319-24. [CrossRef] [PubMed]
24. Kocic G, Vlahovic P, Djordjevic V, Bjelakovic G, Koracevic D, Savic V. Effect of growth factors on the enzymes of purine metabolism in culture of regenerating rat liver cells. Arch Physiol Biochem 1995;103: 715-19. [CrossRef] [PubMed]
25. Balis EM. Adenosine deaminase and malignant cells. Ann NY Acad Sci 1985;451:142-9. [CrossRef] [PubMed]
26. Hirata BKB, Oda JMM, Guembarovski RL, Ariza CB, de Oliveira CEC, Watanabe MAE. Molecular markers for breast cancer: prediction on tumor behavior. Disease Markers 2014;2014:513158. [CrossRef] [PubMed]
27. Kazemi MH, Mohseni SR, Hojjat-Farsangi M, Anvari E, Ghalamfarsa G, Mohammadi H, et al. Adenosine and adenosine receptors in the immunopathogenesis and treatment of cancer. J Cell Physiol 2018;233(3): 2032-57. [CrossRef] [PubMed]
28. Di Virgilio F. Purines, purinergic receptors, and cancer. Cancer research 2012;72:5441-7. [CrossRef] [PubMed]
29. Longhi MS, Robson SC, Bernstein SH, Serra S, Deaglio S. Biological functions of ecto-enzymes in regulating extracellular adenosine levels in neoplastic and inflammatory disease states. Journal of Molecular Medicine 2013;91:165-72. [CrossRef] [PubMed]
30. Sitkovsky MV, Kjaergaard J, Lukashev D, Ohta A. Hypoxia-adenosinergic immunosuppression: tumor protection by T regulatory cells and cancerous tissue hypoxia. Clinical Cancer Research 2008;14:5947-52. [CrossRef] [PubMed]
31. Spychala J. Tumour-promoting function of adenosine. Pharmacol Therapy 2000;87:161-73. [CrossRef] [PubMed]

32. Weisman GA, Lustig KD, Lane E, Huang NN, Belzer I, Friedberg I. Growth inhibition of transformed mouse fibroblasts by adenine Nucleotides occurs via generation of extracellular adenosine. J Biol Chem 1988; 263:12367-72. [CrossRef] [PubMed]
33. Fishman P, Bar-Yehuda S, Vagman L. Adenosine and other low molecular weight factors released by muscle cells inhibit tumor cell activities of adenosine deaminase, 5V-nucleotidase, guanase, and cy-growth. Cancer Res 1998;58:3181-7. [PubMed]
34. Pueyo M, Chen Y, Dangelo G. Regulation of vascular endothelial qrowth factor expression by cAMP in rat aortic smooth muscle cells. Exp Cell Res 1998; 238: 354-8. [CrossRef] [PubMed]
35. Cronstein BN, Kubersky SM, Weissmann G, Hirschhorn R. Engagement of adenosine receptors inhibits hydrogenperoxide release by activated human neutrophils. Clin Immunol Immunopathol 1987;42:4276-85. [CrossRef] [PubMed]
36. Schulte G, Fredholm BB. Signalling from adenosine receptor to mitogen activated protein kinases. Cell Signalling 2003;15:15813-27. [CrossRef] [PubMed]
37. Mahajan M, Tiwari N, Sharma R, Kaur S, Singh N. Oxidative Stress and Its Relationship With Adenosine Deaminase Activity in Various Stages of Breast Cancer. Indian Journal of Clinical Biochemistry 2013; 28(1):51-4. [CrossRef] [PubMed]
38. Zeleznikar RJ, Heyman RA, Graeff RM, Walseth TF, Dawis SM, Butz EA, et al. Evidence for compartmentalized adenylate kinase catalysis serving a high energy phosphoryl transfer function in rat skeletal muscle. J Biol Chem 1990;265:300-11. [CrossRef] [PubMed]
39. Natsumeda Y, Prajda N, Donohue JP, Glover JL, Weber G. Enzymic capacities of purine *de novo* and salvage pathways for nucleotide synthesis in normal and neoplastic tissues. Cancer Res 1984;44:2475-9. [PubMed]
40. Hofbrand AV, Janossy G. Enzyme and membrane markers in leukemia: recent developments. J Clin Pathol 1981;34:254-62. [CrossRef] [PubMed]
41. Bemi V, Tazzini N, Banditelli S, Giorgelli F, Pesi R, Turchi G, et al. Deoxyadenosine metabolism in a human colon-carcinoma cell line (LoVo) in relation to its cytotoxic effect in combination with deoxycoformycin. In J Cancer 1998;75:713-20. [CrossRef] [PubMed]
42. Kate J, Ingh HF, Khan PM, Bosman FT. Adenosine deaminase complexing protein (ADCP) immunoreactivity in colorectal adenocarcinoma. Int J Cancer 1986;15:479-85. [CrossRef] [PubMed]
43. Sanfilippo O, Camici M, Tozzi MG, Turriani M, Faranda A, Ipata L, et al. Relationship between the levels of purine salvage pathway enzymes and clinical/biological aggressiveness of human colon carcinoma. Cancer Biochemistry Biophysics 1994;14(1):57-66. [PubMed]
44. Flamand N, Boudreault S, Picard S, Austin M, Surette ME, Plante H, et al. Adenosine, a potent natural suppressor of arachidonic acid release and leukotriene biosynthesis in human neutrophils. Am J Respir Crit Care Med 2000;161(2):S88-S94. [CrossRef] [PubMed]
45. Veljković A, Stanojević G, Branković B, Pavlović D, Stojanović I, Cvetković T, et al. Parameters of oxidative stress in colon cancer tissue. Acta Medica Medianae 2016;55:32-7. [CrossRef]
46. Erkiliç K, Evereklioglu C. Çekmen M, Özkiris A, Duygulu F, Dogan H. Adenosine deaminase enzyme activity is increased and negatively correlates with catalase, superoxide dismutase and glutathione peroxidase in patients with Behçet's disease: original contributions/clinical and laboratory investigations. Mediators Inflamm 2003;12(2):107-16. [CrossRef] [PubMed]
47. Viviani LG, Piccirillo E, Cheffer A, de Rezende L, Ulrich H, Carmona-Ribeiro AM, et al. Be aware of aggregators in the search for potential human ecto-5'-nucleotidase inhibitors. Molecules 2018;23(8):E1876. [CrossRef] [PubMed]
48. Al-Haj L, Khabar KSA. The intracellular pyrimidine 5'-nucleotidase NT5C3A is a negative epigenetic factor in interferon and cytokine signaling. Sci Signal 2018; 11(518):l2434. [CrossRef] [PubMed]
49. Ikegami T, Natsumeda Y, Weber G. Decreased concentration of xanthine dehydrogenase (EC 1.1.1.204) in rat hepatomas. Cancer Res 1986;46(8):3838-41. [PubMed]
50. Veljkovic A, Hadzi-Djokic J, Sokolovic D, Cukuranovic R, Cukuranovic-Kokoris J, Basic D, et al. Local and Systemic Oxidative Stress in Balkan Endemic Nephropathy Is Not Associated with Xanthine Oxidase Activity. Oxid Med Cell Longev 2020;8209727. [CrossRef] [PubMed]
51. Romagnoli M, Gomez-Cabrera MC, Perrelli MG, Biasi F, Pallard´o FV, Sastre J, et al. Xanthine oxidase-induced oxidative stress causes activation of NF-kappaB and inflammation in the liver of type I diabetic rats. Free Radic Biol Med 2010;49:171-7. [CrossRef] [PubMed]
52. Balamurugan K. HIF-1 at the crossroads of hypoxia, inflammation, and cancer. Int J Cancer 2016;138: 1058-66. [CrossRef] [PubMed]
53. Se-Hyun O, Soon-Youn C, Hyo-Jung C, Hye-Myung R, You-Jin K, Hee-Yeon J, et al. The emerging role of xanthine oxidase inhibition for suppression of breast cancer cell migration and metastasis associated with hypercholesterolemia. FASEB Journal 2019;33(6): 7301-14. [CrossRef] [PubMed]
54. Cummins E, Doherty G, Taylor C. Hydroxylases as therapeutic targets in inflammatory bowel disease. Laboratory Investigation 2013;93(4):378-83. [CrossRef] [PubMed]

Originalni rad **UDC: 616.348-006.6:577.152**
doi:10.5633/amm.2021.0201

ZNAČAJ AKTIVNOSTI ENZIMA KOJI UČESTVUJU U NASTANKU URATA I NJIHOVA VEZA SA OKSIDATIVNIM STRESOM U PROGRESIJI I INVAZIJI KOLOREKTALNOG KARCINOMA

Andrej Veljković[1], Jovan Hadži-Đokić[3], Goran Stanojević[2], Branko Branković[2], Aleksandar Skakić[2], Ivana Damjanović[1], Mladen Stojanović[2], Andrija Šmelcerović[1], Gordana Kocić[1]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2] Univerzitetski klinički centar Niš, Niš, Srbija
[3]Srpska akademija nauka i umetnosti, Beograd, Srbija

Kontakt: Andrej Veljković
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: veljkovicandrej@yahoo.com

Kolorektalni karcinom jedan je od najčešće dijagnostikovanih karcinoma i najčešći uzrok smrtnosti od svih malignih bolesti. I dalje postoji nedostatak ispitivanja patogeneze ove bolesti, uključujući uticaj enzima uratnih puteva na progresivnost i invaziju kolorektalnog karcinoma, te je cilj ovog rada da istraži ulogu aktivnosti adenozin deaminaze (ADA), 5'-nukleotidaze (5'-NT) i ksantin oksidaze (KSO) u odnosu na TBA reaktivne supstance (TBARS), kao markere oksidativnog stresa u progresiji i invaziji humanog karcinoma debelog creva.

Uzeli smo uzorke tkiva karcinoma – zdravo kontrolno tkivo i tkivo koje okružuje tumor od 50 bolesnika sa primarnim karcinomom debelog creva u sva četiri klinička stadijuma bolesti. U 10% homogenatima sprovedeno je istraživanje odgovarajućim metodama.

Aktivnost ADA i KSO u tkivu tumora i tkivu uz tumor bila je značajno veća u poređenju aktivnosti ADA i KSO u zdravom tkivu debelog creva. Aktivnost 5'-NT nije značajno veća u tkivu karcinoma. Najveća aktivnost ADA i KSO je u stadijumima tumora T2 i T3. Najveća koncentracija TBARS je u stadijumima T3 i T4 tumora.

Dobijeni rezultati sugerišu da bi jedan od mogućih uzroka oksidativnog stresa u karcinomu debelog creva mogla biti visoka aktivnost KSO i ADA. To može dovesti u vezu te enzime sa malignom transformacijom epitela debelog creva, kao i sa napredovanjem i metastaziranjem karcinoma debelog creva. Na ovaj način, procena aktivnosti ADA i KSO mogla bi da pomogne u proceni margina prilikom odstranjivanja karcinoma, kako bi se utvrdila opsežnost resekcije debelog creva. Takođe, ovi enzimi mogu imati značaj u dijagnozi, ali i u prognozi bolesti.

Acta Medica Medianae 2021;60(2):05-14.

Ključne reči: *kolorektalni karcinom, adenozin dezaminaza, 5'-nukleotidaza, ksantin oksidaza, oksidativni stres*

Original article

UDC: 611.715:572.7
doi:10.5633/amm.2021.0202

MORPHOMETRIC ANALYSIS OF LARGER OPENINGS OF THE GREATER WING OF THE HUMAN SPHENOID BONE

Miljana Pavlović[1], Ivan Jovanović[1], Milena Trandafilović[1], Vesna Stojanović[1], Milorad Antić[1], Jovana Čukuranović Kokoris[1], Milica Stanković[2]

Analysis of the anatomical characteristics of openings in the greater wing of the sphenoid bone (round, oval and spinous opening – FR, FO and FS) has a clinical significance in surgical and diagnostic procedures. The aim of the study was to examine the morphological and morphometric characteristics of the round, oval and spinous openings in the human skulls. The research was conducted on 20 skulls housed at the Institute of Anatomy of the Faculty of Medicine in Niš. The skulls were numerated, and the openings were photographed against the ruler with a Canon A470 camera. Photo processing and morphometric analysis (measuring the length and width of the FR, FO and FS) were performed using ImageJ software. The average length of the FR on the right was 3.14 ± 0.77 mm and 3.44 ± 0.65 mm on the left, width on the right was 2.38 ± 0.58 mm, 2.61 ± 0.55 mm on the left; length of the FO on the right was 5.88 ± 0.88 mm, 5.50 ± 1.06 mm on the left, width on the right was 2.70 ± 0.58 mm, 2.82 ± 0.68 mm on the left; length of the FS on the right was 1.65 ± 0.27 mm, 1.73 ± 0.49 mm on the left, width on the right was 1.32 ± 0.32 mm, and 1.20 ± 0.39 mm on the left. The t-test of independent samples determined no statistically significant difference neither between the parameters on both sides, nor between the measured parameters of the same openings. A moderate positive correlation existed between FS length and width on the left, FR length and width on the right and between FS widths on both sides; a negligible positive correlation between the length and width of FO on the right and between lengths of the FS on both sides; a weak positive correlation existed between other measured parameters.

Acta Medica Medianae 2021;60(2):15-24.

Key words: *round opening, oval opening, spinous opening, greater wing of the sphenoid bone*

[1]University of Niš, Faculty of Medicine, Department of Anatomy, Niš, Serbia
[2]Institute of Pathology, University Clinical Center Niš, Niš, Serbia

Contact: Miljana Pavlović
81 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: pavlovic_miljana@yahoo.com

Introduction

The greater wing (*ala major*) is a paired extension of the sphenoid bone. Its inner surface forms most of the floor of the middle cranial fossa (1, 2, 3). The greater wing originates from the lateral surface of the body of the sphenoid bone by three roots: front, middle and back root. It contains openings for the passage of vital neurovascular elements. Variants of these openings have been described in the anatomical and radiological literature, which is important both for understanding complex regional neurovascular anatomy and for differentiating normal from potentially abnormal structures (4). Between the front and middle roots of the greater wing, there is the foramen rotundum (FR), a communication between the middle cranial fossa and the pterygopalatine fossa. The maxillary nerve leaves the skull through this opening along with the venous plexus that surrounds it. The cavernous sinus, which lies in the skull, and the pterygoid plexus, which is outside the skull, are connected via the foramen rotundum (5, 6). The opening can communicate with the superior orbital fissure and its contents since it is located below and behind its inner part (2, 7). In a small percentage of cases a lateral rotundal canal, opened to the infratemporal fossa, may be present laterally from the FR. Although the nature of this canal has not been fully elucidated, it is thought that an emissary vein passes through it (8). Between the middle and posterior roots of the greater wing, posterolaterally from the FR and outside from the foramen lacerum, there is the foramen ovale (FO). It is a communication between the middle cranial fossa and the infratemporal fossa

through which the mandibular nerve and occasionally the lesser petrosal nerve exit the skull, while the accessory branch of the maxillary artery enters it (5, 9, 10, 11). Located in the transition zone between intracranial and extra cranial structures, the FO has great clinical significance as an approach during surgical, radiological and radiotherapy treatments (like in trigeminal neuralgia), as well as in various diagnostic procedures (11, 12). In neurosurgery, it is important in terms of micro vascular decompression of the trigeminal nerve (trigeminal rhizotomy). Knowledge of the topography and variation of the FO can be of great importance in the prevention of damage of the trigeminal nerve during mentioned interventions. Knowing the position of this opening is also important in a percutaneous biopsy of cavernous sinus tumors (13). Also, the oval opening is the most common site of the nasopharyngeal cancer spread (11). In addition to the FO, a venous opening (foramen Vesalius) or several small openings for the passage of venous blood vessels may be present (6). If absent, small veins pass through the oval foramen (7). In the outer part of the posterior margin of the greater wing, on the spine of the sphenoid bone, there is the foramen spinosum (FS), placed posterolaterally of the oval opening. It is a communication between the middle cranial fossa and the infratemporal fossa (2, 3, 8). Through the FS, the middle cerebral artery and the meningeal branch of the mandibular nerve enter the skull (4, 5). Proximity of the FS to the FO can make the middle cerebral artery susceptible to iatrogenic injury during percutaneous trigeminal rhizotomy and anesthesia of the mandibular nerve and increase the risk of developing extradural hematomas (14, 15).

Knowing the variability of the size of the mentioned openings, as well as their mutual relations can greatly facilitate numerous diagnostic interventions, and at the same time prevent complications. That's why the aim of the research was to examine the morphological and morphometric characteristics of the FR, FO and FS and to contribute to the knowledge of their representation and possible variations.

Materials and methods

The research was conducted on 20 dried adult human skulls obtained from the Institute of Anatomy, Faculty of Medicine in Niš. All the skulls were numerated. The FR, FO and FS were photographed next to the ruler with a Canon A470 7 Mpix camera. The obtained photographs were processed in the "ImageJ" program for processing and analysis of photographs. Morphometric analysis was performed in the same program (Figures 1, 2 and 3). The values of the length and the width of the FR, FO and FS were processed with the graph-analytical and tabular data processing program "Microsoft Office Excel".

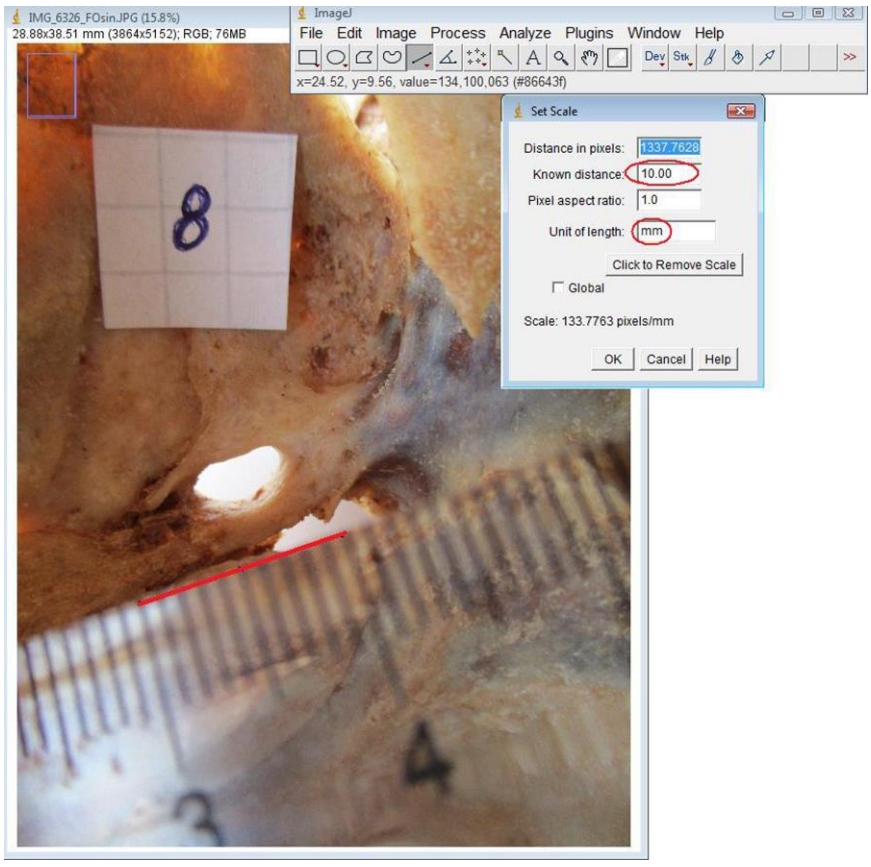


Figure 1. Setting the unit of measurement using ImageJ software

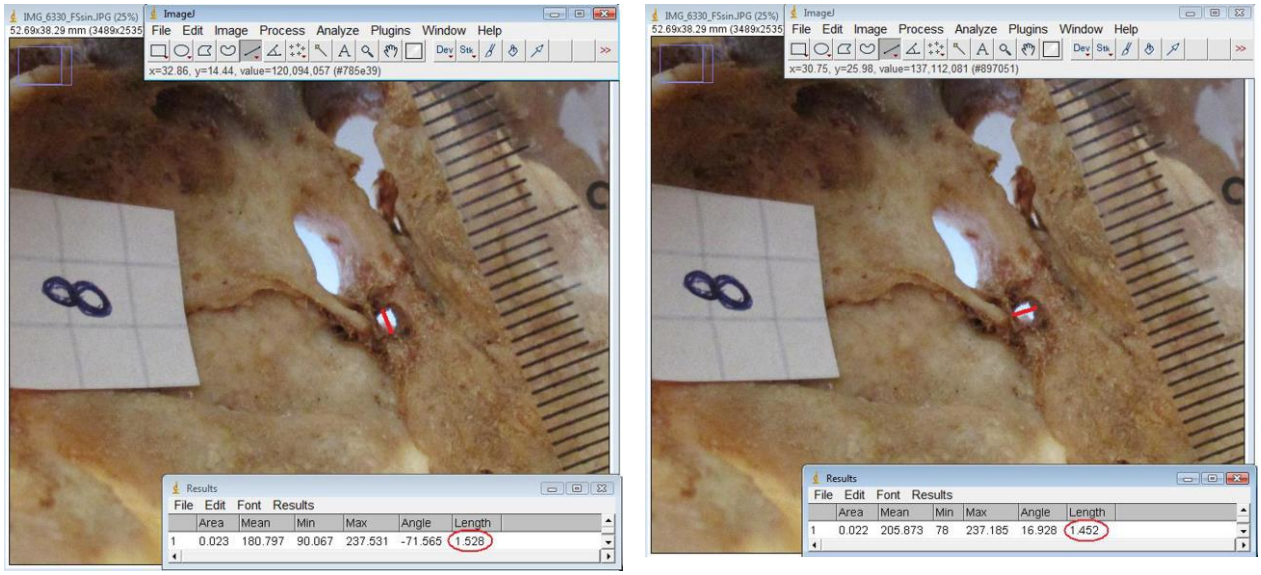


Figure 2. Procedure for measuring the length and width of the foramen spinosum

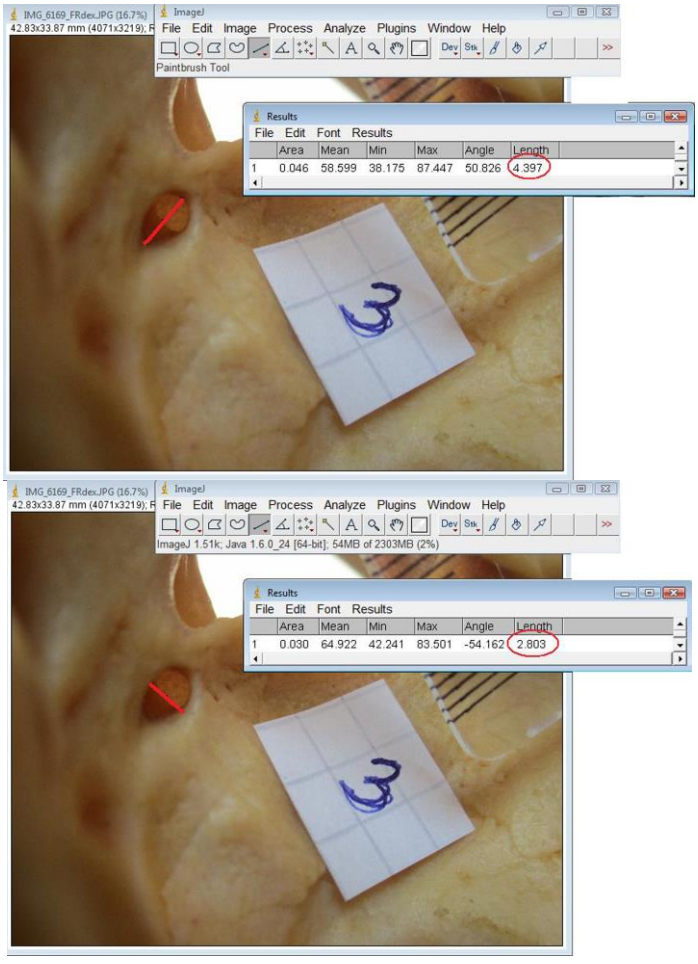


Figure 3. Procedure for measuring the length and width of the foramen rotundum

Results

In our study, the FR, FO and FS were present in all cases and on both sides. The values of the measured parameters and their mean values ($\bar{X}$ ± SD) are shown in the Tables 1 and 2. Statistically significantly reduced or increased values were marked in the tables: all values less than $\bar{X}$ SD in blue and the ones greater than $\bar{X}$ +SD, in red.

The average length of the FR on the left side was 3.44 mm, the maximal length was 4.41 mm, the minimal 2.29 mm. On the right side, the average length was 3.14 mm, the maximal 4.49 mm and the minimal 1.90 mm. The average FR width on the left was 2.61 mm, the maximal 3.63 mm and the minimal 1.48 mm. The average width of the FR on the right side was 2.38 mm, the maximal 3.43 mm and the minimal 1.51 mm. In 75% of cases, the FR was slightly oval in shape, in the rest almost round, with the dimensions which differed just in the decimals of mm. In most cases (65%), the FR was longer on the left, wider on the right (55% of cases).

The average length of the FO on the left side was 5.50 mm, the maximal 7.54 mm and the minimal 3.25 mm. On the right, the average length of the FO was 5.88 mm, the maximal being 8.30 mm and a minimal 4.52 mm. In terms of width, the average value on the left was 2.82 mm, the maximal 4.36 mm and the minimal 1.54 mm. The average width of the FO on the right was 2.70 mm, the maximal 3.72 mm and a minimal 1.42 mm. The right FO were longer in 70% of cases while the left ones were wider in the 75% of cases.

The average length of the FS on the left side was 1.73 mm, the maximal 2.79 mm and the minimal 0.84 mm. On the right, the average length of the FS was 1.65 mm, the maximal 2.27 mm and a minimal of 0.81 mm. The average width of the FS on the left side was 1.20 mm, the maximal 1.92 mm and a minimal 0.54 mm. On the right side, the average width of the FS was 1.32 mm, the maximal 2.07 mm and a minimal 0.78 mm. In 25%, FS were almost round, differing in the decimals of the mm only, while in the rest 75% of cases they were slightly oval in shape. The lengths of FS were equally large on both sides, the widths larger on the right in 65% of cases.

Table 1. Values of measured parameters (mm) on the left side and their mean values ($\bar{X}$ ± SD)

No.	Left side					
	FR length	FR width	FO length	FO width	FS length	FS width
1	4.41	2.57	4.66	2.35	1.40	1.08
2	2.49	2.11	5.10	1.54	1.76	1.10
3	3.66	2.58	5.24	2.78	1.96	1.09
4	2.41	2.17	4.89	2.67	1.53	0.74
5	3.88	2.60	4.09	2.87	1.14	0.78
6	3.79	2.66	5.45	2.27	0.84	0.56
7	3.98	2.39	5.64	2.90	1.07	0.54
8	2.71	1.48	6.14	2.16	1.97	1.33
9	3.33	3.61	3.84	2.43	1.79	1.71
10	4.12	2.92	6.27	4.36	1.67	1.14
11	4.32	3.03	6.91	3.15	1.65	1.43
12	2.29	2.14	3.25	1.91	1.85	1.74
13	3.34	3.09	5.59	3.07	1.84	1.51
14	3.12	1.92	4.91	2.13	1.34	1.00
15	3.15	2.09	6.04	3.53	1.49	0.98
16	3.24	2.33	5.71	3.27	2.25	1.58
17	3.78	2.78	5.81	3.09	2.42	1.12
18	3.68	3.63	7.54	3.20	2.79	1.49
19	2.90	2.93	6.11	3.93	2.49	1.92
20	4.17	3.10	6.90	2.85	1.41	1.17
$\bar{X}$	3.44	2.61	5.50	2.82	1.73	1.20
SD	0.65	0.55	1.06	0.68	0.49	0.39
$\bar{X}$ + SD	4.08	3.16	6.56	3.50	2.23	1.59
$\bar{X}$ - SD	2.79	2.06	4.44	2.14	1.24	0.81

Table 2. Values of measured parameters [mm] on the right side and their mean values ($\overline{X}$ ± SD)

No.	Right side					
	FR length	**FR width**	**FO length**	**FO width**	**FS length**	**FS width**
1	3.90	2.70	6.11	1.58	1.64	0.82
2	2.67	1.87	4.82	1.42	1.64	1.27
3	4.37	3.43	6.21	2.20	1.55	1.40
4	2.68	2.18	4.52	2.14	1.67	0.78
5	2.87	1.93	5.31	2.83	1.97	1.28
6	2.21	1.51	6.33	3.01	0.81	0.85
7	3.34	2.12	5.52	2.61	1.89	1.19
8	1.90	1.62	6.46	2.79	1.79	1.41
9	3.27	2.23	4.96	3.26	1.82	1.24
10	2.26	3.02	6.44	2.52	1.62	1.53
11	3.04	1.68	8.30	3.41	1.69	1.39
12	3.47	2.35	4.54	3.19	1.57	1.56
13	4.49	3.26	6.59	2.81	1.55	1.84
14	2.54	1.95	5.62	2.57	1.71	1.48
15	3.10	2.27	6.21	3.44	1.60	1.41
16	2.64	2.36	6.27	3.72	1.67	1.53
17	4.24	3.42	6.10	2.74	1.40	1.00
18	2.91	2.71	5.09	2.82	1.52	1.11
19	2.56	2.12	6.06	2.64	2.27	2.07
20	4.26	2.79	6.24	2.28	1.60	1.19
$\overline{X}$	3.14	2.38	5.88	2.70	1.65	1.32
SD	0.77	0.58	0.88	0.58	0.27	0.32
$\overline{X}$+SD	3.90	2.96	6.76	3.28	1.92	1.64
$\overline{X}$-SD	2.37	1.79	5.01	2.11	1.38	0.99

Table 3 shows the numbers of skulls with statistically significantly increased (> $\overline{X}$ + SD), or statistically significantly reduced (< $\overline{X}$ - SD) values.

T-test for independent samples revealed absence of statistically significant difference of the corresponding parameters on the right and left sides. These results are shown in the Table 4.

The results of the correlation analysis between the measured parameters of the same openings are shown in the Table 5. A moderate positive correlation existed between the length and width of the FS on the left and between the length and width of the FR on the right side. There was a negligible positive correlation between the length and width of the FO on the right side. Among all other measured parameters, a weak positive correlation was noted.

The results of the correlation analysis among the same measured parameters on different sides are shown in the Table 6. There was a positive correlation between all parameters, but it was of negligible strength for the length of FS between left and right sides, moderate for the width of FS between left and right sides, and also weak for all other parameters of the measured openings.

Table 3. Number of skulls with significantly increased or decreased values of measured parameters (l – length; w – width)

	Left side						Right side					
	FR l	FR w	FO l	FO w	FS l	FS w	FR l	FR w	FO l	FO w	FS l	FS w
> $\overline{X}$+SD	4	3	3	3	4	4	4	4	1	3	2	2
< $\overline{X}$-SD	4	2	3	3	3	4	3	3	4	2	1	4

Table 4. Comparison of measured parameters on the right and left side

Measured parameter		$\bar{X}$ ± SD	p
Left side	FR length [mm]	3.44 ± 0.65	0.187
Right side	FR length [mm]	3.14 ± 0.77	
Left side	FR width [mm]	2.61 ± 0.55	0.204
Right side	FR width [mm]	2.38 ± 0.58	
Left side	FO length [mm]	5.50 ± 1.06	0.223
Right side	FO length [mm]	5.89 ± 0.88	
Left side	FO width [mm]	2.82 ± 0.68	0.540
Right side	FO width [mm]	2.70 ± 0.59	
Left side	FS length [mm]	1.73 ± 0.50	0.510
Right side	FS length [mm]	1.65 ± 0.27	
Left side	FS width [mm]	1.2 ± 0.39	0.301
Right side	FS width [mm]	1.32 ± 0.32	

Table 5. The correlation coefficient between measured parameters of the same openings (l – length; w – width)

Side	Compared parameters	r
Left side	FR l : FR w	0.516
	FO l : FO w	0.539
	FS l : FS w	0.729
Right side	FR l : FR w	0.741
	FO l : FO w	0.288
	FS l : FS w	0.505

Table 6. The correlation coefficient between measured parameters of the same openings on both sides (l – length; w – width)

Parameter	Compared sides	r
FR l	Left side: Right side	0.323
FR w	Left side: Right side	0.387
FO l	Left side: Right side	0.566
FO w	Left side: Right side	0.319
FS l	Left side: Right side	0.226
FS w	Left side: Right side	0.639

Discussion

The greater wing of the sphenoid bone contains several openings. The FR, FO and FS are permanent and connect the middle cranial fossa with the pterygopalatine fossa (FR) and the infra temporal fossa (FO and FS) and convey significant neurovascular elements (16). The studies of the size and shapes of these foramina have not only anatomical but also clinical importance during evaluation of radiologic images, then a profound surgical importance such as in percutaneous trigeminal rhizotomy and transfacial fine-needle aspiration and also a diagnostic importance in tumors and in electroencephalographic analysis of seizures in some types of epilepsy (17). Only scattered reports of the size and variations of these foramina are available in the literature.

The foramen rotundum is located in the root of the greater wing of the sphenoid bone, lateral to the lower part of the superior orbital fissure. It serves as a passage for the maxillary nerve and also contains venous plexus which surrounds the nerve and links the cavernous sinus with the pterygoid plexus lying outside the skull.

In our investigation, the FR was always present and its morphology did not show essential variability. Although its name indicates a round shape, it was most often (in 75% cases) slightly oval in shape. The average dimensions of the FR on the left were 3.44x2.61 mm, while on the right they were 3.14x2.38 mm. According to the average dimensions, the left FR were larger than the right ones. The left FR were longer in 65.00% of cases while the right ones were wider in 55.00% of cases. We did not determine the existence of statistically significant difference between the measured parameters on the left and right side. There was a weak positive correlation between the parameters of the different sides. Other authors have reached similar dimensions in their researches. Thus, Shapiro and Robinson (7) found the dimensions of FR ranging from 3x3 mm to 4x5 mm, Sepahdari and Mong (18) 3.00 mm and Kumar et al. (16) 3.11 mm. There was no statistically significant difference between the parameters of the left and right side, neither in the investigations of the mentioned authors nor in the research of Kocaogullar et al. (19) and Reymond et al. (6). Some researchers have described rare variations in the shape and position of the FR. Double FR was described by Sepahdari and Mong (18), and occurred in cases of double maxillary nerve. Rusu (20) observed a canal lateral to the foramen rotundum that could not clearly be linked to it in 8% of patients. He proposed calling it the lateral rotundal canal. Uncommon congenital asymmetry, enlarged FR was also observed. It is expected in a lesion or a tumor of the maxillary nerve. Anomalous enlargement of the foramen may be distinguished from pathologic erosion by the presence of well-defined margins (7).

The foramen ovale is the largest opening located in the root of the greater wing of the sphenoid bone. It conveys mandibular nerve and the accessory meningeal artery, occasionally lesser petrosal nerve and an emissary vein. The foramen ovale is situated at the transition zone between the intracranial and the extracranial structures, therefore, it is used as a passageway to the intracranial structures during the invasive surgical or diagnostic procedures (12, 17). Variations of the size and shape of the FO could affect transcutaneous needle placement into it or distort anatomic relationships during approaches to the cranial base. Therefore, knowledge of the exact topography, shape and size of this opening is of great clinical importance.

In our study, the average sizes of the FO on the left and right sides were 5.50x2.82 mm and 5.88x2.70 mm, respectively. The length of the opening, in most cases, was larger on the right side (70.00%), and the width was larger on the left side in 75.00% of cases. We did not find statistically significant difference between the measured parameters on the right and left side. The lengths of the FO were greater than the width in all cases, so they were clearly elongated in shape. There was a positive correlation and it was of medium strength for the length of the FO and for the width of this opening, too. Mean FO dimensions in our cases were similar to those in the studies of Reymond et al. (6) and Sepahdari and Mong (18), while in the studies of Ray et al. (12), Osunwoke et al. (21), Nirupma and Anju (22), Patil et al. (13), Murugan and Saheb (23) and Kuppasad et al. (24), they were larger. There was not statistically significant difference between the measured parameters on the right and left side in the researches of Reymond et al. (6), Osunwoke et al. (21), Patil et al. (13) and Kuppasad et al. (24). In contrast, Murugan and Saheb (23) obtained statistically significant differences between the measured values of the FO on the right and left sides. The difference between the length of the FO in the opposite sexes was not statistically significant (6, 12, 23) while the mean width in male skulls was slightly larger than in females. According to the results of one study, FO is narrower on the right than on the left side. The narrow FO can cause pressure on the mandibular nerve which may cause trigeminal neuralgia. In this study, the incidence of trigeminal neuralgia on the right side was shown to be higher (25). Researchers in the USA found statistically significant difference in the length of FO as well as between the average area and perimeter of the FO on both sides (27). Others, comparing the dimensions of FO and FS in newborns and adults, came to the conclusion that the diameter of the FO and FS changes during the growth and development of the child (21).

Variations in the shape of FO were evidenced by a number of investigators with slight differences. Most openings were oval shaped; followed by almond, round and slit like ones. In a small number of cases, the edges of the FO were uneven, with nodules or smaller or larger spines that incompletely divided the opening into sections (11, 12, 17, 22, 23, 24, 26). Variations in the shape of the FO were due to developmental reasons (24) as well as a consequence of the venous blood vessel passing through it (7). The contents of the FO can be partially separated by bony spikes in the cases of doubled foramen ovale (12) or multiple foramen ovale (6). Some other variations as merged FO and FS and crescent-shaped FO were also described (10).

Knowledge of the topography and position variabilities of FO and FR enables avoidance of complications in the cases of surgical procedures with the access through these openings, like during cavernous sinus surgery (28), or in the setting of tumor in the masticator space (13, 18).

The foramen spinosum is a small opening located in the posteromedial part of the root of the greater wing. It transmits the middle meningeal artery and the nervus spinosus. In terms of surgical and anesthetic exploratory maneuvers in the base of the skull, this opening is of great importance, so some researchers have studied its incidence, shape, morphometric details, relations with the FO, as well as the presence of possible anomalies (17, 29).

In our study, FS was present on all skulls, and was single, as in the results of a study by Reymond et al. (6). During morphometry of the FS, we found that its average dimensions on the left and right side were 1.73x1.20 mm, and 1.65x1.32 mm, respectively. The differences between the measured parameters on the right and left side were not statistically significant. Neither Ginsberg et al. (8) nor Osunwoke et al. (21) obtained statistical significance when comparing these parameters. The FS was sometimes absent in a small number of cases, 3.2% (8), 0.8% (6), 4% (10). This happened in the cases when the middle cerebral artery originated from the ophthalmic or internal carotid artery instead of the maxillary artery. The foramen spinosum showed numerous asymmetries and variations in shape and size in the studies. These variations were caused either by incomplete osteogenesis or by aberrant formation of the middle cerebral artery. In our study, the FS was slightly oval in shape in 70.00% of cases, in the study of Saheb et al. (4) it was round in 58% of cases, oval in 38%, and irregular in 4%. The FS may also be incompletely separated from the FO or completely connected to it (7). Double FS has been also seen, in cases when the middle cerebral artery branches before passing through it (10, 17, 30).

Many authors state that variations present on skull-based openings may be the result of an evolutionary process and that it may turn out that the presence of a particular variation is actually normal or frequent in a particular geographic population or ethnic group (31). Perhaps this may explain the differences in the dimensions of FR, FO and FS in our study, as well as in the studies of other researchers.

Knowing of morphological characteristics as well as variations of size and shapes of FR, FO and FS can be of great importance in neurosurgery in terms of planning and performing of diagnostic or surgical interventions such as tumor detection, biopsy and resection, micro vascular decompression of the trigeminal nerve and other transcutaneous methods of treating trigeminal neuralgia.

Conclusion

In this study dimensions (lengths and widths) of the FR, FO and FS were determined. Difference between the lengths and widths of the left and right FR, FO and FS exists but is not statistically significant. Also, correlation between measured dimensions is either moderate or weak.

Acknowledgments

This paper was supported by the projects of the Ministry of Science and Technological Development of the Republic of Serbia (TR 31060, III 41018) and Internal project of the Medical Faculty University of Niš (38/20).

References

1. Krmpotić-Nemanić J, Marušić A. Anatomija čoveka. Drugo izdanje. Zagreb: Medicinska naklada; 2001.
2. Williams PL. Gray's Anatomy: The anatomical basis of clinical practice. 42nd international ed. Edinburgh: Churchill Livingstone/Elsevier imprints; 2020.
3. Čukuranović R, Antić S, Arsić S, Bakić V, Daković Bjelaković M, Jovanović I, et al. Anatomija čoveka. 1. izdanje. Niš: Galaksijanis; 2019.
4. Saheb SH, Khaleel N, Havalder PP, Shruthi BN. Morphological and morphometric study of foramen spinosum. Int J Anat Res 2017;5(4.1):4523-6. [CrossRef]
5. Rosse C, Gaddum-Rosse P. Textbook of anatomy. Fifth ed. Philadelphia: Lippincott - Raven; 1997.
6. Reymond J, Charuta A, Wysocki J. The morphology and morphometry of the foramina of the greater wing of the human sphenoid bone. Folia Morphol (Warsz) 2005;64(3):188-93. [PubMed]
7. Shapiro R, Robinson F. The foramina of the middle fossa: a phylogenetic,anatomic and pathologic study. Am J Roentgenol Radium Ther Nucl Med 1967;101(4): 779-94. [CrossRef] [PubMed]
8. Ginsberg LE, Pruett SW, Chen MY, Elster AD. Skull-base foramina of the middle cranial fossa: Reassessment of normal variation with high-resolution CT. Am J Neuroradiol 1994;15(2):283-91. [PubMed]
9. Snell RS. Clinical anatomy for medical students. Sixth ed. Philadelphia: Lippincott Williams&Wilkins; 2000.
10. Khan AA, Asari MA, Hassan A. Anatomic variants of foramen ovale and spinosum in human skulls. Int J Morphol 2012;30(2):445-9. [CrossRef]
11. Patil GV, Shishirkumar AD, Apoorva D, Thejeswari JS, Sharif J, Sheshgiri C, et al. Morphometry of the foramen ovale of sphenoid bone in human dry skulls in Kerala. IJHSR 2014;4(10):90-3.
12. Ray B, Gupta N, Ghose S. Anatomic variations of foramen ovale. Kathmandu Univ Med J 2005;3(1):64-8. [CrossRef]
13. Patil J, Kumar N, Rao K G M, Ravindra S S, S N S, Nayak B S, et al. The foramen ovale morphometry of sphenoid bone in South Indian population. J Clin Diagn Res 2013;7(12):2668-70. [CrossRef] [PubMed]
14. Saheb SH, Shepur MP, Desai SD, Thomas ST, Maavishettar GF, Haseena S. Study of foramen Vesalius in South Indian skulls. Indian J Med Healthc 2012;1(1):22-4.
15. Srimani P, Mukherjee P, Sarkar M, Roy H, Sengupta SK, Sarkar AN, et al. Foramina in alisphenoid – An observational study on their osseous-morphology and morphometry. Int J Anat Radiol Surg 2014;3(1):1-6.
16. Kumar A, Sehgal R, Roz TS. A morphometric analysis and study of variations of foramina in the middle cranial fossa. J Anat Soc India 2016;65:143-7. [CrossRef]
17. Karan BK, Prashant AB. An anatomical study on the foramen ovale and the foramen spinosum. J Clin Diagn Res 2013;7(3):427-9. [CrossRef] [PubMed]
18. Sepahdari AR, Mong S. Skull base CT: normative values for size and symmetry of the facial nerve canal, foramen ovale, pterygoid canal, and foramen rotundum. Surg Radiol Anat 2013;35(1):19-24. [CrossRef] [PubMed]
19. Kocaogullar Y, Avci E, Fossett D, Caputy A. The extradural subtemporal keyhole approach to the sphenocavernous region: anatomic considerations. Minim Invasive Neurosurg 2003;46(2):100-5. [CrossRef] [PubMed]
20. Rusu MC. Doubled foramen rotundum and maxillary nerve fenestration. Surg Radiol Anat 2011;33(8):723-6. [CrossRef] [PubMed]
21. Osunwoke EA, Mbadugha CC, Orish CN, Oghenmavwe EL, Ukah CJ. A morphometric study of foramen ovale and foramen spinosum of the human sphenoid bone in the southern Nigerian population. J Appl Biosci 2010;26:1631-5.
22. Nirupma G, Anju LR. Foramen ovale - Morphometry and its surgical importance. IJMHS 2013;3:4-6.
23. Murugan M, Saheb SH. Morphometric and morphological study on foramen ovale. Int J Anat Res 2014; 2(4):664-7. [CrossRef]
24. Kuppasad GP, Kundadukkam S, Thittamaranahalli MH, Haradi SR, Amit N. Morphometric and anatomic variations of foramen ovale in human skull and its clinical importance. Asian J Neurosurg 2019;14(4):1134-7. [CrossRef] [PubMed]
25. Gupta N, Rai AL. Foramen ovale-Morphometry and its surgical importance. IJMHS 2013;3(1):4-6.
26. Zdilla MJ, Hatfield SA, McLean KA, Cyrus LM, Laslo JM, Lambert HW. Circularity, Solidity, Axes of a Best Fit Ellipse, Aspect Ratio, and Roundness of the Foramen Ovale: A Morphometric Analysis With Neurosurgical Considerations. J Craniofac Surg 2016;27(1):222-8. [CrossRef] [PubMed]
27. Yanagi S. Developmental studies on the foramen rotundum, foramen ovale and foramen spinosum of the human sphenoid bone. Hokkaido Igaku Zasshi 1987;62(3):485-96. [PubMed]
28. Teul I, Czerwinski F, Gawlikowska A, Konstanty-Kurkiewicz V, Slawinski G. Asymmetry of the ovale and spinous foramina in mediaeval and contemporary skulls in radiological examinations. Folia Morphol (Warsz) 2002;61(3):147-52. [PubMed]
29. Lazarus L, Naidoo N, Satyapal KS. An osteometric evaluation of the foramen spinosum and venosum. Int J Morphol 2015;33(2):452-8. [CrossRef]
30. Karan BK, Surekha DJ, Umarji BN, Patil RJ, Ambali MP. Foramen ovale and foramen spinosum: a morphometric study. Anatomica Karnataka 2012;6(3):68-72.
31. Sharma NA, Garud RS. Morphometric evaluation and a report on the aberrations of the foramina in the intermediate region of the human cranial base: A study of an Indian population. Eur J Anat 2011;15(3): 140-9.

Originalni rad **UDC: 611.715:572.7**
doi:10.5633/amm.2021.0202

MORFOMETRIJSKA ANALIZA VEĆIH OTVORA VELIKOG KRILA KLINASTE KOSTI ČOVEKA

Miljana Pavlović[1], Ivan Jovanović[1], Milena Trandafilović[1], Vesna Stojanović[1], Milorad Antić[1], Jovana Čukuranović Kokoris[1], Milica Stanković[2]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za anatomiju, Niš, Srbija
[2]Institut za patologiju, Univerzitetski klinički centar Niš, Niš, Srbija

Kontakt: Miljana Pavlović
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: pavlovic_miljana@yahoo.com

U korenu velikog krila klinaste kosti nalaze se otvori za prolaz krvnih sudova i živaca (okrugli – FR, ovalni – FO i spinozni – FS otvor). Analiza anatomskih karakteristika i dimenzija ovih otvora ima klinički značaj, zbog njihove pozicije između intrakranijalnih i ekstrakranijalnih struktura i hirurških i dijagnostičkih procedura. Cilj istraživanja bio je da se ispitaju morfološke i morfometrijske karakteristike okruglog, ovalnog i spinoznog otvora na humanim lobanjama. Istraživanje je sprovedeno na 20 lobanja na Institutu za anatomiju Medicinskog fakulteta u Nišu. Lobanje su numerisane, a otvori fotografisani pored lenjira fotoaparatom marke Canon, model A470. Obrada fotografija i morfometrijska analiza (merenje dužine i širine okruglog, ovalnog i spinoznog otvora) izvršene su u programu "ImageJ". Prosečna dužina okruglog otvora desno iznosila je 3,14 mm ± 0,77 mm, levo 3,44 mm ± 0,65 mm; širina okruglog otvora desno iznosila je 2,38 mm ± 0,58 mm, levo 2,61 mm ± 0,55 mm; dužina ovalnog otvora desno 5,88 mm ± 0,88 mm, levo 5,50 mm ± 1,06 mm; širina ovalnog otvora desno 2,70 mm ± 0,58 mm, levo 2,82 mm ± 0,68 mm; dužina spinoznog otvora desno 1,65 mm ± 0,27 mm, levo 1,73 mm ± 0,49 mm; širina spinoznog otvora desno 1,32 mm ± 0,32 mm, levo 1,20 mm ± 0,39 mm. T-testom nezavisnih uzoraka, utvrđeno je da ne postoji statistički značajna razlika među parametrima na desnoj i levoj strani, ni između merenih parametara istih otvora. Srednja pozitivna korelacija postoji između dužine i širine FS na levoj, dužine i širine FR na desnoj strani i između širina FS na desnoj i levoj strani; zanemarljiva pozitivna korelacija postoji između dužine i širine FO na desnoj strani i između dužina FS na desnoj i levoj strani, dok između ostalih merenih parametara postoji slaba korelacija.

Acta Medica Medianae 2021;60(2):15-24.

Ključne reči: *okrugli otvor, ovalni otvor, spinozni otvor, veliko krilo klinaste kosti*

Original article

UDC: 616.611-089.843
doi:10.5633/amm.2021.0203

IMPORTANCE AND POTENTIAL APPLICATION OF MORPHOMETRIC ANALYSIS OF HUMAN GLOMERULI IN CADAVERIC KIDNEY TRANSPLANTATION

Vesna Stojanović[1], Ivan Jovanović[1], Braca Kundalić[1], Sladjana Ugrenović[1], Miljana Pavlović[1], Milena Trandafilović[1], Jovana Čukuranović Kokoris[1], Vladimir Petrović[2]

Cadaveric kidney transplantation is on the constant rise due to decreased mortality of younger individuals. In these transplantations, it is of great importance to determine not only the age limit of recipients, but also the status of donors.

This investigation included 30 tissue samples of human cadaveric kidneys (both genders, aged 20-85). Tissue samples were stained with Mallory's trichrome stain and analyzed by a light microscope. Images were analyzed using ImageJ software. As a result of cluster analysis, 743 glomeruli were classified into 3 groups by morphometric characteristics and into 3 age groups (I with average age of 29, II with 44, III with average of 71 years old). By morphometric characteristics, there were 114 sclerotic glomeruli with the significantly ($p \leq 0.0001$) smallest area and cellularity, and the highest connective tissue percentage in the first group. There were 430 morphologically normal glomeruli with the greatest number of cells/area unit in the second group ($p \leq 0.0001$). In the third group, there were 199 hypertrophic glomeruli with the greatest area, significantly large cellularity and connective tissue area ($p \leq 0.0001$). Out of 114 sclerotic glomeruli, the smallest number belonged to I age group ($p \leq 0.0001$). There were 430 morphologically normal glomeruli in total. Most of them were in II age group ($p \leq 0.0001$). Most of 199 hypertrophic glomeruli were in III age group vs. other two ($p \leq 0.0001$), as well as in II vs. I ($p \leq 0.0001$). Morphometric analysis of morphologically normal glomeruli should be of the greatest importance for transplantation, and not only the assessment of their total number and number of detected manifestly sclerotic glomeruli.

Acta Medica Medianae 2021;60(2):25-34.

Key words: *human glomeruli, kidney transplantation, morphometry*

[1]University of Niš, Faculty of Medicine, Department of Anatomy, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Department of Histology and Embryology, Niš, Serbia

Contact: Milena Trandafilović
81 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: milena.trandafilovic@medfak.ni.ac.rs

Introduction

Kidney transplantation in older patients is associated with an array of ethical dilemma. Previous studies have shown that the older patients in the end stage renal disease gain significant benefit from kidney transplantation (1-3). Relative statistical risk of graft rejection is similar either in patients below or over 65 years of age and it mostly depends on the presence of associated diseases which, in the older patients, often may lead to graft rejection and fatal complications (4). Therefore, the older kidney transplant candidates should be carefully screened for can cer, cardiovascular diseases, peripheral vasculopathy, diabetes mellitus, in order to minimize the risk of early post-transplant morbidity and mortality (3). Apart from age limit of the recipient, it is of great importance to determine the status and age limit of the donor as well, particularly in cases of cadaveric transplantation. There are no age limits for the kidney transplant procedure in the USA currently, which is reflected in the fact that the kidneys of donors over 65 years of age make 13% of total cadaveric kidney transplant number. Due to the lower mortality of young indivuduals, the mean age of cadaveric kidney donors is increasing as well (1). Expanded criteria kidney donor include those either from a brain-dead donor over 60 years of age, or a donor 50 to 59 years of age with at least two of the following features: history of hypertension, terminal serum creatinine > 1.5 mg/dL, or cerebrovascular cause of death. In accordance with it, there are two kidney transplant waiting lists in the USA: one with standard and one with expanded criteria donors.

Expanded criteria donor list is recommended for older recipients with renal failure as the main cause of the disease, as well as for those with difficult vascular access. Kidneys donated by expanded criteria make 17% of all donated kidneys in the USA (5). Eurotransplant Senior Program allows kidney transplantation from donors over 65 years of age to a selected group of nonimmunized 65+ patients undergoing their first transplant.

Kidney ageing process is associated with various factors, such as cytokines, growth factors, proliferation, apoptosis, transcription factors, advanced glycation end products (AGEs) (6-8). Genetic mutations, diet, and living conditions appear to have a key role in ageing process as well (9). Age-related alterations in the kidney are similar to those verified in chronic renal diseases and experimental models with chronic renal failure.

With the medulla being relatively spared, loss of renal mass is primarily expressed in the renal cortex, which is confirmed by the studies that found that half of the total nephron number might be lost till ripe old age.

Age-related increase in sclerotic glomeruli number is empirically confirmed by the clinical data and may be found in many studies. Within the glomeruli, mesangial matrix is progressively being expanded, the arterioles are hyalinized, and while the number of renal corpuscles is decreasing, their area is increasing (10, 11).

Changes associated with the ageing of human glomeruli are progressive decrease of the number of glomeruli, which is directly related to the birth weight, existence of shunts between afferent and efferent arterioles, mesangial matrix expansion followed by the onset of glomerusclerosis, and increased number of globally sclerotic glomeruli (12-17). The key question is: what is the nature of glomerular damage mechanisms, are they immunologically (immune complex accumulation) or non-immunologically associated with hemodynamic factors? From the majority of researchers' standpoint, as pathological substrates we may consider mesangial matrix expansion due to the collagen deposits, vascular changes, and glomerular inflammation caused by immunological mechanisms (18-20).

The aim of our research was to differentiate manifestly sclerotic glomeruli from hypertrophic ones and morphologically non-sclerotic ones, to quantify the presence of mesangial connective tissue and number of glomeruli during ageing, and to investigate the importance of these changes in clinical practice.

Materials and methods

The material was human right kidney tissue of 30 cadavers, obtained during routine autopsies at the Institute of Forensic Medicine in Niš. Their age ranged from 20 to 85 years. During autopsy, kidney damage or congenital anomalies were not observed. Cadavers were without previously diagnosed kidney disease, diabetes, hypertension, or any other systemic disease. Tissue specimens were fixed in 10% buffered formalin for 12 hours and then embedded in paraplast. The tissue was then cut into 5 μm thick sections and routinely stained with Mallory's trichrome stain. Histological slices were analyzed under 400x magnification. Images of histological slices were captured with digital camera (5 megapixels resolution).

Glomeruli were analyzed with ImageJ software (http://rsbweb.nih.gov/ij/) which was spatially calibrated with object micrometer (1:100). The glomerular tuft area (A_G), perimeter (B_G), diameter along main (D_M) and secondary axis (D_m), Feret's diameter (D_F), glomerular connective tissue area (A_{CT}), percentage of connective tissue (CT%) and total number of cells per glomerular area unit (N_n) were measured. Glomerular images were additionally processed for connective tissue area measurement. Glomerular tuft image was first manually selected by polygonal selection tool and extracted from the other parts of histological slice image. Selection of its connective tissue, which was green stained on Mallory's trichrome stained sections, was performed by "Color based tresholding" option. Its application was based on green colored sample of glomerular tuft image. Afterwards, only green stained parts of glomerular tuft remained on image, which was further converted into a binary image. The binary image was used for connective tissue area measurement. Green colored samples were taken at three different localizations in each glomerular tuft image. Connective tissue area was measured for each sample. Average connective tissue area was then calculated from three obtained values for each glomerular tuft. Glomerular connective tissue percentage was obtained from the ratio between glomerular connective tissue area and total glomerular area. Seven cortical, seven columnar and seven juxtamedullary glomeruli were analyzed per one case. Additionally, no more than seven globally sclerotic glomeruli were also analyzed per one case. They served as a positive control during morphometric analysis. Totally, 743 (114 sclerotic and 629 morphologically nonsclerosed) glomeruli were analyzed in all 30 cases. Average values of morphometric parameters were calculated for each of all 30 evaluated cases.

Statistical analysis was performed with NCSS-PASS software (http://www.ncss.com/). Cluster analysis by the k-means method was performed for the classification of glomeruli into age groups according to their morphometric characteristics. One-way ANOVA was used for the comparison of more than two groups. In cases where data did not have normal distribution, Kruskal-Wallis One-way ANOVA was used for the comparison of more than two groups. Statistical significance test was performed for $p < 0.05$.

Cluster analysis was performed twice during this study. Firstly, it was used for the classification of glomeruli into types according to their morphometric characteristics and secondly, for the classification of the evaluated human cases into the groups, according to the percentage of obtained types of glomeruli and their age.

Results

After morphometric analysis of a total of 743 glomeruli, three groups of glomeruli were made. The first group included 114 glomeruli with the lowest values of area, cellularity, and greatest percentage of connective tissue. There were 430 morphologically normal glomeruli with the largest number of cells per area unit in the second group. The third group included 199 glomeruli with the largest area, significant cellularity and percentage of connective tissue (Table 1). All investigated morphometric parameters of glomeruli showed statistically significant alterations (Table 2).

Table 1. Morphometric characteristics of glomeruli groups classified by the cluster analysis

	A_G (μm²)		B_G (μm)		D_M (μm)		D_m (μm)	
Cluster	I (n = 114)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	6712.36	6520.95	302.39	302.31	102.73	100.87	81.97	82.38
SE	173.86	/	3.85	/	1.41	/	1.20	/
95% LCL	6367.90	6148.01	294.76	289.59	99.93	98.80	79.60	78.91
95% UCL	7056.82	7013.99	310.01	314.58	105.53	106.53	84.35	85.87
Cluster	II (n = 430)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	14618.41	14154.06	453.37	450.69	152.07	150.43	121.43	120.54
SE	154.99	/	2.80	/	0.95	/	0.74	/
95% LCL	14313.77	13825.43	447.86	442.54	150.19	148.05	119.99	118.65
95% UCL	14923.05	14697.38	458.87	457.68	153.95	152.03	122.88	121.84
Cluster	III (n = 199)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	22478.81	21894.61	564.24	556.76	189.36	185.69	149.84	149.53
SE	314.27	/	4.69	/	1.53	/	1.30	/
95% LCL	21859.05	21188.06	554.99	546.10	186.36	183.13	147.27	146.19
95% UCL	23098.56	22664.15	573.50	568.77	192.37	189.62	152.40	151.94

	D_F (μm)		A_{CT} (μm²)		CT%		N_n (1/μm²) x 10^{-3}	
Cluster	I (n = 114)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	107.03	105.17	4761.58	4774.59	71.10	71.77	1.7	1.6
SE	1.44	/	128.66	/	0.53	/	0.1	/
95% LCL	104.18	102.06	4506.69	4273.92	70.04	69.86	1.6	1.5
95% UCL	109.87	110.44	5019.23	5019.23	72.16	73.24	1.8	1.8
Cluster	II (n = 430)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	158.87	157.14	3576.19	3591.79	25.26	25.70	6.8	6.9
SE	0.96	/	47.80	/	0.37	/	0.1	/
95% LCL	156.98	154.64	3482.23	3437.98	24.52	24.76	6.7	6.7
95% UCL	160.75	160.00	3670.15	3739.07	25.99	26.11	6.9	7.0
Cluster	III (n = 199)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	197.22	193.01	6774.02	6406.87	30.66	31.49	5.9	5.9
SE	1.58	/	115.15	/	0.45	/	0.1	/
95% LCL	194.11	189.62	6546.94	6207.42	29.76	30.54	5.7	5.8
95% UCL	200.33	197.60	7001.10	6588.96	31.55	32.46	6.0	6.1

Md – median,
SE – standard error,
95% LCL – lower limit of confidence interval,
95% UCL – upper limit of confidence interval

Table 2. Results of One Way ANOVA test of morphometric characteristics of the glomeruli classified into groups

Parameter	One-Way ANOVA			Kruskal-Wallis One-Way ANOVA	
	F	p	Power	H	p
A_G *	799.64	≤ 0.0001	1.00	503.66	≤ 0.0001
B_G *	740.03	≤ 0.0001	1.00	478.13	≤ 0.0001
D_F *	748.78	≤ 0.0001	1.00	469.26	≤ 0.0001
D_M *	712.63	≤ 0.0001	1.00	464.30	≤ 0.0001
D_m *	673.00	≤ 0.0001	1.00	449.39	≤ 0.0001
A_{CT} *	445.72	≤ 0.0001	1.00	416.66	≤ 0.0001
CT% *	1886.46	≤ 0.0001	1.00	337.67	≤ 0.0001
N_n *	1345.94	≤ 0.0001	1.00	365.70	≤ 0.0001

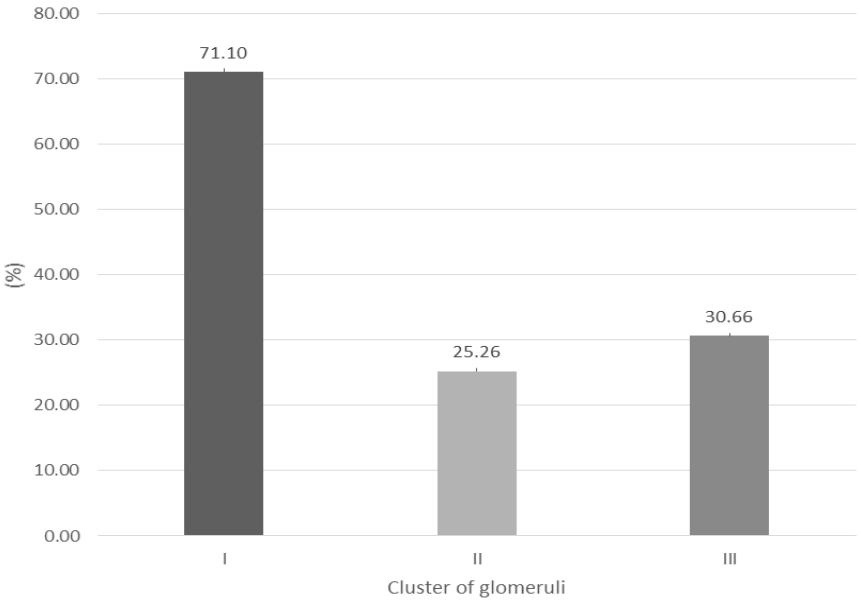
* $p < 0.05$ – positive D'Agostino-Pearson Omnibus normality test

The average values of area, perimeter, diameter along main and secondary glomerular axis, Feret's diameter are considered as morphometric parameters which describe the size and form of the glomerulus.

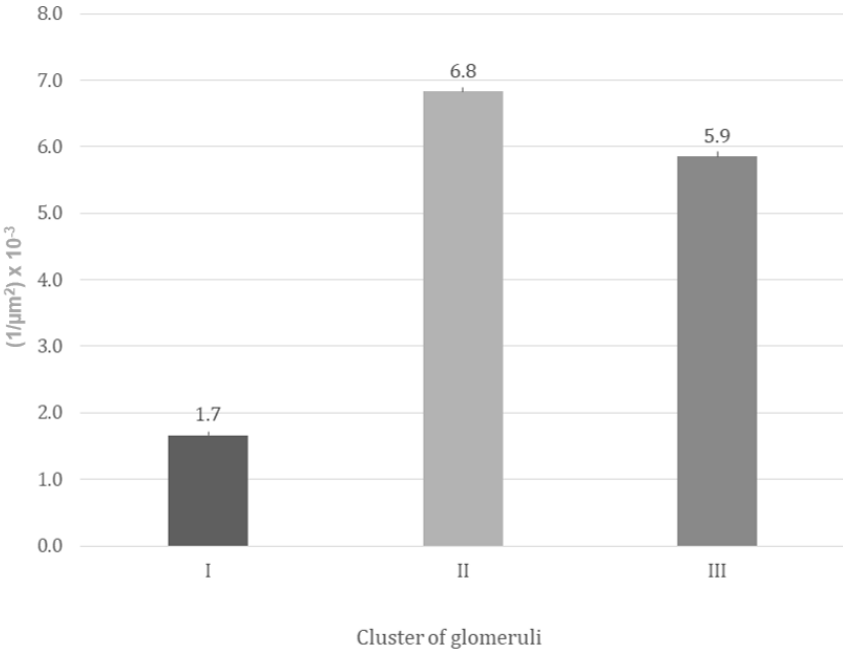
The average area of glomeruli in the first group was 6712.36 μm^2 and it was significantly ($p \leq 0.0001$) lower compared to the second and third group. The mean area in the third group was 22478.81 μm^2, which was a statistically significant increase in comparison with the first and second groups of glomeruli (Table 1; Table 2). The average area (4761.58 μm^2) and percentage (71.1%) of connective tissue within glomeruli had the greatest values in the first group. There was a statistically significant decrease in connective tissue area in the second group vs. the first group (3576.19 μm^2, $p \leq 0.0001$), which made 25.26% of the total glomerular connective tissue in group II. In the third group of glomeruli, the mean connective tissue area was 6406.87 μm^2, or 30.66% of the total glomerular connective tissue, which was significantly lower ($p \leq 0.0001$) vs. the first group (Table 1, Graph 1).

Mean perimeter, diameter along main and secondary glomerular axis, Feret's diameter were statistically significantly lowest in group I of glomeruli ($p \leq 0.0001$). Their values were statistically significantly increasing towards the third group (Table 1, 2).

The average cell nuclei number per area unit of the investigated glomeruli showed the largest increase in group II of glomeruli. It was significantly higher ($p \leq 0.0001$) vs. the first group, where it had the lowest values, as well as vs. the third group of glomeruli (Table 1, Graph 2).



Graph 1. Mean glomerular connective tissue percentage in the group of glomeruli classified by the cluster analysis



Graph 2. Mean cellularity in the group of glomeruli classified by the cluster analysis

According to the changes in morphometric parameters, it is evident that morphologically sclerotic glomeruli, which are the smallest and with highest connective tissue percentage, are present in the first group. Morphologically normal glomeruli in the second group are characterized by a higher cell number and the lowest connective tissue percentage. In the third group, there are morphologically normal glomeruli with the largest area, decreased cell number per are unit, and increased connective tissue percentage, which suggests the presence of hypertrophy.

Further analysis was performed to investigate the representation of the aforesaid group of glomeruli within the age groups. The first group consisted of the youngest cases, six in total, who were aged 24-33 years, average 29. Eleven older cases, aged 40-49 years, average 44, were in the second group. In the third group there were 13 oldest cases aged 65-76 years, average 71 (Table 3). Out of 114 sclerotic glomeruli only two were identified in the first age group, which was significantly lower ($p \leq 0.0001$) than in the second (37 glomeruli) and the third age group (75 glomeruli). There were 430 morphologically normal glomeruli, most of them being in the second age group (162), which was significantly higher number of glomeruli ($p \leq 0.0001$) than in the first (124) and third age groups (144). We found 199 hypertrophic glomeruli, only 2 of them classified in first age group, 69 of them in the second age group, which was significant increase vs. the first group ($p \leq 0.0001$), and 128 glomeruli in the third age group, being statistically significantly higher vs. both younger groups ($p \leq 0.0001$) (Table 4).

These data show a significant increase in the number of sclerotic and hypertrophic glomeruli during ageing, particularly in age group II and III, while morphologically normal glomeruli are most frequent in the first and second age groups. It is at the age of 40-49 when the first changes appear, followed by hypertrophy and glomerulosclerosis, intensifying with age and being the most prominent in the oldest ones.

Gained and expected distribution of clusters of glomeruli within age groups classified by cluster analysis are statistically significantly different ($\chi^2 = 118.91$, d.f. = 4, $p \leq 0.0001$).

Table 3. Age groups of the investigated cases

	Age		**A_G (μm²)**		**B_G (μm)**		**D_M (μm)**		**D_m (μm)**	
Cluster	I (n = 6)									
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	29	27	15605.61	15693.20	463.64	465.11	154.99	157.53	126.43	127.08
SE	2	/	1157.63	/	17.16	/	6.32	/	4.61	/
95% LCL	24	25	12629.84	11055.55	419.53	393.21	138.73	128.21	114.57	108.91
95% UCL	33	34	18581.39	19991.87	507.76	521.23	171.25	176.25	138.28	144.30
Cluster	II (n = 11)									
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	44	45	15982.37	14524.17	466.58	439.88	157.16	148.42	125.03	122.35
SE	2	/	1299.80	/	18.04	/	6.64	/	4.61	/
95% LCL	40	38	13086.23	12873.85	426.38	416.36	142.37	137.36	114.75	112.19
95% UCL	49	50	18878.51	17306.07	506.79	490.13	171.95	166.27	135.30	129.78
Cluster	III (n = 13)									
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	71	71	15340.53	14772.57	456.81	462.46	153.44	152.87	121.01	119.92
SE	2	/	1071.91	/	15.08	/	4.51	/	4.31	/
95% LCL	65	63	13122.69	12551.79	423.97	414.25	143.61	140.12	111.61	109.28
95% UCL	76	78	17558.37	19091.36	489.66	516.56	163.27	170.55	130.40	138.91

	D_F (μm)		**A_{CT} (μm²)**		**CT%**		**N_n (1/μm²) x 10^{-3}**	
Cluster	I (n = 6)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	161.47	164.43	2601.18	2451.77	17.53	15.16	6.9	7.1
SE	6.24	/	133.16	/	2.28	/	0.2	/
95% LCL	145.41	134.60	2258.87	2336.03	11.67	14.69	6.4	6.2
95% UCL	177.52	181.76	2943.48	3012.00	23.38	28.86	7.5	7.4
Cluster	II (n = 11)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	163.44	155.02	4468.52	4280.17	32.11	33.48	6.1	6.0
SE	6.72	/	213.61	/	1.42	/	0.2	/
95% LCL	148.46	144.63	3992.57	3761.00	28.96	30.41	5.7	5.6
95% UCL	178.41	172.25	4944.48	5245.75	35.27	34.91	6.4	6.5
Cluster	III (n = 13)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	160.53	160.78	5496.72	5507.65	40.66	41.58	5.1	5.0
SE	4.56	/	364.47	/	1.19	/	0.2	/
95% LCL	150.61	147.03	4702.62	4347.51	38.08	35.96	4.7	4.5
95% UCL	170.46	176.88	6290.83	6600.06	43.25	44.32	5.6	5.9

Md – median,
SE – standard error,
95% LCL – lower limit of confidence interval,
95% UCL – upper limit of confidence interval

Table 4. Distribution of clusters of glomeruli within the age groups classified by the cluster analysis

Cluster of glomeruli	Age group			Σ
	I	II	III	
I	2	37	75	114
II	124	162	144	430
III	2	69	128	199
Σ	128	268	347	743

Discussion

Structural changes found during normal ageing of the kidney support the concept of decline of renal function along with ageing. Some authors do not consider this as a rule, due to a phenomenon of vascular adaptation which may preserve glomerular filtration by provoking hyperperfusion and hyperfiltration in healthy glomeruli (21-23). Functional alterations are reflected as a decrease of renal functional reserve, associated with limits in renal capacity to adequately answer the challenges of excess or deficit.

Kidney has an ability for adaptation and regeneration. Temporary increase of load is being compensated in kidney by turning on its reserve functional units which are out of function occasionally. If this functional load increase lasts for a long time, it leads to the onset of renal hypertrophy. The best example is compensatory renal hypertrophy, where there are no new nephrons produced, but the diameter and epithelial cell number are being increased, mostly within the wall of the proximal segment of nephron. It is confirmed that, along with increase in kidney size, there is an enlargement of glomeruli as well, whose number stays the same or even get lower (8, 9).

Histological assessment of preimplantation kidney biopsies gained on the basis of expanded criteria donors enables their further use according to the scale for histological kidney sustainability scale (24, 25). In humans, the number of nephrons is determined by genetic and environmental factors and does not increase after birth. Therefore, glomerular adaptation on the higher metabolic demand or decrease in renal mass is associated only with a change in glomerular size (26). So far, studies have shown that "physiological" decline in glomeruli normally begins *in utero* and continues during child's growth and development. In adults aged between 20 and 33, the process may be repaired, therefore 95% of population under 40 have less than 10% of sclerotic glomeruli, while its percentage starts increasing after the age of 50, being 12.5% in average, whilst in patients older than 70 years of age the percentage may reach 30% (9, 17). Most authors consider the presence of glomerulosclerosis as a consequence of a renal failure, if there are more than 10% of sclerotic glomeruli in persons younger than 40 years of age (12, 14, 19). Contrary to that, we cannot precisely define clear boundary between abiotrophic involutional sclerosis caused by ageing from that caused by a renal disease, in persons aged over 40 (18, 23). However, some studies show that the ability of glomeruli to grow in size without any consequent damage progressively decrease during ageing (27, 28).

It is assumed that the implantation of one kidney may be in favor of the onset of hyperfiltration-mediated damage of glomeruli due to the imbalance between mass of the nephron and size of the recipient. Further, it leads to glomerular growth, then to glomerulosclerosis and eventually to progressive renal insufficiency (29). Glomerular adaptation after renal transplantation may be influenced also by present chronic renal failures, post-transplantation injuries, vascular lesions (30), glomerulosclerosis diagnosed after donor kidney biopsies (31), which altogether may affect the outcome of the kidney transplantation. Larger glomerular volume in donor biopsies is related to allograft malfunction (32) and it is suggested that extreme post-transplantation glomerular size is associated with glomerulosclerosis (33). In morphometric study of the kidney tissue obtained from older and young donors by Tan et al. (34), a significant increase of globally sclerotic glomeruli percentage was observed in the older donor group versus the younger donors group. According to Tracy et al. (15) nonsclerosed glomeruli showed increasing volume in the older group compared to the young donors. This resulted in a significant increase of the filtration surface area and single nephron ultrafiltration coefficient. Sclerosing glomerulopathy led to consequent glomerulopenia and compensatory hypertrophy with adaptive hyperfiltration of nonsclerosed glomeruli.

The method for quantification of the glomerular size, area of connective tissue, and cell number within glomeruli that we used might be useful to evaluate donor kidney tissue intended for transplantation. It is known that significant alterations of glomerular morphology are present in older donors. Anyway, similar data about potential donors aged 40-49 years old are very scarce. Our results showed that these examinees might possess a significantly higher number of morphologically normal glomeruli at the first sight, but morphometrically they might belong to the group of hypertrophic glomeruli which are in the initial phase of sclerosis. In our former study we predominantly found the presence of hypertrophic glomeruli in cases over 55 years of age, while those morphologically normal were prevailing in younger ones (28). Such glomeruli are probably in the initial phase of glomerulosclerosis and their predomination in older cases might affect the impairment of their renal functional reserve, as well as the success of the renal transplantation in cases

where renal allograft originated from such older individuals (34-37).

It might be suggested that morphometric assessment of morphologically normal glomeruli should be of greatest importance transplantation-wise, and not only estimation of its total number and number of detected manifestly sclerotic glomeruli (26). Alperovich et al. (38) evaluated mean glomerular volume before and after transplantation using paired pre-implantation and protocol biopsies performed in stable renal allografts. Multiple regression analysis confirmed that glomerular volumes in donors and allografts with chronic nephropathy are independent indicators of glomerular size after transplantation. They detected the increase in glomerular volume after four months, which correlated with creatinine values and indirectly points out that glomerular enlargement is a necessary condition for glomerular adaptation after transplantation.

Glomerular enlargement is reduced in patients with chronic allograft nephropathy. Data from studies on donor biopsies suggested inversely proportional relationship between age and glomerular volume (37). Abdi et al. (32) showed that the increase in glomerular volume in donor biopsies correlated with renal allograft dysfunction. They noticed that the larger glomerular volume in donor biopsies, the smaller post-transplant glomerular size. This result suggests that kidneys with larger glomeruli are already adapted to metabolic needs of a donor and therefore their potential for further adaptation is limited. In stereological studies on a cadaveric material, the inverse ratio between the number and the perimeter of the glomeruli is often described (26). Thus, greater glomerular volume may be considered as a consequence of a decrease in the total number of glomeruli and as such may be predisposing factor for the development of renal disease (39).

Our results suggest that glomerular enlargement may be a necessary precondition for the transplanted kidney to reach adequate renal function, and that higher values of the glomerular area in donors indicate depleted capacity for further functional adaptation of the kidney after transplantation.

Adaptation of glomerular volume either to renal mass loss or increased metabolic needs has been investigated in various experimental and clinical studies. In adults, glomerular volume was doubled after a kidney removal, but mostly without the onset of sclerosis, while glomerular hypertrophy and intraglomerular hypertension developed after a decrease in the number of nephrons may initiate and accelerate the onset of hypertension and progressive renal insufficiency (39). On the contrary, in patients with unilateral renal agenesis, conditions characterized by lower number of nephrons, or oligomeganephronia, the glomerular volume increases five - to eightfold and is associated with glomerulosclerosis (33). This indicates that the capacity for glomerular enlargement in the old age depends on nephron loss, whereby the onset of compensatory glomerular hypertrophy is related to the loss below critical threshold. Our results of increased number of hypertrophic glomeruli in the oldest group supports this assumption.

Conclusion

In accordance with the aforementioned data and our results on cadaveric material, it may be concluded that the donor age alone should not be a problem if a histological analysis of the kidney finds it adequate. Also, if there is a great number of enlarged glomeruli in young donors before transplantation, it may be a reason for possible graft rejection. It is for sure that renal vascular diseases, acute renal failure, obstructive nephropathy, and some systemic diseases are more frequent in the older population. Such conditions may lead to the damage of glomeruli accelerating age-related physiological alterations in the kidney.

Using the new methods for the investigation and classification of glomeruli which morphologically have not shown signs of sclerosis on the basis of area, presence of connective tissue, and cell number within them, the given results may be useful to assess renal function after transplantation.

Acknowledgements

This study was supported by the Ministry of Science and Technological Development, Republic of Serbia (Nos 175092, 175061, 31060 and 41018) and by the Internal projects of the Faculty of Medicine in Niš (No 38/20).

References

1. Silva FG. The aging kidney: a review - part I. Int Urol Nephrol 2005;37(1):185-205. [CrossRef] [PubMed]
2. Macrae J, Friedman AL, Friedman EA, Eggers P. Live and deceased donor kidney transplantation in patients aged 75 years and older in the United States. Int Urol Nephrol 2005;37(3):641-8. [CrossRef] [PubMed]
3. Zhou XJ, Rakheja D, Yu X, Saxena R, Vaziri ND, Silva FG. The aging kidney. Kidney Int 2008;74(6):710-20. [CrossRef] [PubMed]

4. Costello-White R, Ryff CD, Coe CL. Aging and low-grade inflammation reduce renal function in middle-aged and older adults in Japan and the USA. Age (Dordr) 2015;37(4):9808. [CrossRef] [PubMed]
5. Schold JD, Kaplan B, Baliga RS, Meier-Krieshe HU. The broad spectrum of quality in deceased donor kidneys. Am J Transplant 2005;5(4Pt1):757-65. [CrossRef] [PubMed]
6. Percy C, Pat B, Poronnik P, Gobe G. Role of oxidative stress in age-associated chronic kidney pathologies. Adv Chronic Kidney Dis 2005;12(1):78-83. [CrossRef] [PubMed]
7. Peppa M, Uribarri J, Vlassara H. Aging and glycoxidant stress. Hormones (Athens) 2008;7(2):123-32. [CrossRef] [PubMed]
8. Bolignano D, Mattace-Raso F, Sijbrands EJ, Zoccali C. The aging kidney revisited: a systematic review. Ageing Res Rev 2014;14:65-80. [CrossRef] [PubMed]
9. Martin JE, Sheaff MT. Renal ageing. J Pathol 2007; 211(2):198-205. [CrossRef] [PubMed]
10. Rodriguez-Puyol D. The aging kidney. Kidney Int 1998;54(6):2247-65. [CrossRef] [PubMed]
11. Long DA, Mu W, Price KL, Johnson RJ. Blood vessels and the aging kidney. Nephron Exp Nephrol 2005; 101(3):e95-99. [CrossRef] [PubMed]
12. Neugarten J, Gallo G, Silbiger S, Kasiske B. Glomerulosclerosis in aging humans is not influenced by gender. Am J Kidney Dis 1999;34(5):884-8. [CrossRef] [PubMed]
13. Tracy RE, Ishii T. What is 'nephrosclerosis'? lessons from the US, Japan, and Mexico. Nephrol Dial Transplant 2000;15(9):1357-66. [CrossRef] [PubMed]
14. Marcantoni C, Ma LJ, Federspiel C, Fogo AB. Hypertensive nephrosclerosis in African Americans versus Caucasians. Kidney Int 2002;62(1):172-80. [CrossRef] [PubMed]
15. Tracy RE, Parra D, Eisaguirre W, Balanza AT Influence of arteriolar hyalinization on arterial intimal fibroplasia in the renal cortex of subjects in the United States, Peru, and Bolivia, applicable also to other populations. Am J Hypertens 2002;15(12):1064-73. [CrossRef] [PubMed]
16. Stojanović V, Jovanović I, Ugrenović S, Kundalić B, Pavlović M. Histochemical and morphometric analysis of connective tissue in human glomerules during aging. FU Med Biol 2014;16(1):31-5.
17. Stojanović V, Jovanović I, Ugrenović S, Pavlović S. Quantification of sclerotic renal glomeruli during the aging process in humans. Med Pregl 2010;63(11-12):775-8. [CrossRef] [PubMed]
18. Li M, Nicholls KM, Becker GJ. Glomerular size and global glomerulosclerosis in normal Caucasian donor kidneys: effects of aging and gender. J Nephrol 2002; 15(6):614-9. [PubMed]
19. Zheng F, Cheng QL, Plati AR, Ye SQ, Berho M, Banerjee A, et al. The glomerulosclerosis of aging in females: contribution of the proinflammatory mesangial cell phenotype to macrophage infiltration. Am J Pathol 2004;165(5):1789-98. [CrossRef] [PubMed]
20. Colvin MM, Smith CA, Tullius SG, Goldstein DR. Aging and the immune response to organ transplantation. J Clin Invest 2017;127(7):2523-9. [CrossRef] [PubMed]
21. Lindeman RD. Overview: renal physiology and pathophysiology of aging. Am J Kidney Dis 1990;16(4):275-82. [CrossRef] [PubMed]
22. Schmitt R, Cantley LG. The impact of aging on kidney repair. Am J Physiol Renal Physiol 2008; 294(6): F1265-72. [CrossRef] [PubMed]
23. Hoang K, Tan JC, Derby G, Blouch KL, Masek M, Ma I, et al. Determinants of glomerular hypofiltration in aging humans. Kidney Int 2003;64(4):1417-24. [CrossRef] [PubMed]
24. Remuzzi G, Cravedi P, Perna A, Dimitrov B, Turturro M, Locatelli G, et al. Long-term outcome of renal transplantation from older donors. N Engl J Med 2006; 354(4):343-52. [CrossRef] [PubMed]
25. Ruggenenti P, Perico N, Remuzzi G. Ways to boost kidney transplant viability: a real need for the best use of older donors. Am J Transplant 2006;6(11):2543-7. [CrossRef] [PubMed]
26. Hoy WE, Samuel T, Hughson MD, Nicol JL, Bertram JF. How many glomerular profiles must be measured to obtain reliable estimates of mean glomerular areas in human renal biopsies? J Am Soc Nephrol 2006;17(2): 556-63. [CrossRef] [PubMed]
27. Skov K, Nyengaard JR, Patwardan A, Mulvany MJ. Large juxtamedullary glomeruli and afferent arterioles in healthy primates. Kidney Int 1999;55(4):1462-9. [CrossRef] [PubMed]
28. Stojanović VR, Jovanović ID, Ugrenović SZ, Vasović LP, Živković VS, Jocić MV, et al. Morphometric analysis of nonsclerosed Glomeruli size and connective tissue content during the aging process. Scientific World Journal 2012;2012:845046. [CrossRef] [PubMed]
29. Brenner BM, Mackenzie HS. Nephron mass as a risk factor for progression of renal disease. Kidney Int Suppl 1997;63:S124-7. [PubMed]
30. Wang HJ, Kjellstrand CM, Cockfield SM, Solez CK. On the influence of sample size on the prognostic accuracy and reproducibility of renal transplant biopsy. Nephrol Dial Transplant 1998;13(1):165-72. [CrossRef] [PubMed]
31. Gaber LW, Moore LW, Alloway RR, Amiri MH, Vera SR, Gaber AO. Glomerulosclerosis as a determinant of posttransplant function of older donor renal allografts. Transplantation 1995;60(4):334-9. [CrossRef] [PubMed]
32. Abdi R, Slakey D, Kittur D, Burdick J, Racusen L. Baseline glomerular size as a predictor of function in human renal transplantation. Transplantation 1998; 66(3):329-33. [CrossRef] [PubMed]
33. Bhathena DB. Glomerular size and the association of focal glomerulosclerosis in long-surviving human renal allografts. J Am Soc Nephrol 1993;4(6):1316-26. [PubMed]
34. Tan JC, Workeneh B, Busque S, Blouch K, Derby G, Myers BD. Glomerular function, structure, and number in renal allografts from older deceased donors. J Am Soc Nephrol 2009;20(1):181-8. [CrossRef] [PubMed]
35. Tan JC, Busque S, Workeneh B, Ho B, Derby G, Blouch KL, et al. Effects of aging on glomerular function and number in living kidney donors. Kidney Int 2010;78(7):686-92. [CrossRef] [PubMed]
36. Fulladosa X, Moreso F, Narváez JA, Hueso M, Caldés A, Gil-Vernet S, et al. Total glomerular number in stable renal allografts. Transplant Proc 2002;34(1): 343-4. [CrossRef] [PubMed]
37. Rule AD, Amer H, Cornell LD, Taler SJ, Cosio FG, Kremers WK, et al. The association between age and nephrosclerosis on renal biopsy among healthy adults. Ann Intern Med 2010;152(9):561-7. [CrossRef] [PubMed]
38. Alperovich G, Maldonado R, Moreso F, Fulladosa X, Grinyó JM, Serón D. Glomerular enlargement assessed by paired donor and early protocol renal allograft biopsies. Am J Transplant 2004;4(4):650-4. [CrossRef] [PubMed]
39. Hoy WE, Bertram JF, Denton RD, Zimanyi M, Samuel T, Hughson MD. Nephron number, glomerular volume, renal disease and hypertension. Curr Opin Nephrol Hypertens 2008;17(3):258-65. [CrossRef] [PubMed]

Originalni rad **UDC: 616.611-089.843**
doi:10.5633/amm.2021.0203

ZNAČAJ I POTENCIJALNA PRIMENA MORFOMETRIJSKE ANALIZE HUMANIH GLOMERULA U KADAVERIČNOJ TRANSPLANTACIJI BUBREGA

Vesna Stojanović[1], Ivan Jovanović[1], Braca Kundalić[1], Slađana Ugrenović[1], Miljana Pavlović[1], Milena Trandafilović[1], Jovana Čukuranović Kokoris[1], Vladimir Petrović[2]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za anatomiju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Katedra za histologiju i embriologiju, Niš, Srbija

Kontakt: Milena Trandafilović
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: milena.trandafilovic@medfak.ni.ac.rs

Kadaverična transplantacija bubrega u stalnom je porastu, zbog smanjenog mortaliteta mlađih pojedinaca. Tokom procesa ovih transplantacija, od velike je važnosti odrediti ne samo starosnu granicu primaoca, već i status davaoca.

Istraživanje je obuhvatilo 30 uzoraka tkiva ljudskih kadaveričnih bubrega (oba pola, starosti od 20 do 85 godina). Uzorci tkiva obojeni su Mallori trihromskim bojenjem i analizirani pod svetlosnim mikroskopom. Slike su analizirane pomoću softvera ImageJ. Kao rezultat klaster analize, 743 glomerula klasifikovana su u 3 grupe prema morfometrijskim karakteristikama i u 3 starosne grupe (I grupa prosečne starosti 29 godina, II grupa 44 godine, III grupa sa prosekom starosti 71 godina). U prvoj grupi po morfometrijskim karakteristikama bilo je 114 sklerotičnih glomerula sa značajno ($p \leq 0,0001$) najmanjom površinom i calularnošću, a najvećim procentom vezivnog tkiva. U drugoj grupi bilo je 430 morfološki normalnih glomerula sa najvećim brojem ćelija po jedinici površine ($p \leq 0,0001$). U trećoj grupi bilo je 199 hipertrofičnih glomerula sa najvećom površinom, značajno velikom calularnošću i površinom vezivnog tkiva ($p \leq 0,0001$). Od 114 sklerotičnih glomerula najmanji broj pripada I starosnoj grupi ($p \leq 0,0001$). Ukupno je bilo 430 morfološki normalnih glomerula. Većina morfološki normlanih glomerula bila je u II starosnoj grupi ($p \leq 0,0001$). Većina od 199 hipertrofičnih glomerula bila je u III starosnoj grupi, naspram druge dve grupe ($p \leq 0,0001$), kao i u II grupi u odnosu na I grupu ($p \leq 0,0001$). Morfometrijska analiza morfološki normalnih glomerula treba da bude od najveće važnosti za transplantaciju, a ne samo procena njihovog ukupnog broja i broja detektovanih manifestno sklerotičnih glomerula.

Acta Medica Medianae 2021;60(2):25-34.

Ključne reči: *ljudski glomeruli, transplantacija bubrega, morfometrija*

Original article **UDC: 616.915-036.22(497.11)"2017/2018"**
doi:10.5633/amm.2021.0204

MEASLES OUTBREAK IN THE NIŠAVA AND TOPLICA DISTRICTS FROM 2017 TO 2018

Miodrag Stojanović[1,2], Nataša Rančić[1,2], Nikola Milenković[3], Miodrag Perović[2], Ivana Janićijević[2], Marina Kostić[2], Mirko Ilić[2], Marko Stojanović[2], Marko Jovanović[2], Aleksandra Ignjatović[1,2], Marija Apostolović Andjelković[1,2]

Measles was the leading cause of death among children under 5 years of age before the introduction of mandatory vaccination.

The objective of the paper was to describe the epidemiological and clinical characteristics of the affected individuals in the large measles outbreak in 2017-2018 in the Nišava and Toplica Districts.

Descriptive study was done. For the investigation of the outbreak, the general principles of the case definition of the European Union (EU) Commission Decision of 2012 were used. Laboratory investigations of initial patients were conducted at the Center for Control and Prevention of Diseases in the Institute of Public Health Niš, and all specimens were sent for anti-measles IgM/IgG antibody tests to the reference laboratory of the Institute of Virology, Vaccines and Sera "Torlak" in Belgrade.

A total of 1327 (584 males and 743 females) cases were reported from 23 November 2017 to 28 July 2018, when it ended. The average age was 35 years (range from < 1 to 70 years). The highest number of patients (510; 38.4%) were in the 30–39 year age group and the lowest number (34; 2.6%) was in the 15-19 year age group. Infants represented 5.3% of all affected and children from primary schools accounted for 4.4% out of all affected. One-fourth of the outbreak cases (338; 25.5%) were unvaccinated. Only 37 (2.8%) patients received two doses of the combined vaccine against measles, mumps, and rubella (MMR) and 50 (3.8%) received one dose. For the majority of affected cases (902; 68.0%) vaccination status was unknown. Measles-related complications were registered in 962 (72.5%) patients. Complications were the most common in infants (92.9%) and among children 1-6 years of age (88.2%). Malnutrition was the most frequent complication (823 cases; 62.0%) followed by diarrhea (590 cases; 44.5%) and pneumonia (122 cases; 9.2%); encephalitis was reported in 1 case. Measles-related deaths in the observed outbreak were confirmed in four patients (all laboratory-confirmed, three unvaccinated and an immune compromised child). The case-fatality rate of 0.3 per 100 measles cases was determined.

The probable causes of this large measles outbreak were insufficient vaccination and low vaccine coverage with MMR vaccine and accumulation of a high susceptible population. Four measles-related deaths were registered. Monitoring of the vaccination status, high vaccine coverage and effectiveness of MMR vaccine are essential for the prevention of measles outbreaks.

Acta Medica Medianae 2021;60(2):35-43.

Key words: *measles, outbreak, vaccination coverage, complications, deaths*

[1]University of Niš, Faculty of Medicine, Niš, Serbia
[2]Institute for Public Health, Niš, Serbia
[3]University Clinical Center Niš, Niš, Serbia

Contact: Nataša Rančić
81 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: natasa.rancic@medfak.ni.ac.rs

Introduction

Measles is a highly contagious acute respiratory viral illness characterized by fever, malaise, rash, cough, coryza, and conjunctivitis (1). Measles is a vaccine-preventable disease (2) and before the introduction of measles vaccination, measles primarily affected children under the five years of age (3) and mortality due to measles-related complications was high up to 10% (3).

The most common measles-related complications are: diarrhea (in 8% of cases), otitis media (7-9%), pneumonia (1-6%), and encephalitis (1-2 per 1000 cases). Measles-related death occurs in 1-

3 per 1000 cases (4). Measles is still common and often fatal disease in developing countries (5), and incidence has decreased substantially in regions where vaccination has been instituted. Measles occurs predominantly in areas with low vaccination rates, particularly in the developing parts of the world (6).

In 2000, the United States of America (USA) has maintained measles elimination but as measles outbreaks continue to occur globally, the USA remains at risk of imported measles and potential spread (7). Also, imported cases still occur and the highest incidence is in infants, young children and in unvaccinated persons (8). Measles vaccines are live attenuated and are among the most highly effective vaccines available (providing 97% protection with two doses, given at 12 to 15 months and 4 to 6 years of age), with a proven safety record.

In 2000, measles vaccination has prevented an estimated 21 million deaths worldwide (9). Despite these substantial gains, global elimination goals have not been met, and previous studies are now being threatened by a 31% increase in the number of measles cases reported globally between 2016 and 2017.

The measles vaccine was introduced in Serbia in 1971 as a monovalent preparation (10) and the combined against measles, mumps, and rubella (MMR) vaccine has been widely used since 1994 by the National Immunization Program (NIP) in Serbia. The combined MMR vaccine has been given as the first dose from 12 to 15 months of age with additional booster shot before starting school at the age of seven. Since 2010, there have been problems in the distribution and therefore lack of MMR vaccine, which led to the accumulation of highly sensitive unvaccinated or incompletely vaccinated children (10).

For the period 2012-2015, the estimated national vaccine administrative coverage with the first MMR vaccine dose coverage was suboptimal in the Nišava District (ranging between 44.3% for the first dose and 80.4% for the second dose during the period 2007-2016) (11-13).

The objective of the paper was to describe the epidemiological and clinical characteristics of the affected individuals in the measles outbreak in the period from 2017 to 2018 in the Nišava and Toplica Districts.

Materials and methods

Descriptive study was done. Data covering the period from 23 November 2017 to 28 July 2018 were retrospectively analyzed.

Case-Definition

For the investigation of the outbreak, the general principles of the case definition of the European Union (EU) Commission Decision of 2012 were used (14). Laboratory investigations of initial patients were performed at the Center for Control and Prevention of Diseases in the Institute of Public Health Niš. All blood samples collected from measles cases were sent for anti-measles IgM/IgG antibody tests in the reference laboratory of the Institute of Virology, Vaccines and Sera "Torlak" in Belgrade.

Data Analysis

During the measles outbreak, case-based reports provided data for the date of disease onset, gender, and date of birth, age, and date of vaccination against measles, vaccination status, laboratory confirmation, hospitalization, complications, and death. Incidence was calculated per 100,000 inhabitants with the number of measles cases as the numerator and the number of the population by Census 2011 as the denominator. The case fatality rate was calculated as the number of measles-related deaths per 100 cases. Data are presented as frequencies and percentages. Chi squared tests were used to compare characteristics between groups.

The p value was set at $p < 0.05$. Statistical analysis was performed in EPI INFO v7.2.2.6 (CDC, Atlanta, USA).

Laboratory data

The laboratory confirmation of the measles was carried out by detecting measles IgM antibodies in serum samples from the National Reference Laboratory "Torlak". The blood specimens were taken from all case-patients suspected to the measles.

The Ethics Committee of the Faculty of Medicine, University of Niš approved this investigation by Decision Number 12-3782/5 of 13 April 2018.

Results

A total of 1327 cases were reported from the beginning of the measles outbreak on 23 November in 2017 to 28 July 2018 when it ended. The total number of 1045 measles cases (78.7%) were laboratory confirmed, and 282 (21.3%) were epidemiologically connected (Table 1).

The first three measles cases were recorded on the 23 November, 2017. These were unvaccinated children from the city of Niš, who were in the same room at the Pediatric Clinic of the Clinical Center of Niš, and were infected by an imported case. Almost all patients lived in the Nišava and the Toplica Districts or the neighboring rural places and had many contacts with the urban population.

The monthly incidence increased from 15 cases registered in November to a peak of 297 cases in January. The highest number of cases (638) were in the municipalities of Niš (Figure 1).

Laboratory confirmed diagnosis of the disease was found in 78.7% of the patients, mostly in the 15-19 year old age group (85.3%), in older than 30 years old and in infants (80%). The epidemiological relationship was most present in children aged 1-6 years (29.7%) and in persons aged 20-29 years (Table 1).

The number of new cases ranged from 2 to 106 per week. The highest number of cases was

reported from 9th to 11th week of January 2018 (Graph 1). The average number of new measles cases was 40 per week. Majority of patients, 953 (71.7%) were older than 20 years. The median age of the cases was 35 years (Min < 1 year, Max 70 years). The highest number of patients, 510 (38.4%) were in the 30-39 year age group , 306 (23.1%) patients were in the 40-49 year age group, and 212 (16.0%) were children aged 1-6 years. The lowest number (34; 2.6%) were in the 15-19 year age group. Infants represented 5.3% of all affected and children from primary school accounted for 4.4% out of all affected.

In this measles outbreak, females were more affected than males. Of the total, 584 (44.0%) were males and 743 (56.0%) were females (Table 1). Majority of the affected males were from the age group of 15-19 and infants. Most affected females were from the 20-29 year age group. In the age group up to 20 years, males were more affected compared to females, but in age groups older than 20 years, females were more affected than males. We found that there was a statistically significant sex difference in relation to the age categories of the affected individuals ($p < 0.001$).

In relation to occupation, health workers, employees in health institutions, kindergartens, primary and secondary schools were significantly more affected. This can be explained by the fact that they were not vaccinated and that they were more exposed (Table 2).

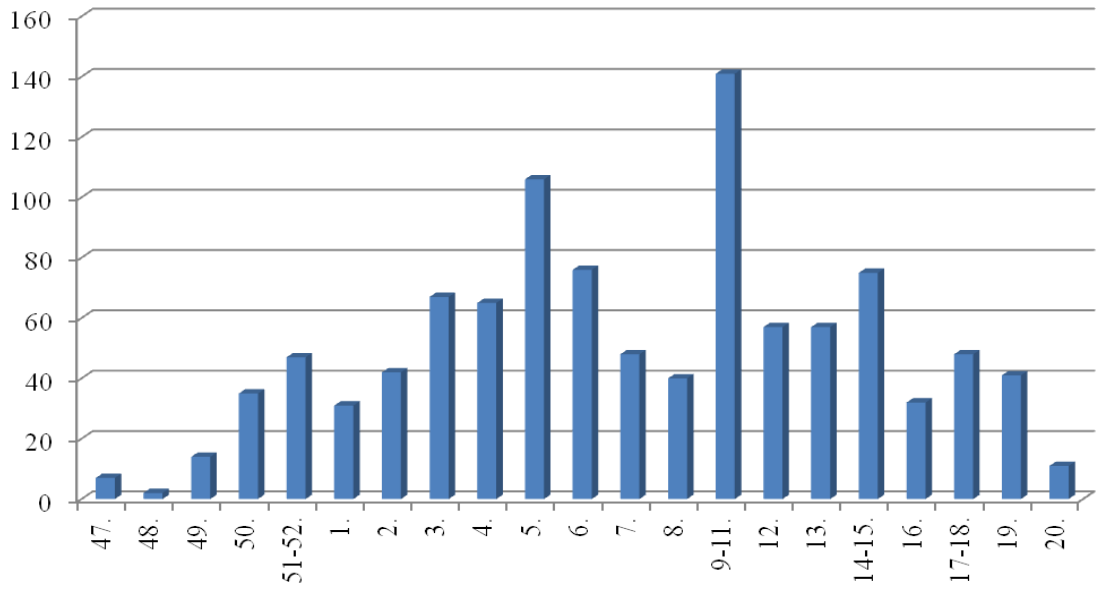
One-fourth of the outbreak cases (338; 25.5%) were not vaccinated against measles and nearly one fifth of them were children under one year of age who were not eligible for vaccination (69 cases, 20.4%). Only 37 (2.8%) patients received a full course of vaccination, two doses of the MMR vaccine and 50 (3.8%) received one dose. For the majority of affected, 902 (68.0%) cases vaccination status was unknown.

The most unvaccinated children were infants (98.6%). The high percentage of unvaccinated children was recorded in the 1-6 year age group (89.6%), and in the 7-14 year age group (62.7%). These findings indicated that children had not been vaccinated or revaccinated. Non-vaccinated patients from all age groups were significantly more numerous than those who had one or two doses of vaccine (Table 1).

The highest number of vaccinated patients with two doses (approximately one-third) was in the 15-19 age group (32.4%) among all age groups. With age, the number of people with unknown vaccination status increased. We found that there was a statistically significant difference in the vaccinal status concerning age categories ($p < 0.001$).

Complications, hospitalization and mortality

Of the total number of measles cases, complications presented in 962 (72.5%) patients. Complications were the most common in youngest patients (92.9%) and among children 1-6 years old (88.2%). With age, the number of complications was statistically significantly decreased ($p < 0.001$) (Table 3).



Graph 1. Distribution of measles cases by the week

Table 1. Demographic, vaccination status and measles-related complications related to age category

Characteristics	Total (n = 1327)		< 1 (n = 70)		1-6 (n = 212)		7-14 (n = 59)		15-19 (n = 34)		20-29 (n = 136)		30+ (n = 816)		p value
Gender															
Male	584	44.0	40	57.1	108	50.9	30	50.8	20	58.8	60	44.1	326	40.0	0.002
Female	743	56.0	30	42.9	104	49.1	29	49.2	14	41.2	76	55.6	490	60.0	
Clinical symptoms															
Rash	1319	99.5	70	100.0	209	98.6	57	96.6	34	100.0	136	100.0	813	99.8	0.065
Temperature	1253	94.5	68	97.1	206	97.2	57	96.6	34	100.0	127	93.4	761	93.4	0.124
Cough	904	68.2	63	90.0	199	93.9	49	83.1	27	79.4	79	58.1	487	59.8	< 0.001
Coryza	573	43.2	53	75.7	174	82.1	42	71.2	16	47.1	52	38.2	236	29.0	< 0.001
Conjunctivitis	574	43.3	52	74.3	163	76.9	41	69.5	17	5.0	41	30.1	260	31.9	< 0.001
Vaccination status															
Unvaccinated	338	25.5	69	98.6	190	89.6	37	62.7	7	20.6	8	5.9	27	3.3	< 0.001
1 dose	50	3.8	0	0.0	10	4.7	1	1.7	1	2.9	4	2.9	34	4.2	
2 dose	37	2.8	0	0.0	0	0.0	5	8.5	11	32.4	14	10.3	7	0.9	
Unknown	902	68.0	1	1.4	12	5.7	16	27.1	15	44.1	110	80.9	748	91.7	
Complications	962	72.5	65	92.9	187	88.2	42	71.2	25	73.5	86	63.2	557	68.3	< 0.001
Diarrhea	590	44.5	44	62.9	127	59.9	31	52.5	13	38.2	52	38.2	323	39.6	< 0.001
Malnutrition	823	62.0	46	65.7	171	80.7	38	64.4	20	58.8	71	52.2	477	58.5	< 0.001
Pneumonia	122	9.2	21	30.0	29	13.7	8	13.6	2	5.9	11	8.1	51	6.2	< 0.001
Encephalitis	1	0.1													
Koplik spots	1	0.1													
Hospitalized	212	16.0	33	47.1	52	24.9	8	13.6	6	17.6	24	17.6	89	11.0	< 0.001
Laboratory findings															
Laboratory confirmed	1045	78.7	56	80.0	149	70.3	43	72.9	29	85.3	107	78.7	661	81.0	0.023
Epidemiologically linked	282	21.3	14	20.0	63	29.7	16	27.1	5	14.7	29	21.3	155	19.0	

Table 2. Demographic, clinical characteristics and vaccination status of the affected individuals in the outbreak

Characteristics	Vaccination status								p-value[1]
	0 dose (n = 38)		1 dose (n = 50)		2 dose (n = 37)		Unknown (n = 902)		
Gender									
Male	170	50.3	17	34.0	21	56.8	376	41.7	0.008
Female	168	49.7	33	66.0	16	43.5	526	58.3	
Clinical symptoms	296	87.6	29	58.0	20	54.1	617	68.4	< 0.001
Rash	199	58.9	17	34.0	9	24.3	365	40.5	< 0.001
Temperature	260	76.9	27	54.0	14	37.8	522	57.9	< 0.001
Cough	65	19.2	0	0.0	0	0.0	57	6.3	< 0.001
Coryza									
Conjunctivitis	1	0.3							
Hospitalized	105	31.3	2	4.0	0	0.0	105	11.7	< 0.001
Laboratory findings									
Laboratory confirmed	249	73.7	39	78.0	26	70.3	731	81.0	0.025
Epidemiologically linked	89	26.3	11	22.0	11	29.7	171	19.0	
Occupation									
Employees in health institutions	7	2.1	8	16.0	6	16.2	106	11.8	< 0.001
Health care personnel	7	2.1	8	16.0	6	16.2	88	9.8	< 0.001
Employees in kindergarten	33	9.8	1	2.0	0	0.0	9	1.0	< 0.001
Employees in Primary schools	28	8.3	1	2.0	5	13.5	24	2.7	< 0.001
Employees in Secondary schools	5	1.5	2	4.0	8	21.6	7	0.8	< 0.001

[1] Chi-squared test

Table 3. Measles-related complication

Characteristics	Complications				p-value[1]
	No (n = 338)		Yes (n = 50)		
Gender					
Male	168	46.0	416	43.2	0.395
Female	197	54.0	546	56.8	
Age					
< 1	5	1.4	65	6.8	< 0.001
1-6	25	6.8	187	19.4	
7-14	17	4.7	42	4.4	
15-19	9	2.5	25	2.6	
20-29	50	13.7	86	8.9	
30+	259	71.0	557	57.9	
Clinical symptoms					
Rash	361	98.9	958	99.7	0.182
Temperature	324	88	929	96.7	< 0.001
Cough	161	44.1	743	77.3	< 0.001
Coryza	85	23.3	488	50.8	< 0.001
Conjunctivitis	70	19.2	504	52.4	< 0.001
Hospitalized	32	8.9	180	18.8	< 0.001
Laboratory findings					
Laboratory confirmed	279	76.4	766	79.6	0.233
Epidemiologically linked	86	23.6	196	20.4	
Occupation					
Employees in health institutions	37	10.1	90	9.4	0.743
Health care personnel	28	7.7	81	8.4	0.740
Employees in kindergarten	5	1.4	38	4.0	0.028
Employees in Primary schools	14	3.8	44	4.6	0.662
Employees in Secondary schools	8	2.2	14	1.5	0.485
Employees at University	6	1.6	14	1.5	1.000

[1] Chi-squared test

Malnutrition was the most frequent complication (823 cases; 62.0%) followed by diarrhea (590 cases; 44.5%), and pneumonia (122 cases; 9.2%); encephalitis was reported in 1 case (> 0.1%). Diarrhea and pneumonia were most common in the youngest patients (62.9% and 30.0%, respectively). Malnutrition was the most common among children aged 1-6 (80.7%). It was found that all complications were statistically significantly different among age categories ($p < 0.001$ for all).

Among all clinical symptoms - cough, corneas and conjunctivitis (signs of catarrhal inflammation of the upper respiratory tract, which is the site of the virus entry) were significantly more frequent in patients of all age groups, while rash as a typical clinical sign of this rash fever and elevated temperatures were less present in affected patients.

Among minor complications, diarrhea and malnutrition were significantly present, and pneumonia was registered among severe complications. Diarrhea and malnutrition were significantly more often observed in infants as well as pneumonia compared to other age groups.

Measles-related complications were significantly more common in unvaccinated patients - both less complicated (diarrhea and malnutrition) and pneumonia as a severe complication. The unvaccinated patients were significantly more frequently hospitalized.

The least complication was in patients who received two doses of vaccine (54.1%). The incidence of complications significantly varies with the vaccine status ($p < 0.001$). All complications were the lowest in people who were fully vaccinated. All individual complications were statistically significantly different in relation to sex ($p < 0.001$, for all). There was no fully vaccinated person who was hospitalized ($p < 0.001$) (Table 2).

Measles-related deaths in the observed period were confirmed in four patients (all laboratory-confirmed, three unvaccinated and an immune compromised children). The case-fatality rate of 0.3 per 100 measles cases was determined.

Control measures

In order to prevent further spread and to control the epidemic, persons with measles were asked to stay at home. The vaccination of unvaccinated children was implemented. Active contact finding of

all suspected and laboratory-confirmed cases of measles in the areas most affected by the outbreak, as well as contact-tracing in hospitals and the community were the priorities.

Discussion

The latest measles outbreak in the Nišava and Toplica Districts of November 2017 with the total of 1327 patients was one of the largest measles outbreaks in Serbia since the introduction of mandatory vaccination against measles in 1971.

In this outbreak, there were 1327 reported cases and four measles-related deaths. We found that there were more affected females than males, the majority of all affected were over 20 years of age and the median age of the patients was 35 years, ranging from < 1 to 70 years. The highest number of patients were in the 30-39 years age group, more than one third. Infants represented 5.3% of all affected and children from primary school represented 4.4% out of all affected.

In measles outbreak in Bulgaria from 2009 to 2011 (15), out of the total number of patients, 51% were males and the median age of the cases was seven years. On the contrary, in the measles outbreak in the Nišava and Toplica District, females represented 56% of all affected and the median age of the patients was 35 years. In the measles outbreak in Bulgaria, infants had the highest age-specific incidence of 5,457 per 100,000 inhabitants, followed by 2,008 in children aged one to four years (15).

In measles outbreak in Italy in 2017, 50.7% of all affected were females and the median age was 27 years and these results were similar to ours (16). In Italy, 88.3% of all cases were unvaccinated, 6.5% received only one dose and 1.6% were fully vaccinated and 3.6% received an unknown number of doses.

We found that 25.5% out of all affected in this measles outbreak were unvaccinated, only 2.8% patients received a full course of vaccination, two doses of the MMR vaccine and 50 (3.8%) received one dose. For the majority of affected, 902 (68.0%) cases, vaccination status was unknown.

In Portugal, the country with high uptake of MMR vaccine, measles outbreaks were registered in two regions from February to May 2017 after 12 years without endemic transmission (17). One hundred fifty-six measles cases were notified and the most confirmed cases occurred in adults, two cases were adolescents, seven cases were children under 10 years and 13 cases were unvaccinated healthcare workers. Among the unvaccinated cases, five were infants under 1 year and thus too young to be vaccinated, the remaining eight cases were adults and three were unvaccinated healthcare workers. Such situation was expected in highly vaccinated communities and might be explained by the fact that MMR was not 100% effective, with about 7.5% and 5.0% non-respondents to the first and second doses (17).

In the measles outbreak in the Nišava and Toplica Districts, the most unvaccinated patients were infants (98.6%) because they were too young to be vaccinated and this finding also indicated that their mothers didn't have antibodies against measles virus or were not vaccinated. Antibodies against measles are transmitted transplacentally, and they are maintained for a long time in the bloodstream of a newborn child.

The high percentage of unvaccinated children was also recorded in the 1-6 year age group, which means that the first dose of MMR vaccine was not taken. Sixty-two point seven percent of unvaccinated children from the age group 7-14 could indicate that children had not been vaccinated or revaccinated or both. We found that there were more males fully vaccinated (56.8%). The vaccine status was statistically significantly different in relation to sex ($p = 0.008$).

In our study of the outbreak in 2017, there were much more laboratory confirmed cases 78.7% and 21.3% were epidemiologically connected.

Most of patients in this measles outbreak were above 30 years of age. The epidemiological shift of disease incidence to the older age groups may potentially increase the rates of serious disease and complications (16). Some studies have attributed this effect to the continuing low vaccination coverage (18-20).

A shift in movement of incidence of measles to older age groups was already observed in the seventies (21-23), so the fact that the largest number of patients in this outbreak was at the age of 20 years and older is not surprising.

The lowest number of patients, above 2% was in the 15 to 19 year age group, the highest number of patients who were vaccinated with two doses were from this age group. Also, most took their last dose less than 10 years ago, so they had a solid immune system as well.

The majority of the patients, more than 65% had unknown vaccination status, about 25% of all reported were unvaccinated, and those who were vaccinated with a single dose or two doses together accounted for about 7% of patients.

Similar data about vaccination status against measles have been reported from recent measles outbreaks (22-25) but the proportion of patients with unknown vaccination status was lower compared with our findings.

The number of notified measles outbreaks especially in Central and Western Europe has been increasing in the last five years, with a reported peak in 2011 (15, 22).

When measles outbreaks occur in a region in which measles has been eliminated, like in the USA (7), they occur in clusters of unvaccinated persons, including those in religious communities (8) such as the Amish, a Christian sect descended from the Swiss Anabaptists, who practice group solidarity and rejection of modern conveniences (19, 20).

In 2016, there was a large measles outbreak in Romania with more than 15,500 cases and a total of 59 deaths by the end of 2018 (21). Slovenia was measles free from 2000 to 2009 and then in 2010, several measles cases were reported in a hospital setting, in 2011 six measles cases were imported from Germany, Italy and Romania, and in November 2014, at the international dog show, measles

outbreak with 44 cases was registered (22). In both outbreaks, in 2011 and in 2014, the most affected were adults from 34 to 51 years of age (22).

In the outbreak of measles in the territory of Nišava and Toplica Districts in the period from March to August 2015, the number of cases was 250 compared to 1327 cases in the outbreak in 2017-2018 (24). Majority of cases in measles outbreak in 2015 were unvaccinated and patients with unknown vaccination status (24) which was similar to the vaccination status of the affected in the 2017-2018 outbreak.

Similarly to the patients in outbreaks in the USA, where in 23 outbreaks in 2014, there were 77% of unvaccinated, 15% of unknown status, 8% vaccinated and 8% were under the age at the time of vaccination (7, 8).

Further, in Serbia and especially in the Nišava and Toplica Districts, vaccination coverage of the predisposed population with MMR vaccine was less than 95% (10-13). According to the official reports, in the territory of Nišava District the lowest coverage was recorded with the first dose of MMR vaccine, only 34.5% in 2013. Then, the lowest coverage with the second dose was in 2014 and in 2015 the minimum coverage in the Republic was recorded both in the case of vaccination and revaccination (10-13).

It is estimated that at the time of the beginning of the 2017-2018 epidemic, in the territory of Nišava and Toplica Districts there were about 7,000 unvaccinated children. The reasons for this high number of the unvaccinated were numerous: MMR vaccine shortage of 2010; the influence of antivaccine lobby and propaganda that linked the MMR vaccination with autism morbidity; the lack of awareness of parents of the importance of immunization against measles in prevention of serious complications and death.

Mandatory vaccination against measles has been conducted in the territory of Nišava and Toplica Districts since 1971, and already in 1972 there was a significant decline in the incidence of measles. Despite the high coverage of vaccination, records show an increase in the incidence of measles and even their outbreaks. The success in coverage of vaccination carried out at the end of the seventies and eighties was 95%, and yet measles outbreaks occurred. Vaccinated patients accounted for about 30% of the total number of patients (24).

In all measles outbreaks in the territory of the Nišava and Toplica Districts since 1972, there was always 1/3 of completely vaccinated people who got sick. This was explained many years later. In the 70s and 80s, it was not obligatory to store vaccines in a cold chain regime as it is now. MMR vaccine contains live attenuated viruses which are very unstable in the higher temperature.

Approximately two thirds of reported measles cases in this outbreak had one or more complications. In the literature, measles-related complications approximately appear in one third of the affected and they are the most common among children younger than 5 years of age and immunocompromised individuals (6). Measles-related complications were the most common reason for hospitalization of the affected in the measles outbreak in 2017 and the most common complications were in infants, pre-school and school children up to 14 years of age.

In Serbia, as previously stated, the first dose of MMR vaccine is required for all children aged 12 to 15 months, and the other is required in the seventh year prior to enrollment in the first grade of primary school (10). Such vaccination schedule exists in most European countries, and already during the seventies and eighties of the XX century many countries like Czechoslovakia, Hungary, France, Belgium carried out vaccination against measles in the period from 14 to 16 months of age because seroconversion was the highest (25). Study from the Netherlands showed that early MMR vaccine administration during an outbreak was safe to protect infants aged 6-14 months against measles (26).

Conclusion

This was the largest measles outbreak in Serbia and the probable causes of this large measles outbreak were insufficient vaccination and low vaccine coverage with MMR vaccine and accumulation of a high susceptible population. There were more than two thirds among affected older than 20 years of age and more females were affected than males. Four measles-related deaths were registered. Monitoring of the immunization status, high vaccine coverage and effectiveness of MMR vaccine are essential for the prevention of measles outbreaks.

References

1. Orenstein WA, Hinman A, Nkowane B, Olive JM, Reingold A. Measles and Rubella Global Strategic Plan 2012–2020 midterm review. Vaccine 2018:36:A1-A34. [CrossRef] [PubMed]
2. Lochlainn MLN, Woudenberg T, van Liera A, Zonnenberg I, Philippi M, de Melker EH et al. A novel measles outbreak control strategy in the Netherlands in 2013–2014 using a national electronic immunization register: A study of early MMR uptake and its determinants. Vaccine 2017;35:5828-834. [CrossRef] [PubMed]
3. Moss WJ, Griffin DE. Measles. Lancet 2012;379:153. [CrossRef] [PubMed]
4. Strebel PM, Papania MJ, Parker Fiebelkorn A, Halsey NA. Measles Vaccine. In: Plotkin SA, Orenstein WA, Offit P, editors. Vaccines. 6 ed. Elsevier Saunders; 2013. pp. 352-387. [CrossRef]
5. Thornton J. Measles cases in Europe tripled from 2017 to 2018. BMJ 2019;364:l-634-l-634. [CrossRef] [PubMed]
6. Rota PA, Moss WJ, Takeda M, de Swart RL, Thompson KM, Goodson JL. Measles. Nat Rev Dis Primers 2016; 2: 16049. [CrossRef] [PubMed]
7. Hall V, Banerjee E, Kenyon C, Strain A, Griffith J, Como-Sabetti K et al. Measles Outbreak — Minnesota April–May 2017. MMWR Morb Mortal Wkly Rep 2017; 66(27):713-17. [CrossRef] [PubMed]
8. Fiebelkorn AP, Redd SB, Gastañaduy PA, Clemmons N, Rota PA, Rota JS et al. A Comparison of Post-elimination Measles Epidemiology in the United States, 2009−2014 versus 2001−2008. J Pediatric Infect Dis Soc 2017;6(1):40-8. [CrossRef] [PubMed]
9. Dabbagh A, Laws RL, Steulet C, Dumolard L, Mulders NM, Katrina Kretsinger K et al. Progress toward regional measles elimination — worldwide, 2000–2017. MMWR Morb Mortal Wkly Rep 2018;67:1323-29. [CrossRef] [PubMed]
10. Rulebook on immunization and drug protection. Republic of Serbia. Official Gazette 55/2017.
11. Institute of Public Health Of the Republic Serbia. Report of Immunization in Serbia in 2015.
12. Institute of Public Health Of the Republic Serbia. Report of Immunization in Serbia in 2014.
13. Institute of Public Health Of the Republic Serbia. Report of Immunization in Serbia in 2013.
14. European Centre for Disease Prevention and Control (ECDC). EUVAC net. Case Definition. Measles. Stockholm: ECDC. Available from: http://ecdc.europa.eu/en/activities/surveillance/euvac/case_definition/Pages/measles.aspx
15. Muscat M, Marinova L, Mankertz A, N Gatcheva N, Mihneva, Santibanez S et al. Surveillance and outbreak report; The measles outbreak in Bulgaria, 2009–2011: An epidemiological assessment and lessons learnt. EuroSurveill 2016;21(9):pii=30152. [Crossref] [PubMed]
16. Filia A, Bella A, Del Manso M, Baggieri M, Magurano F, Rota MC. Ongoing outbreak with well over 4,000 measles cases in Italy from January to end August 2017 – what is making elimination so difficult? Euro-Surveill. 2017;22(37):pii=30614. [Crossref] [PubMed] Available from: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.37.30614
17. George F, Valente J , Augusto GF, Silva AJ , Pereira N , Fernandes T et al. Measles outbreak after 12 years without endemic transmission, Portugal, February to May 2017. Euro Surveill 2017;22(23):pii=30548. [CrossRef] [PubMed]
18. Knol MJ, Urbanus AT, Swart EM, Mollema L, W L Ruijs WL, van Binnendijk RS et al. Large ongoing measles outbreak in a religious community in the Netherlands since May 2013. Euro Surveill 2013;18(36): pii=20580. [CrossRef] [PubMed]
19. Gastañaduy PA, Budd J, Fisher N, Redd SB, Fletcher J, Miller J et al. A Measles Outbreak in an Under-immunized Amish Community in Ohio. N Engl J Med 2016;375:1343-54. [CrossRef] [PubMed]
20. Hukic M, Ravlija J, Karakas S, Mulaomerovic M, Dedeic Ljubovic A, Salimović-Besic I et al. An ongoing measles outbreak in the Federation of Bosnia and Herzegovina, 2014 to 2015. Euro Surveill 2015; 20(9):pii=21047. [CrossRef] [PubMed]
21. Dascalu S. Measles Epidemics in Romania: Lessons for Public Health and Future Policy. Front Public Health 2019 Apr 25;7:98. [CrossRef] [PubMed]
22. Grgič-Vitek M, Frelih T, Ucakar V, Fafangel M, Markocic OJ, Prosenc K. et al. An outbreak of measles associated with an international dog show in Slovenia, November 2014. Euro Surveill 2015; 20(3): pii=21012. [CrossRef] [PubMed]
23. Pegorie M, Shankar K, Welfare WS, Wilson RW, Khiroya C, Munslow G et al. Measles outbreak in Greater Manchester, England, October 2012 to September 2013: epidemiology and control. Euro Surveill 2014;19(49):pii=20982. [CrossRef] [PubMed]
24. Veličković Z, Ilić M, Rančić N. Epidemija malih boginja na teritoriji Nišavskog i Topličkog okruga. Acta med Median 2016:55(2):71-5. [CrossRef]
25. Navarro E, Mochón MM, Galicia MD, Marín I, Laguna J. Study of a measles outbreak in Granada with preventive measures applied by the courts, Spain, 2010 to 2011. Euro Surveill 2013;18(43):pii=20612. [CrossRef]
26. van der Maas NA, Woudenberg T, Hahné SJ, de Melker HE. Tolerability of Early Measles-Mumps-Rubella Vaccination in Infants Aged 6–14 Months During a Measles Outbreak in The Netherlands in 2013–2014. J Infect Dis 2016;213:1466-71. [CrossRef] [PubMed]

Originalni rad **UDC: 616.915-036.22(497.11)"2017/2018"**
doi:10.5633/amm.2021.0204

EPIDEMIJA MALIH BOGINJA U NIŠAVSKOM I TOPLIČKOM OKRUGU OD 2017. DO 2018. GODINE

Miodrag Stojanović[1,2], Nataša Rančić[1,2], Nikola Milenković[3], Miodrag Perović[2], Ivana Janićijević[2], Marina Kostić[2], Mirko Ilić[2], Marko Stojanović[2], Marko Jovanović[2], Aleksandra Ignjatović[1,2], Marija Apostolović Anđelković[1,2]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Institut za javno zdravlje Niš, Niš, Srbija
[3]Univerzitetski klinički centar Niš, Niš, Srbija

Kontakt: Nataša Rančić
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: natasa.rancic@medfak.ni.ac.rs

Male boginje su pre uvođenja obavezne vakcinacije bile glavni uzrok umiranja dece mlađe od 5 godina.

Cilj rada bio je da prikaže epidemiološke i kliničke karakteristike obolelih u epidemiji malih boginja, koja je počela 23. novembra 2017. godine i trajala sve do 28. jula 2018. godine, na teritoriji Nišavskog i Topličkog okruga.

Primenjena je deskriptivna metoda, kojom su retrospektivno analizirani podaci od novembra 2017. godine do jula 2018. godine. Primenjena je definicija slučaja, koju je dala Komisija Evropske unije (EU) 2012. godine. U Institutu za javno zdravlje Niš, uzimana je krv za određivanje antitela i svi uzorci su slati u referentnu ustanovu Zavod za vakcine i serume "Torlak" u Beogradu, gde su testirani na prisustvo IGM i IgG antitela. Statistička analiza izvršena je u programskom paketu EPI INFO v7.2.2.6.

Od 23. novembra 2017. godine do 28. jula 2018. godine, ukupno je obolelo 1327 osoba (584 muškarca i 743 žene), a prosečan uzrast obolelih bio je 35 godina. Najviše obolelih bilo je iz dobne grupe od 30 od 39 godina, 510 osoba (38,4%), a najmanje iz dobne grupe od 15 do 19 godina, 34 osobe (2,6%). Deca mlađa od jedne godine bila su zastupljena sa 5,3%, a deca iz osnovnih škola sa 4,4%, u odnosu na ukupan broj obolelih. Više od jedne četvrtine obolelih nije bilo vakcinisano, 338 osoba (25,5%); samo su 37 (2,8%) bolesnika primili dve doze kombinovane vakcine protiv malih boginja, zauški i rubele (MMR), a 50 osoba (3,8%) vakcinisano je samo jednom dozom. Za 902 (68,0%) bolesnika vakcinalni status nije bio poznat. Komplikacije su zabeležene kod 962 osobe (72,5%) i najčešće su bile kod dece mlađe od jedne godine (92,9%) i kod dece predškolskog uzrasta (88,2%). Najčešće komplikacije bile su: malnutricija (kod 823 osobe, 62,0%), dijareja (kod 590 osoba , 44,5%), pneumonija (kod 122 osobe, 9,2%), a encefalitis je zabeležen samo kod jednog deteta. Smrt zbog komplikacija potvrđena je kod 4 bolesnika; svi su imali laboratorijsku potvdu oboljenja, troje nije bilo vakcinisano, a jedno dete bilo je imunokompromitovano. Letalitet je iznosio 0,3 na 100 obolelih.

Mogući uzrok ove velike epidemije bili su nedovoljna imunizacija i mali obuhvat MMR vakcinom osetljive populacije. Zabeležena su 4 smrta ishoda kod obolelih. Nadzor nad imunizacijom, visok vakcinalni obuhvat osetljive populacije, kao i efikasnost MMR vakcine, osnovni su preduslovi za prevenciju malih boginja.

Acta Medica Medianae 2021;60(2):35-43.

Ključne reči: *male boginje, epidemija, vakcinalni status, vakcinalni obuhvat, komplikacije*

Original article

UDC: 616.314.16-007.23-07
doi:10.5633/amm.2021.0205

ULTRASTRUCTURAL ANALYSIS OF EXTERNAL APICAL ROOT RESORPTION

Aleksandar Mitić[1], Vladimir Mitić[2], Jelena Popović[1], Stefan Dačić[1], Radomir Barac[1], Kosta Todorović[3]

Primary teeth mineralized tissue resorption is considered physiological, while this process in persons with permanent dentition is obligatory pathological. External root resorption is the consequence of multinuclear clastic cell activity which starts at the root surface and spreads further into cementum all the way to the dentin. In normal conditions there is a dynamic balance between osteoblastic and osteoclastic cell activity that maintain a physiological state of root and bone. Due to the disbalance of cells activity, under the influence of different factors, root resorption occurs. The aim of the present study was to perform ultrastructural analysis of pathologically resorbed apical root cementum and dentin formed after traumatic occlusion. The study was conducted on 18 extracted teeth from male patients aging from 54 to 73 years with internal and external pathological root resorption. The resorbed root surface (dentine structure) was analyzed using scanning electron microscope. In all studied samples occlusal surface enamel cracks, as a consequence of traumatic occlusion, were found. Ultrastructural analysis of the dentine surface in the peripheral parts of the root revealed the smooth surface of the resorbed apical root surface, described as "eggshell", with clear demarcation line separating preserved from the resorbed dentine. Also, wavy multi-layered resorption with irregular structure could be seen. Based on the scanning electron microscopic analysis of the apical root dentin one can conclude that the main cause of the external pathological resorption of the apical root, occurring due to traumatic occlusion, is aseptic inflammation.

Acta Medica Medianae 2021;60(2):44-50.

Key words: *traumatic occlusion, external root resorption, SEM analysis*

[1]University of Niš, Faculty of Medicine, Department of Restorative Dentistry and Endodontics, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Department of Pediatric and Preventive Dentistry and Orthodontics, Dental Clinic, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Department of Maxilofacial and Oral Surgery, Niš, Serbia

Contact: Aleksandar Mitić
81 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: alek.mitic@yahoo.co.uk

Introduction

Tooth resorption represents a process, seen in different physiological and pathological conditions, which can lead to irreversible dentine, cementum and alveolar bone loss in both vital and nonvital teeth (1, 2). Primary teeth mineralized tissue resorption is considered physiological, while this process in persons with permanent dentition is obligatory pathological (3). This process depends on the interaction between different inflammatory, clastic and mineralized tissue cells. Besides a great number of cells (monocytes, macrophages, osteoclasts, etc.) that control this process, systemic and local factors (cytokines, prostaglandins, bacteria, etc.) are involved in this process as well (4).

Pathological resorption can be internal and external. Internal resorption is a pathological process that leads to mineralized tooth tissue loss due to tooth destruction from within, where this process starts from pulp and spreads towards the surface (5).

The etiology of external resorption has two stimulatory components (i) mechanical/chemical and (ii) infection/pressure that affect osteoclasts. External root resorption is the consequence of multinuclear clastic cell, localized in the periodontal ligament and bone, activity, which starts at the root surface and spreads further into cementum all the way to the dentin (6). The mineralized tissue of the permanent denture is hard to resorb since it is well protected by predentin and odontoblasts found in

root canals and cementoblasts found on the root surface. When predentin or precementum is mineralized and the mechanical damage of precementum occurs, osteoclastic cells migrate to the mineralized surface in order to resorb it (1, 7).

Based on the clinical and histological manifestations, there are numerous root resorption classifications, however the authors present the following external resorption classification as it is the most frequent one:

(i) superficial root resorption;
(ii) inflammatory resorption, called cervical/apical;
(iii) ankylosis;
(iv) replacement resorption and
(v) transient apical disorders (8).

According to Andersons classification from 1988, there are three types of external resorption: superficial, external ankylosis-resorption and external inflammatory resorption. Recently, Hulsman et al. also described three types of external resorption: progressive inflammatory, cervical (extra-canalicular invasive resorption) and replacement resorption (9).

External root resorption can be found in both vital and nonvital teeth and is most frequently revealed on routine radiographic image as an asymptomatic condition (1). Pathological resorptions can be diagnosed using radiographic images only when they are large, with lesion diameters of 2 mm and at least 1 mm separated from the superficial cortex. Numerous external resorptions are painless and pass unnoticed by patients until the pulpous or periodontal tissue gets inflamed (1). Deep cavity occurring during resorption can lead to heat tenderness of the tooth due to pulp vicinity.

Osteoclasts are multinuclear cells, originating from multipotent hematopoietic stem cells, involved in bone resorption. Their origin is much closer to immune then to connective tissue cells (2). Using specific receptors osteoclast adhere to root bone and cementum, thus forming an isolated area in which they secrete proteolytic enzymes, that degrade protein matrix, and acids that "meltdown" the mineral composition of the bone and other structures. Under physiological conditions, when there are no traumatic and/or pathological tooth changes, there is a dynamic balance between osteoblastic and osteoclastic activity that maintains the root bone homeostasis. Different factors can disturb this balance leading to root resorption (10).

Osteoclast polarity is regulated by their actin cytoskeleton and in contact with the mineralized extracellular matrix, this cytoskeleton forms a zone without cellular organelles within the cell (clear zone), allowing the cell to form a direct contact with cementum via cell membrane. The clear zone is surrounded by a numerous finger-like cell membrane invaginations (podosomes) that are known as a ruffled border. Bellow this border the resorption process is occurring, where the resorbed surface within the clear zone, isolated from the extracellular surface, is forming acidic microenvironment for mineralized tissue resorption (8).

Multinuclear clastic cells responsible for the bone and root resorption do not possess the receptors for direct binding of parathormone (PTH), thus the clast cell stimulation by PTH is indirect. Both PTH and PTH related protein (PTHrP) bind to osteoblasts and increase the receptor activator of nuclear factor-kB ligand (RANKL) expression, which further binds to a RANK receptor of osteoclast precursors, activating these cells to fuse and form osteoclasts (2, 8).

The multinuclear clastic cells of the periodontal ligament also express RANKL that binds the same way as described previously. The intracellular signal pathway includes TRAF6 that activates protein complex NF-B, protein kinase c-Src and JNK-cell cycle regulators. If the OPG would block RANKL, clastic cells would undergo apoptosis rather than their activation. The relation RANKL-RANK is mandatory for clastic cell survival (8).

Osteoprotegerin (OPG) is a secreted glycoprotein belonging to TNF receptor family and has different biological functions including tissue remodulation. OPG is a powerful competitive inhibitor of osteoclastic bone resorption (11).

Recently, it was discovered that besides proinflammatory cytokines IL-1 and IL-2, TNF-α significantly contributes to the development of osteoclasts and multinuclear cells with dentine resorption potential (1). According to Komine et al., human TNF-α significantly stimulates mononuclear preosteoclast cells (POC) in the presence of conditioned osteoblast cell medium and contributes to hematopoietic cell differentiation to POC. The TNF-α induce POC to form multinuclear cells, which further express dentin resorbing potential. Extremely low levels of TNF-α in POC increase calcitonin receptor cathepsin K mRNA. Both RANKL and TNF-α effects on osteoclast development are inhibited by OPG.

Transformation of macrophage-like clastic cells leads to the formation of multinuclear clastic cells that are almost identical to osteoclasts. Several mediators produced by various cells can be involved in the development of multinuclear cells from their precursors. These multinuclear resorbing cell activators/stimulators include PTH, PTHrP, IL-1, IL-6, IL-11, PDGF, 1α,25-dihydroxy vitamin D3, glucocorticoids, and substance P, while on the other hand calcitonin, estrogen, interferon, IL-4, IL-8, IL-10, IL-18, and corticosteroids are known to inhibit osteoclast/odontoclast cells (8).

Cytokines (IL-1, IL-6, and TNF-α as proinflammatory ones) bind to pattern recognition receptors (PRR) and via secondary messengers, e.g. tyrosine-kinase, activate cells to start their clastic activity.

The aim of the present study was to perform ultrastructural analysis of pathologically resorbed apical root cementum and dentin formed after traumatic occlusion by using scanning electron microscope.

Materials and methods

The study was conducted on 18 extracted teeth from male patients, aged 54 to 73 years, with internal and external pathological root resorption

(Figure 1). The diagnosis was made according to the radiographic images (type and process localization), where in all studied patients with external root resorption the abrasion of the occlusal surface due traumatic occlusion was found.

Figure 1. Radiographic image of a tooth with external root resorption

The resorbed root surface (dentine structure) was analyzed using JEOL-JSM-5300 scanning electron microscope (SEM) by a single researcher.

Sample preparation included teeth preservation in sterile saline at 4 ^{O}C, without any fixative. Occlusal surfaces (2-3 mm thick) of the tooth crowns were circularly cut by the thinnest diamond borer. The roots were cut transversely using shafts in order to separate the apical part of the root. In order to eliminate superficial debris, generated by cutting, the samples were rinsed with distilled water and dried using a compressed air. First, the occlusal sur-face was separated using separating forceps, followed by transversal separation of the roots (apex third) down the cut gutters. Each sample was mounted on a special holder and covered with gold in a vacuum evaporator before they were analyzed under SEM.

Results

Results obtained using SEM are shown in Figures 2-7. In all investigated samples superficial occlusal enamel cracks, as a consequence of traumatic occlusions, were found (Figure 2). Ultrastructural analysis of the dentine root surface appeared as smooth surface of the resorbed apex, described as "eggshell" (Figure 3). At the root peripheral parts, a clear demarcation line separating the preserved from the resorbed dentin could be seen (Figure 4). The irregularity of resorbed dentine surface can be seen in Figures 6 and 7. At the level of the middle and apical third (proximal side) of the root, cellular cementum was observed (Figure 5).

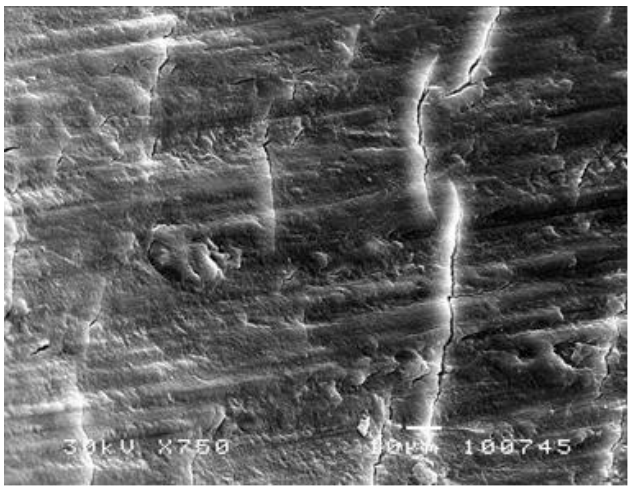


Figure 2. Occlusal enamel surface with cracks due to traumatic occlusion

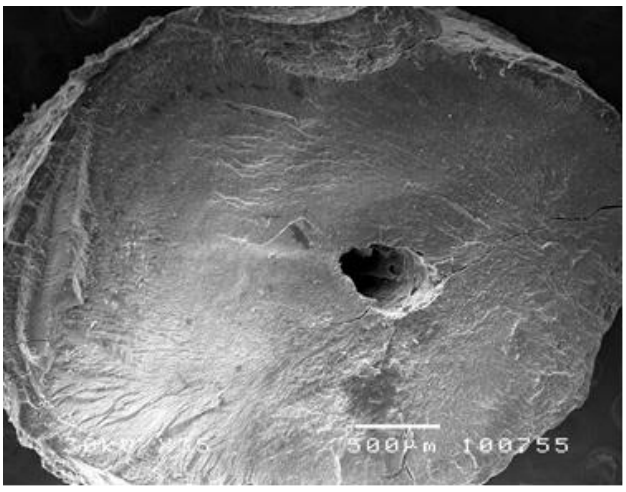


Figure 3. External resorption of root apex with broad, irregular and funnel-like apical foramen

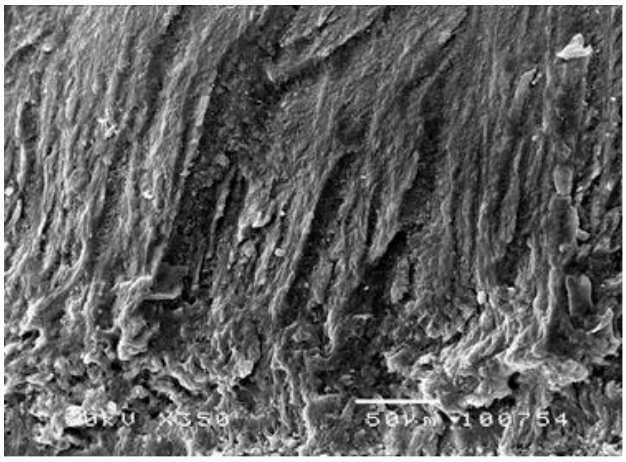


Figure 4. Demarcation border between resorbed and preserved peripheral dentine

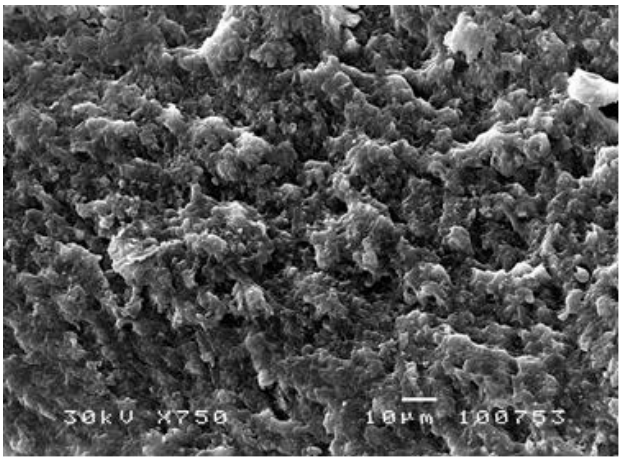


Figure 5. Cellular cementum at the approximal side of the root at the level of the apical and mid third

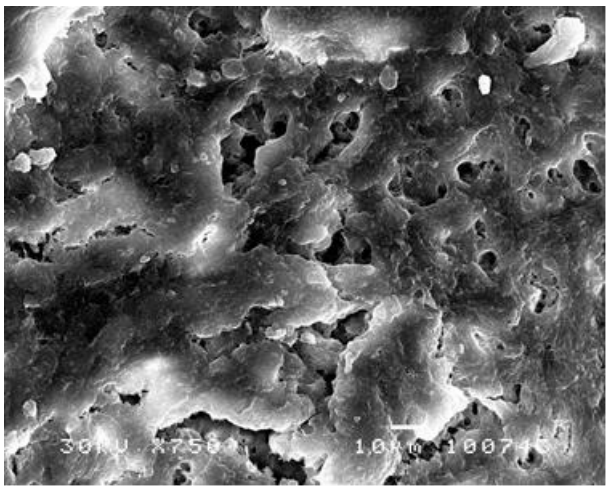


Figure 6. Resorbed irregularly structured dentine surface near apical foramen

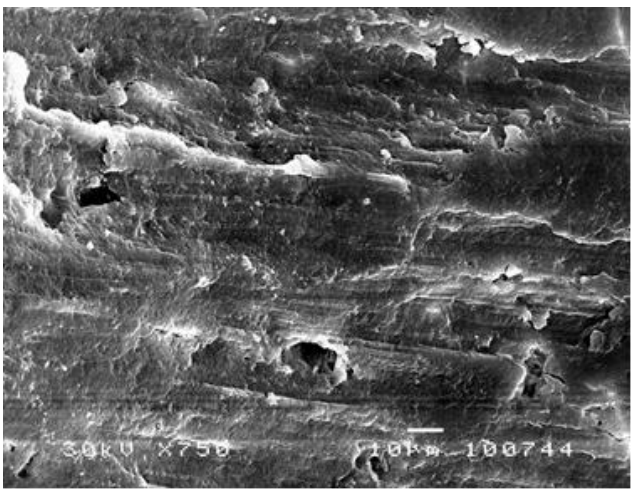


Figure 7. Irregular structure of resorbed dentine surface at the peripheral parts of root apex

Discussion

Osteoclasts and other cells (e.g. macrophages, monocytes and osteocytes) have the ability of mineralized tissue resorption. They from erosions, Howship's lacunae, by liberating demineralizing agents and enzymes, allowing phagocytes to ingest the degraded products (13). In some of the lacunae examined on the teeth in the present study dentine tubules could be seen, which points to the fact that the process is affecting the dentin as well. Substances that are liberated from the surrounding tissue cells, such as osteoclast activating factor, macrophage chemotactic factor and prostaglandins are intensifying the process of mineralized tissue resorption (5).

Different causes of root resorption are attributed to pressure, inflammation, neoplastic processes and some systemic conditions (1). Clastic cells in different physiological and pathological conditions have different potential for root resorption and the variations in the type of resorption can be expected. Active external resorption can be found in patients two months after orthodontic treatment. The areas of resorption could be seen on the entire surface and lead to the loss of normal smooth surface appearance.

In the early phases of the orthodontic treatment, this causes premolar buccal leaning of the crown and with its apex leaning lingually producing zones of tension and compression on opposite sides of the crown and root. In the present study, the

resorption process observed under SEM was not focused on that part. There are numerous cases of root resorption, except in advanced phase, seen as a consequence of inflammation due to orthodontists' therapy (13). In the present study, all premolar resorption was limited to the small cementum surface, probably due to the short time period of tooth exposure to mechanical force. The size and location of the resorption were not uniform in the studied sample, thus one cannot conclude that the local factors are playing a key role in root resorption caused by orthodontic treatment. It is very important to know that even in these phases of orthodontic treatment, the cementum resorption is occurring, although these processes are not always visible on radiographic images. Inflammatory resorption of the apical parts of the root can be seen in teeth with apical periodontitis and is probably more frequent than reported (15-17). Ferlini found, in his microscopic analysis, that in teeth with chronic apical periodontitis resorption is occurring in the foramen region (18). In most of the cases root resorption can be detected on radiographic images, however, in everyday clinical practice, these images are not sufficiently precise for establishing the diagnosis of resorption defects as a consequence of apical periodontitis.

In the present study, the formed lacunae are spreading on the teeth surface, rather than affecting deeper parts (layers). In some lacunae, dentine tubules could be seen, which implicates that the process is affecting dentin as well. Only in the cases of severe resorption, changes in the foramen contours, which appeared irregular, were seen (Figure 2). In accordance with the results of the present study, Rosa Neto et al., found apical root surface to be irregular, eroded, with cementum-dentine resorption (19).

External root resorption can be seen as root contour irregularity located in the neck of the tooth (cervical resorption) at any level of external root surface, or at the apex followed by surrounding bone resorption (19). Root apex is susceptible to resorption due to anato-morphological variations in cementum-dentine connection structure, as well as due to a number and Sharpey's fibers attachments. The mentioned structures with some others as well, form a barrier that prevents the clastic cell activity (21). In the present study, the apical region appeared funnel-shaped, which is in accordance with previous research (22). According to the present findings, the resorption areas are localized at the apex with clear demarcation line which separates healthy from the affected area, pointing to the fact that pressure which creates lesions can be a significant factor in this process (Figure 3).

Different epidemiological research related to root resorption is allowing us to see a bigger picture and enable us to perform adequate and "timely" diagnosis, and treatment of the tooth with external root resorption (23).

Resorption areas are unreachable for the chemical-mechanical treatment, thus they remain the resident places for different microorganism (24, 25). This information should be beared in mind when treating root canals since the presence of external root resorption can be the cause of therapeutic failure.

For the treatment of external resorption 2% solution of chlorhexidine and calcium hydroxide powder are used, due to their relatively low cytotoxic potential and stable antibacterial properties. The usage of sodium hypochlorite is not recommendable since in the case of root resorption it can pass through apical foramen and cause periapical irritation (23). Chlorhexidine increases dentin pH, inhibits acidic hydrolase, arriving from osteoclasts, activity in periodontal tissue and at the same time inhibits alkaline phosphates. It also acts as antiseptic with a prolonged activity which increases antibacterial activity of calcium hydroxide (23).

External root resorption is a process that involves the activity of multinuclear clastic cells originating from periodontal ligaments. The process is spreading from the surface, in a form of infiltration, into a cementum and dentin at different root levels (2).

The most commonly affected teeth are upper molars and incisors, which is explained by the fact that these teeth are under greater occlusal pressure, especially when the occlusion is not adequate. Additionally, in these teeth, crown abrasion as a consequence of occlusal pressure could be seen (8). The present study revealed that the teeth affected by external apical root resorption were secondary mandibular premolars, which is not in agreement with the previous findings. However, such conclusion related to the frequency of the affected teeth, in this case, is not possible due to small sample size.

The literature data related to RANKL-RANK-OPG system response to traumatic occlusion, which could be observed as a mechanical stress that would trigger inflammatory mechanism within the pulp, are scarce. It is known that aseptic inflammatory response is the organisms' response to any mechanical stress including the traumatic occlusion (13). Having in mind that there are no data related to this topic, one can assume, but not claim, that the external root resorption is the consequence of stress caused by traumatic occlusion.

The disturbance in pulp circulation arriving from a traumatic occlusion initiates immunological mechanisms and causes clast cell differentiation, where the entire immunological mechanism is under the control of proinflammatory cytokines (4). Proinflammatory cytokines are binding to pattern recognition receptors (PRR), which include TRAF6 that activates protein complex NF-B, protein kinase c-Src and JNK-cell cycle regulators. If the OPG would block RANKL clastic cells would undergo apoptosis rather than their activation. The relation RANKL-RANK is mandatory for clastic cell survival (8).

Conclusion

In our population, external root resorption is not a very common clinical phenomenon and the data relating to its prevalence, accurate and fast diagnosis are a predisposition for successful endodontic treatment. Based on the scanning electron microscopic analysis of the apical root dentin, one can conclude that the main cause of the external

pathological resorption of the apical root, occurring due to traumatic occlusion, has immunopathogenic background. Aseptic pulp inflammation that is a consequence of mechanical stress, an initial factor in pro-inflammatory cytokine production (IL-1, IL-6, and TNF-α), leads to tissue resorption at various parts of the root.

References

1. Bartok RI, Văideanu T, Dimitriu B, Vârlan CM, Suciu I, Podoleanu D. External radicular resorption: Selected cases and review of the literature. J Med Life 2012;5: 145-8. [PubMed]
2. Felippe WT, Ruschel MF, Santos Felippe G, Pozzobon MH, Santos Felippe MC. SEM evaluation of the apical external root surface of teeth with chronic periapical lesion. Aust Endod J 2009;35:153-7. [CrossRef] [PubMed]
3. Sreeja R, Minal C, Madhuri T, Swati P, Vijay W. A scanning electron microscopic study of the patterns of external root resorption under different conditions. J Appl Oral Sci 2009;17:481-6. [CrossRef] [PubMed]
4. de Silveira PF, Boessio Vizzotto M, Montagner F, Dias da Silveira HL, Dias da Silveira HE. Development of a new in vitro methodology to simulate internal root resorption. J Endod 2016;42:978-83. [CrossRef] [PubMed]
5. Bouysson M, FH. Lepp, C. Zerossi, editors. Resorptions dentaires et biologie osseuse, Sciences et Lettres: Liege; 1965.
6. Andreasen JO. External root resorption: its implication in dental traumatology, paedodontics, periodontics, orthodontics and endodotics. Int Endod J 1985;18: 109-18. [CrossRef] [PubMed]
7. Saoud TMA, Mistry S, Kahler B, Sigurdsson A, Lin LM. Regenerative endodontic procedures for traumatized teeth after horizontal root fracture, avulsion, and perforating root resorption. J Endod 2016;42:1476-82. [CrossRef] [PubMed]
8. Ne RF, Witherspoon D, Gutmann JL. Root resorption. Quintessence Int 1999;30:9-25. [PubMed]
9. Hulsmann M, Schafer E. Resorption. In: Hulsmann M, Schafer E. editors. Problems in endodontics, eiology, diagnosis and treatment, London: Quintessence; 2009, p. 421-34.
10. Yusof WZ, Ghazali MN. Multiple external root resorption, JADA 1989;118:453-55. [CrossRef] [PubMed]
11. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309-19. [CrossRef] [PubMed]
12. Komine M, Kukita A, Kukita T, Ogata Y, Hotokebuchi T, Kohashia O. Tumor necrosis factor-α cooperates with receptor activator of nuclear factor κB ligand in generation of osteoclasts in stromal cell-depleted rat bone marrow cell culture. Bone 2001;28:474-83. [CrossRef] [PubMed]
13. Gunraj MN. Dental root resorption. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:647-53. [CrossRef] [PubMed]
14. Trope M, Chivian N. Traumatic injuries. In: Choen S, Burns RC. editors. Pathways of the pulp 7th ed., Maryland Heights: Mosby Inc; 1998. p. 572-82.
15. Tronstad L, Barnett F, Riso K, Slots J. Extraradicular endodontic infections. Endod Dent Traumatol 1987;3: 86-90. [CrossRef] [PubMed]
16. Hammarström L, Lindskog S. General morphological aspects of resorption of teeth and alveolar bone. Int Endod J 1985;18:93-9. [CrossRef] [PubMed]
17. Ehnevid H, Jansson L, Lindskog S, Weintraub A, Blomlof L. Endodontic pathogens: propagation of infection through patent dentinal tubules in traumatized monkey teeth. Endod Dent Traumatol 1995;11:229-34. [CrossRef] [PubMed]
18. Ferlini J. Estudo radiográfico e microscópico das reabsorções radiculares na presença de periodontites apicais crônicas (microscopia ótica e eletrônica de varredura). PhD thesis, Universidade de São Paulo, Bauru, 1999.
19. Rosa Neto JJ, Esberard RM, Consolaro A. Estudo em microscopia eletrônica de varredura do ápice radicular e do limite apical de obturação em dentes portadores de lesão periapical crônica. Rev Dent Press Biol Oral 2002;1:18-28.
20. Bergmans L, Van Cleynenbreugel J, Verbeken E, Wevers M, Van Meerbeek B, Lambrechts P. Cervical external root resorption in vital teeth. X-ray microfocustomographical and histopathological case study. J Clin Periodontol 2002;29:580-5. [CrossRef] [PubMed]
21. Opačić V, Živković S. Učestalost eksternih resorpcija na korenu zuba, Srpski arhiv 2004;132:152-6. [CrossRef] [PubMed]
22. Malueg LA, Wilcox LR, Johnson W. Examination of external apical root resorption with scanning electron microscopy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82:89-93. [CrossRef] [PubMed]
23. Opačić-Galić V, Živković S. Endodotska terapija patoloških resorpcija korena zuba. Stom Glas S 2004;51: 130-5. [CrossRef]
24. Lomçali G, Sen BH, Çankaya H. Scanning electron microscopic observations of apical root surfaces of teeth with apical periodontitis. Dent Traumatol 1996; 12:70-6. [CrossRef] [PubMed]
25. Leonardo MR, Rossi MA, Silva LAB, Ito Y, Bonifácio KC. SEM evaluation of bacterial biofilm and microorganisms on the apical external root surface of human teeth. J Endod 2002;28:815-8. [CrossRef] [PubMed]

Originalni rad **UDC: 616.314.16-007.23-07**
doi:10.5633/amm.2021.0205

ULTRASTRUKTURNA ANALIZA EKSTERNE RESORPCIJE APEKSA KORENA ZUBA

Aleksandar Mitić[1], Vladimir Mitić[2], Jelena Popović[1], Stefan Dačić[1], Radomir Barac[1], Kosta Todorović[3]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za bolesti zuba i endodonciju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Katedra za preventivnu i dečiju stomatologiju i ortopediju vilica, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Katedra za maksilofacijalnu i oralnu hirurgiju, Niš, Srbija

Kontakt: Aleksandar Mitić
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: alek.mitic@yahoo.co.uk

Resorpcija mineralizovanog tkiva mlečnih zuba smatra se fiziološkim procesom, dok je ovaj proces kod osoba sa stalnom denticijom uvek patološki. Eksterna resorpcija korena posledica je multinuklearne aktivnosti klastnih ćelija, koja započinje na površini korena i širi se dalje u cement sve do dentina. U normalnim uslovima postoji dinamička ravnoteža između aktivnosti ćelija osteoblasta i osteoklasta, koje održavaju fiziološko stanje korena i kosti. Zbog dis-balansa aktivnosti ćelija, pod uticajem različitih faktora, dolazi do resorpcije korena. Cilj ovog rada je ultrastrukturna analiza patološki resorbovanog cementa i dentina na apeksu korena zuba, koji su posledica traumatske okluzije. Studija je sprovedena na 18 izvađenih zuba muškaraca starih od 54 do 73 godine, sa internom i eksternom patološkom resorpcijom korena zuba. Resorbovana površina korena zuba (dentinska struktura) analizirana je na skening elektronskom mikroskopu. Kod svih posmatranih uzoraka uočene su naprsline na okluzalnoj površini gleđi, kao posledica traumatske okluzije. Ultrastrukturnom analizom dentinske površine u perifernim delovima korena, uočena je glatka površina resorbovanog apeksa, slična "ljusci jajeta". Na periferiji korena zuba uočljiva je jasna demarkaciona linija, koja odvaja očuvani od resorbovanog dentina. Uočava se i slojevita, talasasta resorpcija iregularne strukture. Na osnovu analize skening elektronske mikroskopije dentina na apeksu korena zuba, može se zaključiti da značajnu ulogu u etiologiji patološke eksterne resorpcije apeksa korena zuba, pod dejstvom traumatske okluzije, ima aseptična inflamacija.

Acta Medica Medianae 2021;60(2):44-50.

Ključne reči: *traumaska okluzija, eksterna resorpcija korena, SEM analiza*

Original article

UDC: 616-097:616.322-002.2
doi:10.5633/amm.2021.0206

EXPRESSION OF CD68 ANTIGEN IN CHRONICALLY DISEASED HUMAN PALATINE TONSIL

Braca Kundalić[1], Vesna Stojanović[1], Miljana Pavlović[1], Vladimir Živković[1], Milena Trandafilović[1], Jovana Čukuranović Kokoris[1], Milorad Antić[1], Ivana Graovac[1]

Based on the pathohistological examination of tonsillar tissue, chronic tonsillitis can be classified as chronic hypertrophic tonsillitis (CHT) and recurrent tonsillitis (RT). CD68 is a glycoprotein ubiquitously expressed on the cells of the monocyte-macrophage lineage, as well as on the dendritic cells. Macrophages and dendritic cells are major initiators, effectors, and regulators of immune response in the palatine tonsil. The aim of this paper was to examine microanatomical distribution of CD68-immunopositive cells and to determine their numerical areal density in morphological compartments of palatine tonsils with CHT and RT, in order to show the possible differences in antigen-presentation potential between these two pathological conditions. As a material we used tonsils taken after tonsillectomy, from patients of both sexes, aged 10-29 years: six tonsils with RT and nine tonsils with CHT. The quantification of CD68-immunopositive cells by "ImageJ" software was performed on 5 μm thick serial paraffin tissue slices, which were stained immunohistochemically, by using monoclonal anti-CD68 antibody. The results of morphometrical analysis showed presence of CD68-immunopositive cells in all morphological compartments of tonsils with RT and CHT, being higher in number in RT compared with CHT. Statistically significant difference in numerical areal density of the CD68-immunopositive cells was found in the germinal centers of lymphoid follicles (RT > CHT), and interfollicular lymphoid tissue (CHT > RT). The difference in the number of CD68-immunopositive cells might imply the different mechanisms involved in the infiltration of tonsillar tissue with CD68-immunopositive cells, as well as the different antigen-presenting potential in these two conditions.

Acta Medica Medianae 2021;60(2):51-56.

Key words: *CD68 antigen, morphometry, chronic tonsillitis, macrophage, dendritic cell*

[1]University of Niš, Faculty of Medicine, Department of Anatomy, Niš, Serbia

Contact: Braca Kundalić
81 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: braca.kundalic@medfak.ni.ac.rs

Introduction

Palatine tonsil is the organ of the immune system that significantly contributes to the local and general immunity, due to its specific anatomic location and histological structure. As a part of Waldeyer's tonsillar ring, it is responsible to initiate both the cellular and humoral immune response against the antigens entering the organism trough the oral cavity (1, 2). The parenchyma of palatine tonsils contains both T- and B-lymphocytes, as well as antigen presenting cells, which are specifically distributed into four morphological compartments: crypt epithelium, subepithelial lymphatic tissue, lymphoid follicles (germinal center and mantle zone) and interfollicular lymphoid tissue (3-5). Lymphoid follicles are structurally and functionally divided into mantle zone and germinal center. The mantle zone contains mostly small B memory lymphocytes, while in the germinal center there are large dividing B lymphoblasts (centroblasts) and their differentiated non-dividing forms called centrocytes, specific subset of T helper cells, germinal center dendritic cells and follicular dendritic cells (4, 6-8).

Chronic inflammations are the most common pathological conditions of the palatine tonsil. However, the exact pathogenetic mechanisms leading to the chronic inflammation of the palatine tonsils are not yet elucidated (3, 9). Based on the pathohistological examination of tonsillar tissue, chronic tonsillitis can be classified as chronic hypertrophic tonsillitis (CHT) and recurrent tonsillitis (RT). CHT is characterized by enlarged palatine tonsils and hypertrophy and hyperplasia of lymphoid follicles, while in RT palatine tonsils contain lymphoid follicles with active germinal centers, fibrosis in interfollicular lymphoid tissue and thin crypt epithelium (10).

CD68 is glycoprotein expressed on the cells of the monocyte-macrophage lineage, as well as on dendritic cells. Unlike macrophages that are part of the innate immune system, dendritic cells are components of the acquired immunity and have the ability of its modulation (11). The main function of macrophages in germinal centers is the phagocytosis of cellular remains after the division of B lymphoblasts and plasma cells, and of those localized in subepithelial tissue is the antigen presentation to T-lymphocytes (2, 12). The sole role of dendritic cells is the antigen presentation to T-lymphocytes (11).

After penetrating the crypt epithelium the antigens reach the subepithelial tissue and interfollicular lymphoid tissue where they are being caught and processed by macrophages and dendritic cells, and subsequently presented via MHC II molecules to CD4+ T-lymphocytes (4, 13). T helper lymphocytes stimulate the divisions of germinal center B cells which give rise to two populations: antibody-expressing B memory cells and antibody-producing plasma cells (14). Dendritic cells also have the ability to activate naïve T cells and to initiate, coordinate, and regulate adaptive immune responses (15, 16).

The aim of the paper was to examine microanatomical distribution of CD68-immunopositive cells and to determine their numerical areal density in morphological compartments of palatine tonsils with CHT and RT, in order to show the possible differences in the antigen-presentation potential between these two pathological conditions.

Materials and methods

The research was performed at the Department of Anatomy and Department of Histology of the Faculty of Medicine, University of Niš, and at the Clinic for Ear, Throat and Nose of the University Clinical Center of Niš, Serbia.

The material was obtained following the ethical guidelines and consisted of palatine tonsils taken after the tonsillectomies of patients of both genders: 5 tonsils with RT (patients aged 10-29 years) and 5 tonsils with CHT (patients aged 18-22 years).

The tonsils were fixated in 10% buffered formaldehyde and were routinely processed to paraffin blocks. The paraffin blocks were cut on Leica microtome in order to obtain 5μm thick tissue sections that were subsequently stained with hematoxylin-eosin and immunohistochemically by using antibody against CD68 antigen (GeneTex, GTX41865, 1:100). As a visualization system for immunohistochemistry was used EnVisionFLEX, High pH (Agilent).

The numerical areal density (N_A) is the parameter showing the average number of cells in mm^2 of the tissue. We were determining the numerical areal density of CD68-immunopositive cells in different morphological compartments of palatine tonsils with CHT nad RT: crypt epithelium and subepithelial lymphoid tissue, germinal centers of lymphoid follicles, mantle zones of lymphoid follicles and interfollicular lymphoid tissue. The values are obtained by using formula $N_A = (N/A)*1000000$ (N – number of cells on the examined visual field, A – area of the examined visual field in μm^2).

The images of the tonsillar tissue were obtained by using Olympus BX50 (Olympus, Japan) microscope equipped with Leica DFC 295 camera (Leica Microsystems, Germany). All images were taken under the magnification of the objective x40. For the numbering of cells and determining the area of the examined visual field we used Image J software. Fifty visual fields per the morphological compartment were examined in each group (CHT or RT), after the calibration of the images.

The obtained values for N_A were compared between the examined groups by using Mann-Whitney test.

Results

CD68 immunopositive cells were found to be present in all examined morphological compartments of palatine tonsils with CHT and RT (Figures 1, 2).

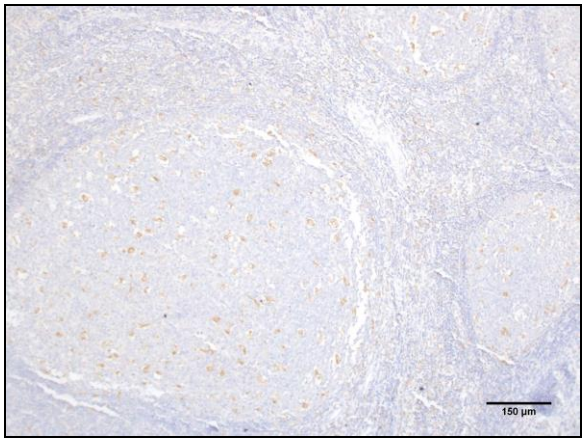


Figure 1. Distribution of CD68-immunopositive cells in the palatine tonsil with chronic hypertrophic tonsillitis x100

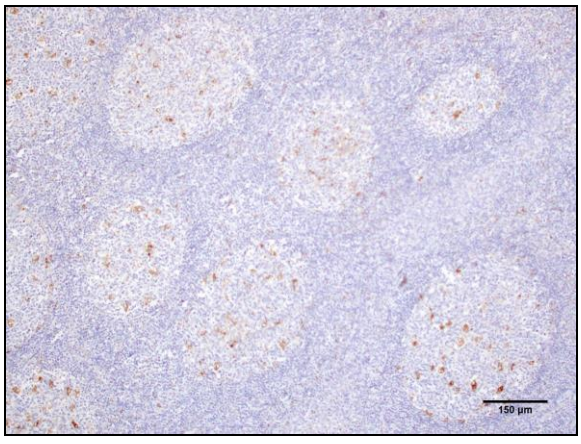


Figure 2. Distribution of CD68-immunopositive cells in the palatine tonsil with recurrent tonsillitis x100

The results of CD68-immunopositive cells numerical areal density in morphological compartments in palatine tonsils with CHT and RT are presented in Table 1.

Numerical areal density of CD68-immunopositive cells shows statistical significance between the germinal centers and interfollicular lymphoid tissue of palatine tonsils with RT and CHT.

Table 1. Average values of numerical areal density (N_A) of CD68-immunopositive cells in morphological compartments of palatine tonsils with CHT and RT

Morphological compartment of palatine tonsil	CHT n = 5	RT n = 5	p
Crypt epithelium and subepithelial connective tissue	185.17 ± 47.1	227.54 ± 48.32	0.3
Germinal center	276.16 ± 85.18	477.58 ± 27.9	0.018
Mantle zone	179.72 ± 47.68	210.17 ± 45.72	0.106
Interfollicular lymphoid tissue	262.40 ± 36.84	197.67 ± 23.49	0.001

RT – recurrent tonsillitis,
CHT – chronic hypertrophic tonsillitis,
n – number of palatine tonsils per examined group

Discussion

Our results show that the CD68-immunopositive cells are distributed in all morphological compartments of the palatine tonsil, which is in accordance with the findings of the other authors (12, 17-19). Statistically significant difference in numerical areal density of these cells was found in germinal centers of lymphoid follicles and interfollicular lymphoid tissue, which differs from the reports of Gorfien et al. that found the difference in the number of these cells only in interfollicular areas in tonsils with RT and CHT (17). Stent et al. reported that CD68 antigen was expressed in all dendritic cell populations and macrophages in cell cultures obtained from human palatine tonsils after tonsillectomy. Interestingly, their findings suggest that S-100 antigen, usually associated with macrophages and dendritic cells, was not expressed in subpopulation of dendritic cells that were CD11c negative (20). Yamamoto et al. reported the presence of S-100-immunopositive cells in all morphological compartments of palatine tonsils with RT, tonsillar hyperplasia and tonsils with focal infection (19). They examined the numerical areal density of these cells in crypt epithelium and interfollicular lymphoid tissue, and found the statistically significant difference in the number of these cells in crypt epithelium of tonsils with tonsillar hyperplasia and tonsils with focal infection. Although these results cannot be directly compared with the results of our study, it is noteworthy to mention that they reported 611 ± 231 S-100-immunopositive cells by mm^2 in crypt epithelium in RT, while our findings suggest the number of 227.54 ± 48.32 CD68-immunopositive cells in mm^2 in both crypt epithelium and subepithelial connective tissue in tonsils with RT.

The previous studies reported that the number of macrophages increases in the diseased palatine tonsils, compared to the healthy ones (17). However, regardless the higher numbers, the increase in number of macrophages in superficial and crypt epithelium of the chronically diseased palatine tonsils is not followed by the increased expression of RFD7 antigen that is a characteristic of mature phagocyte cells, which might imply that these cells are still functionally immature and inactive. Also, the number of dendritic cells decreases in these compartments, as well as the expression of RFD1 antigen which is connected with functional activation and antigen-presenting potential of dendritic cells (17). The functional immaturity of macrophages and dendritic cells, possibly caused by chronic inflammation, might represent one of the reasons of local immunosuppression that occurs in the chronically inflamed tonsillar tissue (17, 21). T- and B- lymphocytes in palatine tonsils with CHT, although increased in number due to the bacterial load and hypertrophy of tonsillar tissue, are not adequately immunocompetent and the experiments performed in vitro showed that these lymphocytes are relatively unresponsive to the stimulation by antigens (12).

Chen et al. examined tonsils with the RT and the TH (tonsillar hyperplasia) by combining beta-galactosidase staining, connected with the cellular senescence, and immunohistochemical staining with CD68. Their results showed the increased number of senescent CD68-immunopositive cells in germinal centers and mantle zones in both examined groups (22). Macrophages and dendritic cells are the major initiators, effectors and regulators of immune system, and the senescence of these cells is characterized by increased inflammatory cytokine production and impairment of chemotaxis and phagocytosis (22, 23). The impairment of these three functions might be responsible for the hyperplasia of lymphoid follicles in TH and CHT, as well as for increased

overload of pathogens in palatine tonsils especially found in RT.

The crypt epithelium and subepithelial connective tissue represent the main site of entry and contact of different antigens with M cells, macrophages and dendritic cells of the palatine tonsil (18). The previous studies showed the decreased ability of M cells to uptake the antigens, as well as the functional immaturity of macrophages and dendritic cells in this morphological compartment in hronic tonsillitis (12, 17, 21, 22, 24). Although the reasons for this local immuno-suppression are not yet completely elucidated, there is an increased number of evidences that some bacteria *(Pseudomonas aeruginosa, Streptococcus pneumoniae*), viruses (Epstein-Barr), bacterial bio-films and recurrent infections cause the cellular senescence probably via cellular oxidative stress, and lead to the morphological changes of the crypt epithelium (hyperkeratosis, cryptitis) (17, 22, 25-27). Lymphocytes and antigen presenting cells re-present the axis of the immune response in palatine tonsils and every change in their functional activity impairs the immunological potential of this organ. Bearing in mind the importance of epithelial com-partment for the function of the palatine tonsil, the future studies should focus on the possibly surface- and crypt epithelium-related mechanisms involved in the impairment of lymphocyte functions and in-adequate activation of macrophages and antigen presenting cells.

Conclusion

The results of our study showed that CD68-immunopositive cells were present in all morphological compartments of the tonsils with RT and CHT. Numerical areal density of the CD68-immunopositive cells was significantly higher in the germinal centers of lymphoid follicles with the RT compared to the CHT, and in the interfollicular lymphoid tissue in the CHT compared to the RT. The difference in the number of CD68-immunopositive cells might imply the different mechanisms involved in the infiltration of tonsillar tissue with CD68-immunopositive cells, as well as the different anti-gen-presenting potential in these two conditions.

Acknowledgments

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (under the projects no. 41018 and no. 43012), and by the Faculty of Medicine, University of Niš, Serbia (under the project no. 38/20).

References

1. Thorbecke GJ, Silberberg I, Flotte TJ. Langerhans cells as macrophages in skin and lymphoid organs. J Invest Dermatol 1980;75:3243. [CrossRef] [PubMed]
2. Reichel O, Mayr D, Winterhoff J, De La Chaux R, Hagedorn H, Berghaus A. Tonsillotomy or tonsillectomy? – a prospective study comparing histological and immunological findings in recurrent tonsillitis and tonsillar hyperplasia. Eur Arch Otorhinolaryngol 2007; 264:277-84. [CrossRef] [PubMed]
3. Scadding KG. Immunology of the tonsil: a review. J Roy Soc Med 1990;83:104-7. [CrossRef] [PubMed]
4. Nave H, Gebert A, Pabst R. Morphology and immunology of the human palatine tonsil. Anat Embryol 2001;204:367-73. [CrossRef] [PubMed]
5. Noussious G, Xanthopoulos J, Zaraboukas T, Vital V, Konstantinidis I. Morphological study of development and functional activity of palatine tonsils in embryonic age. Acta Otorhinolaryngol Ital 2003;23:98-101. [PubMed]
6. Feuillard J, Taylor D, Casamayor-Palleja M, Johnson GD, MacLennan IC. Isolation and characteristics of tonsil centroblasts with reference to Ig class switching. Int Immunol 1995;7(1):121-30. [CrossRef] [PubMed]
7. Aguzzi A, Kranich J, Krautler NJ. Follicular dendritic cells: origin, phenotype, and function in health and disease. Trends Immunol 2014;35(3):105-13. [CrossRef][PubMed]
8. Gars E, Butzmann A, Ohgami R, Balakrishna JP, O'Malley DP. Life and death within germinal centres: a double-edged sword. Ann Diagn Pathol 2020;44: 151421. [CrossRef] [PubMed]
9. Zhang PC, Pang YT, Loh KS, Wang DY. Comparison of histology between reccurent tonsillitis and tonsillar hypertrophy. Clin Otolaryngol 2003;28:235-9. [CrossRef] [PubMed]
10. Surjan JR, Brandtzaeg P, Berdal P. Immunoglobulin systems in human tonsils II. Patients with chronic tonsillitis or tonsilar hyperplasia: quantification of Ig-producing cells, tonsilar morphometry and serum Ig concentrations. Clin Exp Immunol 1978;31:382-90.
11. Ferenbach D, Hughes J. Macrophages and dendritic cells: what is the difference? Kidney Int 2008;74(1):5-7. [CrossRef] [PubMed]
12. Mogoantă CA, Ioniță E, Pirici D, Mitroi M, Anghelina F, Ciolofan S, et al. Chronic tonsillitis: histological and immunohistochemical aspects. Rom J Morphol Embryol 2008;49(3):381-6. [PubMed]
13. Brandtzaeg P, Halstensen TS. Immunology and immunopathology of tonsils. In: Galioto GB, editor. Advances in otorhinolaryngology, a clinical oriented update. 47th ed. Basel: Karger;1992. p. 64-75. [CrossRef] [PubMed]
14. Quiding JM, Granstrom G, Nordstrom I, Holmgren J, Czerkinsky C. Induction of compartmentalized B-cell responses in human tonsils. Infect Immun 1995;63: 853-7. [CrossRef] [PubMed]
15. Patente TA, Pinho MP, Oliveira AA, Evangelista GCM, Bergami-Santos PC, Barbuto JAM. Human dendritic cells: Their heterogeneity and clinical application potential in cancer immunotherapy. Front Immunol 2018;9:3176. [CrossRef] [PubMed]
16. Vangeti S, Gertow J, Yu M, Liu S, Baharom F, Scholz S, et al. Human Blood and Tonsil Plasmacytoid Dendritic Cells Display Similar Gene Expression Profiles but Exhibit Differential Type I IFN Responses to Influenza A Virus Infection. J Immunol 2019;202(7): 2069-81. [CrossRef] [PubMed]
17. Gorfien JL, Noble B, Brodsky L. Comparison of the microanatomical distributions of macrophages and dendritic cells in normal and diseased tonsils. Ann Otol Rhinol Laryngol 2001;110(2):173-82. [CrossRef] [PubMed]
18. Ruco LP, Uccini S, Stoppacciaro A, Pilozzi E, Morrone S, Gallo A, et al. The lymphoepithelial organization of the tonsil: an immunohistochemical study in chronic recurrent tonsillitis. J Pathol 1995;176(4):391-8. [CrossRef] [PubMed]
19. Yamamoto Y, Okato S, Takahashi H, Takeda K, Magari S. Distribution and morphology of macrophages in palatine tonsils. Acta Otolaryngol Suppl 1988;454:83-95. [CrossRef] [PubMed]
20. Stent G, Reece JC, Baylis DC, Ivinson K, Paukovics G, Thomson M, et al. Heterogeneity of freshly isolated human tonsil dendritic cells demonstrated by intracellular markers, phagocytosis, and membrane dye transfer. Cytometry 2002;48(3):167-76. [CrossRef] [PubMed]
21. Hart DNJ, Starling GC, Calder VL, Fernando NS. B7/BB-l is a leucocyte differentiation antigen of human dendritic cells induced by activation. Immunology 1993;79:616-20. [PubMed]
22. Chen S, Wang WW, Wang Y, Li YQ, Zhu LX. Cellular senescence in recurrent tonsillitis and tonsillar hypertrophy in children. Int J Pediatr Otorhinolaryngol 2020;133:110004. [CrossRef] [PubMed]
23. van Beek AA, Van den Bossche J, Mastroberardino PG, de Winther MPJ, Leenen PJM. Metabolic Alterations in Aging Macrophages: Ingredients for Inflammaging? Trends Immunol 2019;40(2):113-27. [CrossRef] [PubMed]
24. Surjan L. Reduced lymphocyte activation in repeatedly inflamed human tonsils. Acta Otolaryngol (Stockh) 1980;89:187-94. [CrossRef] [PubMed]
25. Li H, Luo YF, Wang YS, Yang Q, Xiao YL, Cai HR, et al. Using ROS as a Second Messenger, NADPH Oxidase 2 Mediates Macrophage Senescence via Interaction with NF-κB during *Pseudomonas aeruginosa* Infection. Oxid Med Cell Longev 2018;2018:9741838. [CrossRef] [PubMed]
26. Kwon IS, Kim J, Rhee DK, Kim BO, Pyo S. Pneumolysin induces cellular senescence by increasing ROS production and activation of MAPK/NF-kappaB signal pathway in glial cells. Toxicon 2017;129:100-12. [CrossRef] [PubMed]
27. Abu Bakar M, McKimm J, Haque SZ, Majumder MAA, Haque M. Chronic tonsillitis and biofilms: a brief overview of treatment modalities. J Inflamm Res 2018;11: 329-37. [CrossRef] [PubMed]

Originalni rad **UDC: 616-097:616.322-002.2**
doi:10.5633/amm.2021.0206

EKSPRESIJA ANTIGENA CD68 U HRONIČNO OBOLELOM HUMANOM NEPČANOM KRAJNIKU

Braca Kundalić[1], Vesna Stojanović[1], Miljana Pavlović[1], Vladimir Živković[1], Milena Trandafilović[1], Jovana Čukuranović Kokoris[1], Milorad Antić[1], Ivana Graovac[1]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za anatomiju, Niš, Srbija

Kontakt: Braca Kundalić
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: braca.kundalic@medfak.ni.ac.rs

Hronični tonzilitis se prema patološkom nalazu tkiva krajnika može podeliti na hronični hipertrofični (HHT) i rekurentni tonzilitis (RT). CD68 predstavlja ubikvitarni glikoprotein, koji se eksprimira na ćelijama monocitno-makrofagne linije i dendritičnim ćelijama. Makrofagi i dendritične ćelije glavni su inicijatori, efektori i regulatori imunog odgovora u nepčanom krajniku. Cilj ovog istraživanja bio je da se istraži mikroanatomska distribucija ćelija pozitivnih na CD68, kao i da se odredi njihova numerička arealna gustina u morfološkim odeljcima nepčanih krajnika sa HHT i RT, da bi se odredile moguće razlike u antigenoj prezentaciji između ova dva stanja. Za materijal su korišćeni krajnici dobijeni nakon tonzilektomije, od bolesnika oba pola, starosti između 10 i 29 godina. Šest krajnika bilo je sa RT, a devet sa HHT. Kvantifikacija ćelija pozitivnih na CD68 rađena je putem programa "ImageJ", na parafinskim isečcima debljine 5 μm, koji su imunohistohemijski bojeni monoklonalnim anti-telom na CD68. Rezultati morfometrijske analize pokazali su prisustvo ćelija pozitivnih na CD68 u svim morfološkim odeljcima krajnika sa HHT i RT, pri čemu ih je više bilo u potonjoj grupi. Statistički značajno veća razlika u numeričkoj arealnoj gustini ćelija imunopozitivnih na CD68 nađeno je u germinativnim centrima limfnih folikula (RT > CHT) i interfolikularnom limfnom tkivu (CHT > RT). Ova razlika u broju imunopozitivnih ćelija na CD68 može ukazati na postojanje različitih mehanizama, koji utiču na infiltraciju tkiva krajnika ovim ćelijama, kao i na različit potencijal za prezentaciju antigena u ova dva stanja.

Acta Medica Medianae 2021;60(2):51-56.

Ključne reči: *antigen CD68, morfometrija, hronični tonzilitis, makrofag, dendritična ćelija*

Original article

UDC: 618.19-006.6(497.16)
doi:10.5633/amm.2021.0207

THE QUALITY OF LIFE OF BREAST CANCER FEMALE PATIENTS IN MONTENEGRO

Mitar Saveljić[1,2], Damir Peličić[2,3], Batrić Vukčević[2,4], Tahir Kalač[4], Gora Miljanović[5]

Breast cancer is a common malignant disease in the Balkan region. The aim was to determine whether there is a difference in the parameters of perception of health and quality of life in Montenegrin female patients after surgery, chemotherapy and radiation therapy for breast cancer in relation to age, the type of treatment, and the attitudes towards preventive diagnostic measures.

The research was carried out on a sample of 200 women diagnosed with breast cancer in health institutions in Montenegro. Authentically designed questionnaire relying on three questionnaires (Functional Assessment of Cancer Therapy: General (FACT-G), its breast cancer-specific type (FACT-B), and the instrument designed by the European Organisation for Research and Treatment of Cancer (EORTC-QLQ C30)) was used.

The lowest quality of life was seen in patients aged 60–64 years. The patients frequently reported that they felt sad, lost hope or worried about family members suffering from breast cancer. The quality of life was lower in patients who stated that they did not understand their disease well. The history of breast cancer surgery was not significantly related to the quality of life, similarly to the history of chemotherapy (or the time since last chemotherapeutical session). However, the analysis of the time since last radiation therapy course yielded statistical significance; in a sense that the quality of life was lowest in the group of patients who underwent radiation therapy.

These results should inspire the clinicians to educate the patients and to provide psychological support during the treatment.

Acta Medica Medianae 2021;60(2):57-63.

Key words: *breast, cancer, quality of life*

[1]Emergency Department, Clinical Center of Montenegro, Podgorica, Montenegro
[2]University of Montenegro, Faculty of Medicine, Podgorica, Montenegro
[3]Center of Science, Clinical Center of Montenegro, Podgorica, Montenegro
[4]Clinical Center of Montenegro, Podgorica, Montenegro
[5]Academy of Professional Studies Belgrade, Department of Health School, Belgrade, Serbia

Contact: Damir Peličić
Ljubljanska 1, 20000 Podgorica, Montenegro
E-mail: damir.pelicic@t-com.me

Introduction

Breast cancer is the most common type of cancer in females worldwide, accounting for 25% of all malignancies worldwide and causing 20% of all deaths in the female population (1). It is one of the most common causes of cancer death in Europe, second only to lung cancer (2). About 45,000 new cases of female breast cancer and 18,000 deaths occur annually in South-Eastern European countries (3). Montenegro has roughly 600,000 inhabitants (with nearly half of the population female), and a successful mammography screening program active since 2016.

The development of screening and therapeutical strategies increased the likelihood of breast cancer survival (2, 4), thus inspiring research on the quality of life of breast cancer sufferers and survivors. The quality of life stems from the state of physical and mental health, the degree of personal freedom, social and economic factors, personal beliefs and relationships with the environment (5). The main indicators of health in cancer patients are autonomy and functional capacity, regarded not only as physical health, but also as the emotional and psychological state of the patient, the environmental and social circumstances (6). The burden of breast cancer-a life-threatening chronic diagnosis with long-term therapy-presents as a transitional experience

which may result in anxiety or depression, subsequently affecting the quality of life (7).

The aim of this study was to determine the quality of life of breast cancer female patients in Montenegro. Through an investigation of specific issues related to the physical, emotional and functional state of the patients, the self-perceived quality of life scores were analyzed. Possible differences in the quality of life were examined in relation to the age of the patients, the type of treatment, the attitudes towards the preventive diagnostic measures, as well as the understanding of their own illness. Given the fact that there are no studies on this subject in the Balkan region, the results might inspire the clinicians in the developing countries to make an effort in providing the psychological support during the process of breast cancer treatment.

Materials and methods

A cross-sectional study was performed on a sample of 200 breast cancer female patients treated during the period between June and September of 2015.

The patients included in the study suffered from histologically diagnosed malignant breast disease treated with surgery, chemotherapy or radiotherapy. Informed consent was signed by all the patients included in the study. The exclusion criteria were: the presence of other malignant and/or significant systemic disease, psychiatric disorders, alcohol, and substance abuse.

A questionnaire was designed specifically for this research, inspired by three instruments: the Functional Assessment of Cancer Therapy: General (FACT-G, version 4) and the version of FACT specific for breast cancer (FACT-B, version 4), as well as the instrument designed by the European Organisation for Research and Treatment of Cancer (EORTC): EORTC QLQ-C30. The possible answers to the questions related to the quality of life were ranged from 1 ("constantly") to 5 ("never"). With 37 questions, the total score ranges from 37 (all the questions responded "constantly") to 185 (all the questions responded "never"). Certain questions are reverse-scored so that a higher total score indicates a better quality of life.

The standard protocol of descriptive statistics was used, including the Student's t-test and ANOVA. A ***p*** value below 0.05 was considered statistically significant.

Results

The mean total score of self-perceived quality of life in the entire sample was 98.95 ± 11.21 (with a minimum of 64 and a maximum of 132). The majority of women fell into the category of 55-59 years of age (22.1%), followed by women of 45-49 years of age (20.8%), and finally, women aged 50-54 (19.5%). The age structure was significantly different among various age groups ($p = 0.012$), with the lowest quality of life in women aged 60-64 (Table 1).

The history of breast cancer surgery was not significantly related to the quality of life ($p = 0.349$), similar to the history of chemotherapy (or the time since last chemotherapeutical session) ($p = 0.811$). However, the analysis of the time since last radiation therapy course yielded statistical significance; in a sense that the quality of life was lowest in the group of patients who underwent radiation therapy 3-5 years ago ($p = 0.036$) (Table 1).

The quality of life of women with various attitudes towards preventive diagnostic measures (breast ultrasound, mammography, gynecological exams) was not significantly different. However, the participants who stated that they do not understand their illness well showed a significantly lower quality of life (Table 2).

Table 3 shows the scores of specific questions regarding the quality of life. It is evident that the majority of women did not complain of physical symptoms such as pain, malaise, nausea, etc. On the other hand, a significant percentage of women complained of psychological issues such as feeling sad, losing hope, not being satisfied with the way they were dealing with cancer, and worrying about a family member possibly suffering from breast cancer. The results indicated that the majority of patients were satisfied with the support they received from their family and friends, as well as their overall activities (besides the questions regarding sexual attractiveness and the satisfaction with sex life-where a significant number of women showed dissatisfaction) (Table 3).

Table 1. The age structure and the quality of life total scores of the patients included in the study relative to the age groups and the received therapy

	Age groups						
	40–44	45–49	50–54	55–59	60–64	≥ 65	Total
Number of patients (%)	21 (10.4)	42 (20.8)	39 (19.5)	44 (22.1)	29 (14.3)	26 (13)	200 (100)
Mean ± standard deviation (minimum – maximum) of the total scores	106.75 ± 11.27 (87-123)	101.81 ± 8.62 (84-121)	95.53 ± 7.87 (84-107)	98.24 ± 9.08 (74-110)	90.73 ± 14.52 (64–115)	103.5 ± 12.86 (87-132)	64-132 (98.95 ± 11.21)
Surgical treatment performed (tumorectomy or mastectomy)							
	Number of patients (%)	Minimum – maximum (mean ± standard deviation)					**p**
Yes	169 (84.4)	64 – 115 (99.72 ± 9.917)					0.349
No	31 (15.6)	84 – 132 (95.75 ± 15.618)					
Time since last chemotherapy session							
< 6 months	44 (22.1)	78 – 114 (99.75 ± 9.333)					0.811
6-12 months	31 (15.6)	85 – 111 (97.73 ± 8.253)					
1-3 years	49 (24.7)	74 – 115 (99.47 ± 11.292)					
3-5 years	21 (10.4)	64 – 121 (96.17 ± 15.467)					
> 5 years	44 (22.1)	84 – 123 (100.79 ± 9.034)					
No chemotherapy	10 (5.2)	78 – 106 (96 ± 9.957)					
Time since last radiotherapy session							
< 6 months	31 (15.6)	85 – 132 (100.35 ± 12.175)					0.036
6-12 months	31 (15.6)	92 – 101 (96.25 ± 3. 775)					
1-3 years	55 (27.3)	74 – 115 (98.25 ± 13.572)					
3-5 years	21 (10.4)	64 – 105 (92.42 ± 11.65)					
> 5 years	42 (20.8)	84 – 123 (101.48 ± 8.394)					
No radiotherapy	21 (10.4)	78 – 114 (95.63 ± 11.057)					

Table 2. The quality of life total scores relative to the attitudes towards preventive diagnostic procedures and the understanding of own illness

Do you agree that regular breast ultrasound exams are important for female health?			
	Number of patients (%)	Minimum – maximum (mean ± standard deviation)	**p**
Strongly agree	171 (85.7)	64 – 132 (99.23 ± 8.182)	0.772
Agree	26 (13)	87 – 107 (97.8 ± 11.731)	
Disagree	3 (1.3)	92	
Do you agree that regular mammography exams are important for female health?			
Strongly agree	169 (84.4)	64 – 132 (98.65 ± 11.356)	0.156
Agree	18 (9.1)	89 – 107 (100.43 ± 7.656)	
Disagree	3 (1.3)	123	
I do not know	10 (5.2)	87 – 106 (95.25 ± 9.743)	
Do you agree that regular gynaecological exams are important for female health?			
Strongly agree	166 (83.1)	64 – 132 (99.28 ± 11.876)	0.772
Agree	23 (11.7)	86 – 106 (98.22 ± 6.2)	
I do not know	10 (5.2)	64 – 132 (99.49 ± 11.138)	
Do you agree that regular check-up exams contribute to successful treatment?			
Strongly agree	153 (76.6)	84 – 132 (96.71 ± 11.751)	0.58
Agree	44 (22.1)	105 – 115 (106.15 ± 11.138)	
I do not know	3 (1.3)	64 – 132 (98.95 ± 11.206)	
How do you estimate your understanding of your own illness?			
Well	156 (77.9)	95 – 132 (110.5 ± 13)	0.012
Unsatisfactory	44 (22.1)	64 – 123 (97.25 ± 10.656)	

Table 3. Self-perceived quality of life regarding specific issues addressed through the administered questionnaire

Specific issues	Percentage of given answers				
	Never	Sometimes	Often	Very often	Constantly
Experiencing lack of energy	24.7	54.5	15.6	2.6	2.6
Nausea	55.8	35.1	5.2	1.3	2.6
Overall pain	45.5	44.2	5.2	1.3	3.9
Pain in specific regions of the body	23.4	62.3	2.6	6.5	5.2
Feeling malaise	49.4	44.2	2.6	1.3	1.3
Shortness of breath	61	28.6	7.8	1.3	1.3
Experiencing treatment side effects	45.5	45.5	5.2	2.6	1.3
One or both arms being soft or swollen	53.2	27.3	6.5	2.6	10.4
Being forced to stay in bed	64.9	27.3	3.9	2.6	1.3
Being unable to perform household chores	48.1	44.2	1.3	3.9	1.3
Feeling sad	20.5	22.7	6.8	22.7	27.3
Losing hope in the battle with own illness	7.8	20.8	15.6	26	29.9
Anxiety	71.8	19.5	2.6	3.9	2.6
Fear of death	28.6	55.8	13	1.3	1.3
Fear of condition worsening	59.7	22.1	15.6	1.3	1.3
Annoyed by hair loss	49.3	29.9	4.5	9	7.5
Worried about family members suffering from the same illness	23.4	35.1	10.4	14.3	16.9
Worried about stress affecting the current illness	18.2	37.7	18.2	16.9	9.1
Annoyed by weight change	37.7	26	14.3	10.4	11.7
Satisfactory dealing with own illness	28.9	59.2	9.2		2.6
Being able to feel feminine	6.5	22.1	18.2	13	40.3
Being conscious of the current dressing style	15.6	19.5	13	6.5	45.5
Feeling sexually attractive	30.4	36.2	14.5	11.6	7.2
Feeling satisfied with sex life	92.4	3.8			3.8
Enjoying things usually done for fun	1.3	14.3	16.9	19.5	48.1
Satisfactory current quality of life	5.2	9.1	16.9	27.3	41.6
Feeling close to the friends	2.6	5.2	18.2	22.1	51.9
Feeling emotional support from the family	1.3	5.2	14.3	18.2	61
Feeling emotional support from the friends	1.3	6.5	19.5	18.2	54.5
Family accepting the disease	3.9	2.6	10.4	14.3	68.8
Satisfied with the way the family communicates regarding my disease	1.3	13	9.1	18.2	58.4
Feeling close with the partner/strongest supporter	2.6	10.4	9.1	16.9	61
Being able to work individually	41.6	40.3	13	1.3	3.9
Feeling content with household work	2.6	9.1	18.2	23.4	46.8
Being able to enjoy life	2.6	24.7	11.7	29.9	31.2
Accepting the disease	1.3	10.4	11.7	27.3	49.4
Good quality sleep	1.3	20.8	15.6	29.9	32.5

Discussion

The study presented herein presented several interesting findings regarding the self-perceived quality of life of breast cancer patients in Montenegro:

1. The lowest self-perceived quality of life was seen in women aged 60-64.

2. The majority of patients claimed that they often felt sad, lost hope in their battle against the illness, or worried about a family member suffering from breast cancer.

3. Lower quality of life was seen in women who reported that they did not understand their disease well.

The screening program in Montenegro resulted in a relatively large number of patients diagnosed in the treatable phase of the disease, meaning that a significant number of patients underwent long-term therapy. The results indicate that the major complaints of breast cancer patients in Montenegro are related to the psychological aspect of the disease. A similar conclusion was reached in a study by Carelle et al., which showed that breast cancer patients regarded the nonphysical effects of chemotherapy as more important than the physical symptoms. The most frequent complaints made by the patients included in their study were related to the effect of chemotherapeutical treatment on the family or partners of the patients, as well as hair loss and fatigue. Additionally, their study showed that the patient complaints regarding chemotherapy shifted from nausea, emesis, and apprehension of treatment difficulties to the worries about fatigue and functional aspects of life (8). Costa et al. analyzed 400 breast cancer survivors using EORTC QLQ-C30 (as well as its Breast Cancer-Specific version) and the Karnofsky Performance Scale, showing a positive correlation between the functional capacity and the quality of life. Furthermore, their results indicate that women with distant metastases have a lower functional capacity and quality of life, as expected (9). Hsu et al. performed a follow-up study on 535 women suffering from early or locally advanced breast cancer, reporting that their overall quality of life was similar to the quality of life of the cancer-free control group. The patients analyzed in this study showed a significant improvement in their quality of life during the first year after the diagnosis, and this trend was continued during long-term follow-up. Interestingly, a significant cognitive deficit was seen in breast cancer long-term survivors in comparison with controls. The authors hypothesize that this finding may be contributed to the long-term effects of cancer treatment, or it may reflect unmasking the previously present and undiagnosed congitive issues (4).

The study conducted by Kaminska et al. on 85 patients treated with breast-conserving treatment and 94 patients treated with mastectomy showed that the patients aged 30-45 were more worried about their prognosis, the disease affecting their family and the relationship with their partner. Also, their study showed that highly educated patients who underwent breast-conserving therapy showed a superior social and physical functioning (7). This is in accordance with the results of the study presented herein-lack of understanding of their own disease leads the patients to a poorer quality of life. Boman et al. showed that the learning process in breast cancer patients relied mainly on the bodily experiences and the events occurring during the process of diagnosing and treating the disease. The authors describe three main themes which encompass the process of learning and understanding from the perspective of a breast cancer patient: interacting with a diversity of information, concealed and expressed understandings, and struggling to understand and manage the new life situation (10).

Hamer et al. found that breast cancer patients receiving chemotherapy had a lower quality of life and a greater symptom burden than patients without chemotherapy. On the other hand, patients with ductal carcinoma in situ and with early-stage cancer who underwent radiation treatment had a greater quality of life than the patients not receiving radiation therapy (11). Marino et al. showed that patients receiving chemotherapy for breast cancer more commonly report "severe problems" with physical well-being and had a lower breast cancer-specific quality of life; however, there were no differences in the functional, social and family well-being in comparison with the patients who did not receive chemotherapy (12). Tiezzi et al. found that chemotherapy worsened the physical functioning of the patient (13). The study presented herein did not show any significant differences in the quality of life regarding chemotherapy or radiation therapy. However, a relatively small number of patients not receiving these types of treatment (10 and 21, respectively) may have influenced the lack of statistical significance.

A satisfying result of this study is related to a significant number of patients receiving psychological support from their environment (family and friends). Namkoong et al. published the results of two randomized clinical trials performed by the National Cancer Institute as an analysis of expressed and received emotionally supportive messages in breast cancer patients within 2 months of diagnosis. In their study, perceived bonding was positively related to all four coping strategies (active coping, positive reframing, planning and humor). The authors emphasize the significance of perceived bonding as well as the patients' power to provide emotional support to others, thus strengthening shared group bonds (14).

The present study has several limitations. There was neither specific analysis of the type of radiotherapy or chemotherapy, nor there was any precise information on the extent of the disease (locoregional or distant metastases). Also, there was no analysis of the time passed since diagnosing cancer and administering the questionnaire, or any baseline psychological testing before receiving any treatment for breast cancer. A similar study should be repeated in a prospective manner (with the aforementioned variables which were omitted in this study) with a matched control group in order to further investigate the quality of life of breast cancer patients in the developing European countries.

Conclusion

Breast cancer is a serious, life-threatening condition requiring long-term therapy. The treatment of breast cancer in developing countries is challenged with social and economic circumstances. The study indicates that breast cancer patients in Montenegro complain about the psychological issues more frequently than the physical symptoms. Furthermore, the understanding of their own illness is positively related to the quality of life. The results should inspire the health professionals in developing countries to provide psychological support and educate the patients in order to facilitate the treatment of breast cancer.

References

1. Kelsey JL, Bernstein L. Epidemiology and prevention of breast cancer. Annu Rev Public Health 1996;17:47-67. [CrossRef] [PubMed]
2. Carioli G, Malvezzi M, Rodriguez T, Bertuccio P, Negri E, La Vecchia C. Trends and predictions to 2020 in breast cancer mortality in Europe. Breast 2017;36: 89-95. [CrossRef] [PubMed]
3. Dimitrova N, Znaor A, Agius D, Eser S, Sekerija M, Ryzhov A, et al. Breast cancer in South-Eastern European countries since 2000: Rising incidence and decreasing mortality at young and middle ages. Eur J Cancer 2017;83:43-55. [CrossRef] [PubMed]
4. Hsu T, Ennis M, Hood N, Graham M, Goodwin PJ. Quality of life in long-term breast cancer survivors. J Clin Oncol 2013;31(28):3540-8. [CrossRef] [PubMed]
5. Cummins RA. Personal income and subjective well-being: a review. J Happiness Stud 2000;1:133-58. [CrossRef]
6. Dialla PO, Chu WO, Roignot P, Bone-Lepinoy MC, Poillot ML, Coutant C, et al. Impact of age-related socio-economic and clinical determinants of quality of life among long-term breast cancer survivors. Maturitas 2015;81(3):362-70. [CrossRef] [PubMed]
7. Kamińska M, Ciszewski T, Kukiełka-Budny B, Kubiatowski T, Baczewska B, Makara-Studzińska M, et al. Life quality of women with breast cancer after mastectomy or breast conserving therapy treated with adjuvant chemotherapy. Ann Agric Environ Med 2015; 22(4):724-30. [CrossRef] [PubMed]
8. Carelle N, Piotto E, Bellanger A, Germanaud J, Thuillier A, Khayat D. Changing patient perceptions of the side effects of cancer chemotherapy. Cancer 2002; 95(1):155-63. [CrossRef] [PubMed]
9. Costa WA, Eleutério J Jr, Giraldo PC, Gonçalves AK. Quality of life in breast cancer survivors. Rev Assoc Med Bras 2017;63(7):583-9. [CrossRef] [PubMed]
10. Engqvist Boman L, Sandelin K, Wengström Y, Silén C. Patients' learning and understanding during their breast cancer trajectory. Patient Educ Couns 2017; 100(5):795-804. [CrossRef] [PubMed]
11. Hamer J, McDonald R, Zhang L, Verma S, Leahey A, Ecclestone C, et al. Quality of life (QOL) and symptom burden (SB) in patients with breast cancer. Support Care Cancer 2017;25(2):409-19. [CrossRef] [PubMed]
12. Marino JL, Saunders CM, Emery LI, Green H, Doherty DA, Hickey M. How does adjuvant chemotherapy affect menopausal symptoms, sexual function, and quality of life after breast cancer? Menopause 2016; 23(9):1000-8. [CrossRef] [PubMed]
13. Tiezzi MF, de Andrade JM, Romão AP, Tiezzi DG, Lerri MR, Carrara HA, et al. Quality of Life in Women With Breast Cancer Treated With or Without Chemotherapy. Cancer Nurs 2017;40(2):108-16. [CrossRef] [PubMed]
14. Namkoong K, McLaughlin B, Yoo W, Hull SJ, Shah DV, Kim SC, et al. The effects of expression: how providing emotional support online improves cancer patients' coping strategies. J Natl Cancer Inst Monogr 2013;(47):169-74. [CrossRef] [PubMed]

Originalni rad **UDC: 618.19-006.6(497.16)**
doi:10.5633/amm.2021.0207

KVALITET ŽIVOTA ŽENA OPERISANIH OD KARCINOMA DOJKE U CRNOJ GORI

Mitar Saveljić[1,2], Damir Peličić[2,3], Batrić Vukčević[2,4], Tahir Kalač[4], Gora Miljanović[5]

[1]Urgentni centar, Klinički centar Crne Gore, Podgorica, Crna Gora
[2]Univerzitet Crne Gore, Medicinski fakultet, Podgorica, Crna Gora
[3]Centar za nauku, Klinički centar Crne Gore, Podgorica, Crna Gora
[4]Klinički centar Crne Gore, Podgorica, Crna Gora
[5]Akademija strukovnih studija Beograd, Odsek visoka zdravstvena škola, Beograd, Srbija

Kontakt: Damir Peličić
Ljubljanska 1, 20000 Podgorica, Crna Gora
E-mail: damir.pelicic@t-com.me

Karcinom dojke je česta maligna bolest na Balkanu. Cilj rada bio je utvrditi da li postoje razlike u parametrima percepcije zdravlja i kvaliteta života među crnogorskim bolesnicama nakon operacija, radioterapije i hemioterapije zbog karcinoma dojke. Pomenuti ishodi ispitivani su u odnosu na uzrast, vrstu tretmana i stav prema preventivnim dijagnostičkim merama.

Istraživanje je sprovedeno na uzorku od 200 žena sa karcinomom dojke, lečenim u zdravstvenim ustanovama u Crnoj Gori. Korišćen je autentično dizajnirani upitnik baziran na tri prethodno dizajnirana upitnika (Functional Assesment of Cancer Therapy: General (FACT-G); njegova vrsta, specifična za karcinom dojke (FACT-B), kao i instrument dizajnirani su od strane Evropske organizacije za istraživanje i lečenje raka (EORTC-QLQ C30)).

Najniži kvalitet života nađen je kod bolesnica starosti od 60 godina do 64 godine. Bolesnice su često navodile da se osećaju tužno, da gube nadu ili da brinu da će neko iz njihove porodice oboleti od raka dojke. Kvalitet života bio je niži među bolesnicama koje su navele da ne razumeju svoju bolest dobro. Vreme operacije karcinoma dojke nije bilo značajno povezana sa kvalitetom života, slično vremenu hemioterapije (ili vremenu od posljednje hemioterapijske sesije). Međutim, analiza vremena od posljednjeg kursa zračenja dala je statističku značajnost, u smislu da je kvalitet života bio najniži u grupi bolesnica koje su bile podvrgnute zračnoj terapiji.

Ovi rezultati treba da inspirišu kliničare da edukuju bolesnike i pruže im psihološku podršku u toku lečenja.

Acta Medica Medianae 2021;60(2):57-63.

Ključne reči: *dojka, karcinom, kvalitet života*

Review article

UDC: 616.33-008.6:615.243.015.3
doi:10.5633/amm.2021.0208

REBOUND PHENOMENON OF PROTON PUMP INHIBITOR THERAPY

Daniela Benedeto Stojanov[1], Goran Koraćević[1], Dragan Stojanov[1], Maja Koraćević[1], Nebojša Ignjatović[1]

Proton pump inhibitors (PPIs) are the most potent drugs for suppressing gastric acid secretion. They are used in the treatment of acid-peptic disorders, including peptic ulcer disease, gastroesophageal reflux disease, Zollinger Ellison syndrome, in the eradication of *Helicobacter pylori* infection and ulcer prophylaxis. In the pharmacotherapy of these disorders, they have significantly suppressed the use of H2 blockers, like other, older groups of antisecretory drugs.

Long-term PPI therapy leads to moderate hypergastrinemia (increased gastrin secretion) in 20-25% of patients. This hypergastrinemia results in rebound acid hypersecretion (RAHS) in 30-40% patients, who abruptly discontinue PPI. Most patients who abruptly discontinue PPI have symptoms of dyspepsia and gastroesophageal reflux, most commonly heartburn and a burning sensation in the esophagus.

Therefore, care should be taken to properly discontinue PPI and reduce the dose of the drug before complete discontinuation. A less effective acid blocker (H2 blocker) can be switched, since H2 receptor blockers cause less pronounced hypergastrinemia and hyperplasia of enterochromaffin-like cells (ECL cells) compared to PPI.

Acta Medica Medianae 2021;60(2):64-68.

Key words: *proton pump inhibitor, rebound, hypergastrinemia, gastric acid hypersecretion*

[1]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Daniela Benedeto Stojanov
6/6 Todora Milovanovića St., 18000 Niš, Serbia
E-mail: dbenedetostojanov@gmail.com

Introduction

At present, there is plentiful evidence to suggest that short-term PPI therapy is well effective and relatively safe. Therefore, PPIs have become the drug of choice in all conditions accompanied by increased gastric acid secretion (1). Thus, in 2001 and 2008 they were the second group of drugs prescribed and dispensed in the United States (1). They significantly suppress the use of H2 blockers, as other, older groups of antisecretory drugs.

Indications for the use of PPI are short term treatment of gastroesophageal reflux disease (GERD) and long term treatment of severe esophagitis and Barrett's esophagus, dyspepsia caused by acid hypersecretion, Zollinger Ellison syndrome, healing of gastric and duodenal peptic ulcer disease, eradication of Helicobacter pylori infection (in combination with antibiotic therapy), prophylaxis of gastroduodenal lesions associated with the use of non-steroidal anti-inflammatory drugs (NSAID), aspirin or antiplatelet agents, as well as in critical patients in intensive care units (2).

Long-term PPI therapy increases the risk of developing "rebound" hypersecretion of gastric acid. Abrupt discontinuation of PPI in these patients can lead to a worsening of disease symptoms, even above the intensity of initial symptoms (3).

Regulation of gastric secretion

The proton pump (H+/K+ ATPase) located in the canalicular membrane of gastric parietal cells has a key role in acid secretion. It enables the transport of hydrogen ions into the gastric lumen (3). Three types of receptors have been identified on parietal cells whose activation leads to stimulation of acid secretion: receptors for acetylcholine, histamine and gastrin. Gastrin is the main hormonal mediator of the gastric phase of acid secretion released by antral neuroendocrine G-cells into peripheral blood in response to a variety of physical and neurohumoral stimuli such as gastric distension, histamine, presence of amino acids, and vagal stimulation. Gastrin stimulates histamine synthesis (via an increase in expression of histidine decarboxylase) and histamine secretion from fundic enterochromaffin-like (ECL)

cells (4). Histamine diffuses to interact with the H2-receptors and stimulate parietal cells to secrete HCL. This activation cascade usually named gastrin–ECL axis is considered as the main stimulatory pathway of gastric acid secretion. Gastrin might directly promote acid secretion to some extent inducing H+/K+-ATPase activation directly on parietal cells. This mechanism is considered to be less extensive (4). Vagal stimulation of acid secretion is mediated by acetylcholine which stimulates the parietal cells directly by binding to M3 receptors. Acetylcholine and gastrin stimulation leads to an increase in cytosolic calcium (Ca2+). Histamine induces the activation of adelynate cyclase which converts ATP to cyclic adenosine monophosphate (cAMP). An increase of cytosolic calcium (Ca2+) followed by accumulation of cAMP activates cAMP-dependent protein kinases and phosphorylation cascades which activate proton pump transport (H+/K+ ATPase). Activation of proton pump results in the exchange of intracellular H+ with extracellular K+-gastric acid secretion.

The inhibiting negative feedback prevents excessive gastric acid secretion which is potentially harmful to the integrity of the gastric mucosa. The main negative regulator of gastric acid secretion is somatostatin. It is produced in the antral mucosa by D cells in response to several stimuli. Gastrin is one of the stimuli which induce secretion of somatostatin by antral D cells and in turn inhibits secretions of gastrin from G cells. This constitutes the so-called gastrin-somatostatin axis which takes part in gastric levels and acid secretion. Another stimulus, low antral pH is considered as the most important inducer of somatostatin release which inhibits further gastrin secretion from G cells. Gastric food content or neutral gastric secretion inhibits somatostatin secretion (5).

Pharmacokinetics and pharmacodynamics of proton pump inhibitors

PPIs are acid-resistant capsules or tablets, which in inactive form pass through the esophagus and stomach. They are resorbed in the small intestine and reach the systemic circulation, and then by diffusion into the secretory canaliculus of parietal cells (6). The pH in the canalicular system of parietal cells is very low and activates PPI. After being activated, PPIs inhibit the active proton pump (H+/K+ ATPase) by covalent binding. Longer duration of the acid secretion inhibition, even after the PPIs levels in the blood have decreased, is enabled by this covalent binding. The duration of the inhibitory activity of PPIs is variable. The loss of covalently bound PPIs and pump turnover affect the inhibitory activity duration (7). The PPIs effect is most prominent when the proton pumps are active which occurs after a meal. This is the reason why patients should take PPIs before a meal (8).

PPIs inhibit acid secretion and lead to hypoacidity (higher pH level). The increase in pH inhibits antral D cells from somatostatin secretion which inhibits the negative feedback of gastrin secretion from antral G cells. The secretion of gastrin from the antral G cells is increased which is followed by an increase in gastrin blood concentration. Some increase in gastrin blood levels develops in patients who have been on long-term PPI therapy. Only a small number of them develop hypergastrinemia which defines gastrin levels higher than the upper limit of the reference range for fasting blood gastrin (9). Gastrin has a hypertrophic effect on the gastric mucosa and causes enterochromaffin-like (ECL) hyperplasia. The increase in gastrin is most pronounced in the first few months, but also up to 1-2 years in patients on PPI long-term treatment (10, 11).

Rebound phenomenon of proton pump inhibitors

After abrupt discontinuation of PPI treatment, a rebound phenomenon of acid hypersecretion can develop. It is believed that the rebound acid hypersecretion (RAHS) phenomenon results from hypertrophic effects of gastrin on ECL cells, which leads to an increase in acid production following discontinuation of PPIs therapy. The increased acid production causes rebound symptoms which may be followed by new inappropriate PPI prescribing (12). In 30-40% of patients who abruptly discontinue PPI therapy, there will be rebound acid hypersecretion and rebound symptoms. Most patients have rebound symptoms of dyspepsia and GERD: heartburn and a burning sensation in the esophagus (12).

Cellular hyperplasia leads to reversible excessive secretion of gastric acid and can last for weeks. There is no consensus on how long this hypersecretion lasts on average. It is estimated that it is a period of 6-8 weeks, but also up to 26 weeks.

The incidence of a rebound phenomenon depends on the intensity and duration of the drug and how long it has been applied, the individual sensitivity of the patient (severity of the primary disease and the present comorbidity), and the application of other co-therapy (13).

A gastric carcinogenic effect may be a consequence of long-term hypergastrinemia. (14). PPI therapy with secondary hypergastrinemia and ECL cell hyperplasia on a long term basis may lead to ECL cells neoplasia. In numerous case reports it has been described that gastric polyp formation with subsequent development of ECL carcinoids and carcinomas may appear in patients on long-term PPI therapy (15-18). It is debatable whether gastric cancer is induced by gastrin alone or gastrin acts as a co-factor with once triggered premalignant changes.

Proper dosing of proton pump inhibitors

Proton pump inhibitors are often inappropriately prescribed inconsistent with recommendations and guidelines, whether there is a poor indication, an overdose, or an excessive duration of therapy. For example, in therapeutic guidelines, short-term use of PPI in a duration of eight weeks is recommended for GERD and mild esophagitis to heal the inflammation and lose symptoms (19).

It has been reported that 51% of 901 Danish primary care patients on long-term PPI therapy had uninvestigated symptoms. This cross-sectional study

showed that 22% of these patients received PPI regardless of normal upper GI endoscopy (20).

If PPIs are prescribed without an appropriate indication or for longer than necessary, the dose and/or frequency of administration should be reduced. A proper way to quit PPI therapy seems to be halving the PPI dose for a month or two, and then ceasing PPI or switching to a less effective acid suppressant (H2 blocker). Antacids or H2 blockers should be prescribed to control rebound symptoms, for another 1-3 weeks after discontinuation of PPI (3). H2 blockers cause less pronounced hypergastrinemia and ECL cell hyperplasia compared with PPIs.

Studies have shown that about 30% of patients can discontinue long-term PPI therapy (21). The dose of PPI may be lower in up to 80% of patients on long term therapy (11).

If there is an appropriate indication for PPIs, PPIs should be continued with the lowest effective dose. Indications for long-term PPI therapy are severe esophagitis (LA grade C or D), Barret's esophagus, documented history of bleeding GI ulcer, chronic NSAIDs use with bleeding risk factors, Zollinger-Ellison syndrome (22).

Proper prescribing of PPI requires a documented indication, plan of the treatment duration, and patient control plan to decide on the need for further treatment.

Conclusion

Proton pump inhibitors are relatively safe drugs, with rare serious side effects.

However, it should be pointed out that these drugs are overprescribed, and it should be emphasized that they should be given only to patients with clear indications.

Long-term use of PPIs carries certain risks, insofar as their use is abruptly discontinued due to the possible rebound phenomenon of gastric hypersecretion and the appearance of rebound symptoms of dyspepsia and reflux disease.

Therefore, care should be taken to properly discontinue the use of PPIs by reducing the dose and frequency of drug administration before complete cessation, and if necessary, we can switch to H2 blockers.

References

1. Rašić J, Rašić D, Janićijević Hudomal S, Nestorović V. Inhibitori protonske pumpe-primena i bezbednost. Biomedicinska istraživanja 2013;4: 48-56. [CrossRef]
2. Halfdanarson OO, Pottegard A, Bjornsson ES, Lund SH, Ogmundsdottir MH, Steingrimsson E, et al. Proton-pump inhibitors among adults: A nationwide drug-utilization study. Therap Adv Gastroenterol 2018;11: 1-11. [CrossRef] [PubMed]
3. Pasina L, Urru SA, Mandelli S, Giua C, Minghetti P. Evidence-based and unlicensed indications for proton pump inhibitors and patients' preferences for discontinuation: a pilot study in a sample of Italian community pharmacies. J Clin Pharm Ther 2016;41:220-223. [CrossRef] [PubMed]
4. Chu S, Schubert ML. Gastric secretion. Curr Opin Gastroenterol 2013;29:636-41. [CrossRef] [PubMed]
5. Bloom SR, Mortimer CH, Thorner MO, Besser GM, Hall R, Gomez-Pan A, et al. Inhibition of gastrin and gastric-acid secretion by growth-hormone release-inhibiting hormone. Lancet 1974;2:1106-9. [CrossRef] [PubMed]
6. Shin JM, Kim N. Pharmacokinetics and pharmacodynamics of the proton pump inhibitors. J Neurogastroenterol Motil 2013;19:25-35. [CrossRef] [PubMed]
7. Gedda K, Scott D, Besancon M, Lorentzon P, Sachs G. Turnover of the gastric H+,K(+)-adenosine triphosphatase alpha subunit and its effect on inhibition of rat gastric acid secretion. Gastroenterology 1995;109: 1134-41. [CrossRef] [PubMed]
8. Hatlebakk JG, Katz PO, Camacho-Lobato L, Castell DO. Proton pump inhibitors: Better acid suppression when taken before a meal than without a meal. Aliment Pharmacol Ther 2000; 14: 1267–1272. [CrossRef][PubMed]
9. Helgadottir H, Lund SH, Gizurarson S, Metz DC, Bjornsson ES. Predictors of Gastrin Elevation Following Proton Pump Inhibitor Therapy. J Clin Gastroenterol 2020;54:227-34. [CrossRef] [PubMed]
10. Lundell L, Vieth M, Gibson F, Nagy P, Kahrilas PJ. Systematic review: The effects of long-term proton pump inhibitor use on serum gastrin levels and gastric histology. Aliment Pharmacol Ther 2015;42:649-63. [CrossRef] [PubMed]
11. Helgadottir H, Metz DC, Lund SH, Gizurarson S, Jacobsen EI, Asgeirsdottir GA, et al. Study of Gender Dierences in Proton Pump Inhibitor Dose Requirements for GERD: A Double-Blind Randomized Trial. J Clin Gastroenterol 2017;51:486-93. [CrossRef] [PubMed]
12. Waldum HL, Qvigstad G, Fossmark R, Kleveland PM, Sandvik AK. Rebound acid hypersecretion from a physiological, pathophysiological and clinical viewpoint. Scand J Gastroenterol 2010;45:389-94. [CrossRef] [PubMed]
13. Koraćević M, Lalić J, Nedeljković S, Koraćević G. Rebound phenomenon-important and ubiquitous in pharmacotherapy. Acta Medica Medianae 2018;57: 148-52. [CrossRef]
14. Waldum HL, Hauso O, Brenna E, Qvigstad G, Fossmark R. Does long-term profound inhibition of gastric acid secretion increase the risk of ECL cell-derived tumors in man? Scand J Gastroenterol 2016; 51:767-73. [CrossRef] [PubMed]
15. Tran-Duy A, Spaetgens B, Hoes AW, deWit NJ, Stehouwer CD. Use of Proton Pump Inhibitors and Risks of Fundic Gland Polyps and Gastric Cancer: Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2016;14:1706-19. [CrossRef] [PubMed]
16. Brusselaers N, Wahlin K, Engstrand L, Lagergren J. Maintenance therapy with proton pump inhibitors and risk of gastric cancer: A nationwide population-based cohort study in Sweden. BMJ Open 2017;7, e017739. [CrossRef] [PubMed]
17. Cheung KS, Chan EW, Wong AYS, Chen L, Wong ICK, Leung WK. Long-term proton pump inhibitors and risk of gastric cancer development after treatment for Helicobacter pylori: A population-based study. Gut 2018;67:28-35. [CrossRef] [PubMed]
18. Cavalcoli F, Zilli A, Conte D, Ciafardini C, Massironi S. Gastric neuroendocrine neoplasms and proton pump inhibitors: Fact or coincidence? Scand J Gastroenterol 2015;50:1397-1403. [CrossRef] [PubMed]
19. Katz PO, Gerson LB, Vela MF. Guidelines for the diagnosis and management of gastroesophageal reflux disease. Am J Gastroenterol 2013;108:308-28. [CrossRef] [PubMed]
20. Reimer C, Bytzer P. Clinical trial: Long-term use of proton pump inhibitors in primary care patients-A cross sectional analysis of 901 patients. Aliment Pharmacol Ther 2009;30:725-32. [CrossRef] [PubMed]
21. Zwisler JE, Jarbol DE, Lassen AT, Kragstrup J, Thorsgaard N, Schaffalitzky de Muckadell OB. Placebo-Controlled Discontinuation of Long-Term Acid-Suppressant Therapy: A Randomised Trial in General Practice. Int J Fam Med 2015;175436. [CrossRef] [PubMed]
22. Strand DS, Kim D, Peura DA. 25 Years of Proton Pump Inhibitors: A Comprehensive Review. Gut Liver 2017; 11: 27-37. [CrossRef [PubMed]

Pregledni rad **UDC: 616.33-008.6:615.243.015.3**
doi:10.5633/amm.2021.0208

FENOMEN NAGLE OBUSTAVE TERAPIJE INHIBITORIMA PROTONSKE PUMPE

Daniela Benedeto Stojanov[1], Goran Koraćević[1], Dragan Stojanov[1], Maja Koraćević[1], Nebojša Ignjatović[1]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Daniela Benedeto Stojanov
Todora Milovanovića 6/6, 18000 Niš, Srbija
E-mail: dbenedetostojanov@gmail.com

Inhibitori protonske pumpe (IPP) najpotentniji su lekovi za suprimiranje sekrecije želudačne kiseline. Primenjuju se u terapiji acido-peptičkih poremećaja, uključujući peptičku ulkusnu bolest, gastroezofagusnu refluksnu bolest, Zollinger–Elissonov sindrom, infekciju izazvanu bakterijom *Helicobacter pylori* i profilaksu ulkusa. U farmakoterapiji ovih poremećaja, u značajnoj meri je potisnuta upotreba H2 blokatora, kao drugih, starijih grupa antisekretornih lekova.

Dugotrajna terapija IPP dovodi do umerene hipergastrinemije (pojačana sekrecija gastrina) kod 20% do 25% bolesnika. Ova hipergastrinemija rezultira povratnom hipersekrecijom želudačne kiseline (*rebound* fenomen) kod 30% do 40% bolesnika, koji su naglo prekinuli IPP. Većina bolesnika, koja je naglo prekinula IPP, ima simptome dispepsije i gastroezofagusnog refluksa, najčešće gorušicu i osećaj gorenja u jednjaku.

Zbog toga treba voditi računa o pravilnom prekidu upotrebe IPP i smanjiti dozu leka pre potpunog ukidanja. Može se preći na manje efikasan blokator kiseline (H2 blokator), s obzirom na to da blokatori H2 receptora izazivaju manje izraženu hipergastrinemiju i hiperplaziju ćelija sličnih enterohromafinu (ECL ćelija) u poređenju sa IPP.

Acta Medica Medianae 2021;60(2):64-68.

Ključne reči: *inhibitori protonske pumpe, rebound, hipergastrinemija, želudačna hipersekrecija*

Case report

UDC: 616.12-089.819.5-073.7
doi:10.5633/amm.2021.0209

OPTIMIZATION OF PERCUTANEOUS CORONARY INTERVENTION WITH OPTICAL COHERENCE TOMOGRAPHY

Zoran Perišić[1], Nenad Božinović[1], Mihajlo Lazarević[1]

Optical coherence tomography (OCT) is a method which provides precise insight into the morphology of the coronary arteries. The application of OCT can evaluate atherosclerotic plaques, stoutness of fibrous cap, and also gives precise illustration of the stent position in the coronary artery.

A 72 year old woman was received with acute myocardial infarction with ST segment elevation in anterolateral leads and immediately was sent to catheterization lab for PCI. During the procedure, we found occluded left circumflex artery and after predilatation two stents were implanted. Flow rate of stents was very slow and signs of stent thrombosis were registered. The flow rate was not restored after post-dilatation and thrombus aspiration. Due to rapid worsening of condition of the patient, the surgery was ceased, and we continued therapy with GP inhibitors, anticoagulant and antithrombotic therapy. After 7 days, coronary angiography was repeated and OCT system was used. We found substantial apposition of stents with some struts at up to 0.5 mm distance from the coronary artery wall. Post-dilatation was done with the larger balloon sized according to the arterial diameter, and subsequently the thrombosis disappeared and struts were close to the coronary artery wall. The patient was discharged from hospital with dual antiplatelet therapy in a good condition.

OCT is a new diagnostic method which provides exceptionally easy and precise identification of unexpanded stents in coronary arteries, which present main mechanical cause for stent thrombosis after PCI.

Acta Medica Medianae 2021;60(2):69-76.

Key words: *optical coherence tomography, percutaneous coronary intervention, stent apposition, coronary thrombosis*

[1]University Clinical Center Niš, Cardiology Department, Niš, Serbia

Contact: Zoran Perišić
12A Sterijina St., 18000 Niš, Serbia
E-mail: perisic.zoran@me.com

Introduction

First ever percutaneous coronary intervention (PCI) was performed by Andreas Ghruncig in 1977 and since than a new era of nonsurgical therapy for coronary artery disease started. Development of materials technology and techniques for PCIs led to safe coronary diagnostics and therapy even in centers without support of cardiosurgery which resulted in expansion and opening of huge number of cath labs through the world. As coronary angiography became the gold standard for visualization of coronary arteries, new additional diagnostic procedures were developed in order to make better assessment of severity and importance of lesions: flow measurement through the coronary artery (flow fractional reserve - FFR), intravascular ultrasound (IVUS) and few years ago, development of optical coherence tomography. Both IVUS and OCT provide visual assessment of inner side of coronary arteries which overcomes limitations of coronary angiography. These limitations often understate or overstate coronary artery stenosis.

Optical coherence tomography is a diagnostic method in interventional cardiology which provides almost histological resolution in observation and analysis of coronary arteries by the use of light (near infrared spectrum). In this way, even a small details in coronary pathology as atherosclerotic plaque specificity, analysis of stent position and periprocedural blood vessel injuries can be seen. OCT is much more sensitive than intravascular ultrasound, which was inviolable method for analysis of changes in coronary arteries until now (1, 2).

OCT measures intensity of reflected light waves and converts these optical echoes to high resolution two dimensional images, analogue to ultrasound. As the speed of light is much faster than the speed of sound, latency of reflected light wave cannot be direct so interference is used. That means that light signal is divided into two parts: referent

signal, which has a known instance, and causal signal, which comes from the tissue. The reflected signal from the tissue is compared to the light signal that comes through known distance.

High resolution image of blood vessel is made based on comparison of these two signals (signal interference). The use of light instead the use of ultrasounds has consequences. Image resolution is ten times higher compared to IVUS, but because of that there is less and limited penetration into tissue. It is necessary that the blood vessel is free of blood for a short period of time and filled with contrast because the erythrocytes could cause multiple scattering and deformation of the light signal.

OCT can be used in multiple indications (3, 4, 5):

- to check and determine characteristics of atherosclerotic plaque with the possibility to identify a high risk of plaque rupture;
- evaluation of vulnerable plaque;
- thrombus detection;
- evaluation of stent after PCI;
- assessment of acute effects in coronary artery stenting;
- follow up in covering and endothelialization of struts;
- stent apposition identification;
- follow up and assessment of restenosis;
- periprocedural lesion assessment.

If the distance from endoluminal surface of stent is bigger than the sum of strut and polymer thickness, that is called malapposition or stent struts apposition. Existence of stent apposition is significant because those struts are a site of early and late stent thrombosis and their coverage is poor and happens later or it can even fail. Stent apposition is possible when the artery size is underestimated or when the implanted stent is smaller than the diameter of the blood vessel. In acute coronary syndrome, especially in ST segment myocardial infarction (STEMI) there is often the presence of a thrombus that is adherent to the vessel wall. This can give wrong impression of the size of the coronary vessel. No matter the reason for stent apposition, if apposition is not corrected, stent thrombosis is almost certain when anticoagulation therapy is stopped despite double antiaggregation therapy. Angiography cannot detect stent apposition, so IVUS and OCT are the only known certain methods by which stent apposition can be identified for now.

There is a description of the first OCT in Serbia in further text.

Case presentation

A female patient, 72 years old was admitted to the Cardiology Clinic as an emergency with acute inferolateral myocardial infarction with ST elevation. She had only arterial hypertension as a risk factor (well regulated, for 5 years). As pain occurred for more than 3 hours from the symptom onset, and patient was admitted to the primary PCI (pPCI) capable center, decision to perform coronary angiography was made in order to determine further therapy. She was treated by STEMI protocol directed to pPCI (ASA 300 mg, clopidogrel 300 mg, unfractionated heparin 5000 IU). At the moment of coronary angiography, her blood pressure was 95/65 mm Hg and heart rate 40/min so temporary pace maker was first placed.

Left coronary artery angiography revealed occluded proximal part of the circumflex artery (TIMI flow 0) and left anterior descending artery had no changes (Figure 1).

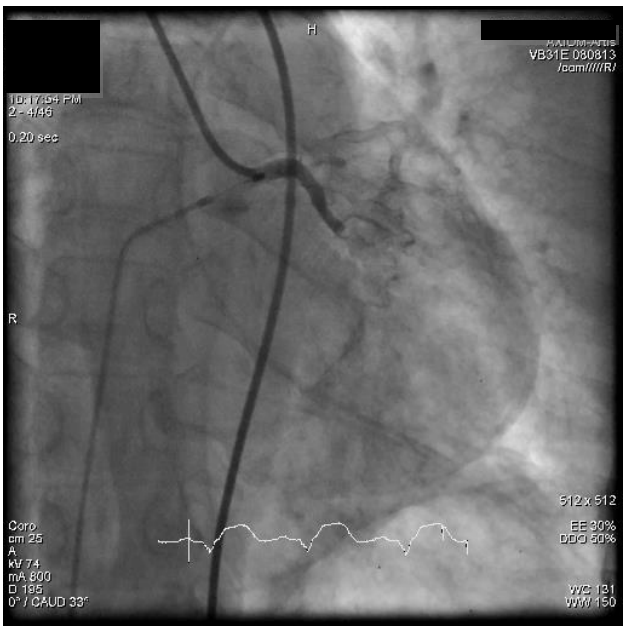


Figure 1. The occluded circumflex artery at the beginning of the procedure (TIMI 0 flow)

PCI was immediately started and angiography of right coronary artery was not done due to worsening of the patient's condition. Coronary wire was easily passed through thrombus (Runthough Hypercoat, Terumo, Japan) and predilatation by using balloon Powerline 2.0x15 mm (Biosensors, Singapore) was done. The artery was opened and the distal part of the artery was visualized (TIMI 2 flow). The surgeon decided to implant stents in the artery: Tsunami Gold 2.5x18 mm (Terumo, Japan) distally, insufflated at 16 atmospheres. The proximal part of this stent was overlapped with ML Vision 2.75x23 mm stent (Abbott, USA) insufflated at 16 atmospheres.

Despite implanted stents, the flow through the circumflex artery was not good and stent thrombosis developed (TIMI 1 flow). Post-dilatation with balloon Sprinter NC 2.5x15 mm was done (Medtronic, USA) in all segments of both stents, but the flow was not improved (Figure 2). Unsuccessful thromboaspiration was performed by the use of Diver (Invatec; USA) aspiration catheter. PCI was stopped due to rapid worsening of the patient's arterial blood pressure (50/35 mmHg), signs of hypoperfusion and threatening cardiogenic shock. Intraaortic balloon pump was placed as a hemodynamic support, tirofiban was started and she was transferred to ICU.

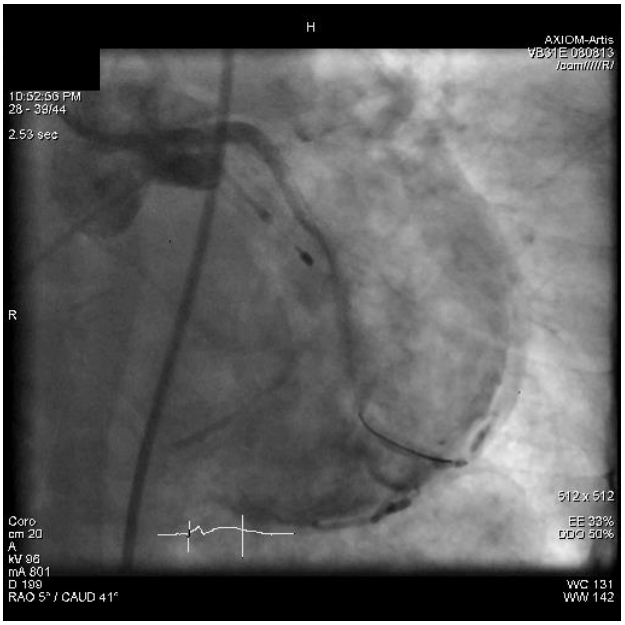


Figure 2. The circumflex artery after PCI (TIMI 1 flow)

In the next 24 hours patient became stable and blood pressure and heart rate were within reference range. Temporary pacemaker and intraaortic balloon pump were turned off. When tirofiban therapy ended, low molecular weight heparin was started (enoxaparin, 50 mg every 12 hours subcutaneously) with double antiaggregation therapy, statin and symptomatic treatment. She was treated for five days and then she was sent again to the cath lab for right coronary artery angiography and to estimate stent position with the use of optical coherence tomography (OCT). Multiplate test revealed good inhibition of thrombocyte aggregation and effectiveness of anticoagulant therapy.

The right coronary artery had no morphologic changes. Angiography of the left coronary artery revealed patent circumflex artery (TIMI3 flow) but with opacifications within the artery at the level of implanted stents which rose suspicion of thrombus not big enough to compromise flow through the artery. We decided to use OCT (LightLab Imaging, USA) in order to evaluate the presence of stent apposition and eventual need for another PCI.

Coronary wire Balance MiddleWeight (Abbott Vascular, USA) was introduced in the artery but the placement of OCT catheter over it was unsuccessful. We concluded that the wire passed beneath the struts so the tip of the wire was reshaped and then the wire was successfully placed in the artery. First OCT capture was done at the length of 56 mm which covered part of the circumflex artery distally to the placed stent all the way to its ostium.

Struts of both stents were seen very well and stent apposition was seen immediately from 6th millimeter of the distal stent all the way to the ostium of the artery, meaning that struts do not lay over the artery wall but there is a space for at least double of strut thickness.

At some places, distance of stent struts from the artery wall was greater than half a millimeter.

The most apposed struts had huge thrombotic masses around them and in a stented part of the artery thrombotic masses were also present, displaced by guidewire (Figures 3 and 4).

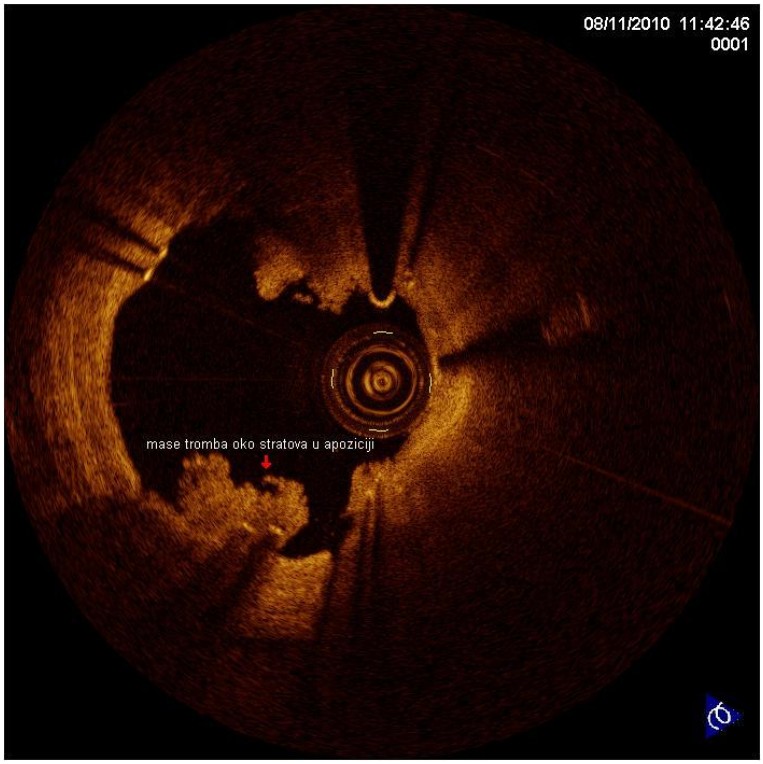


Figure 3. OCT image shows thrombotic masses around the struts in apposition

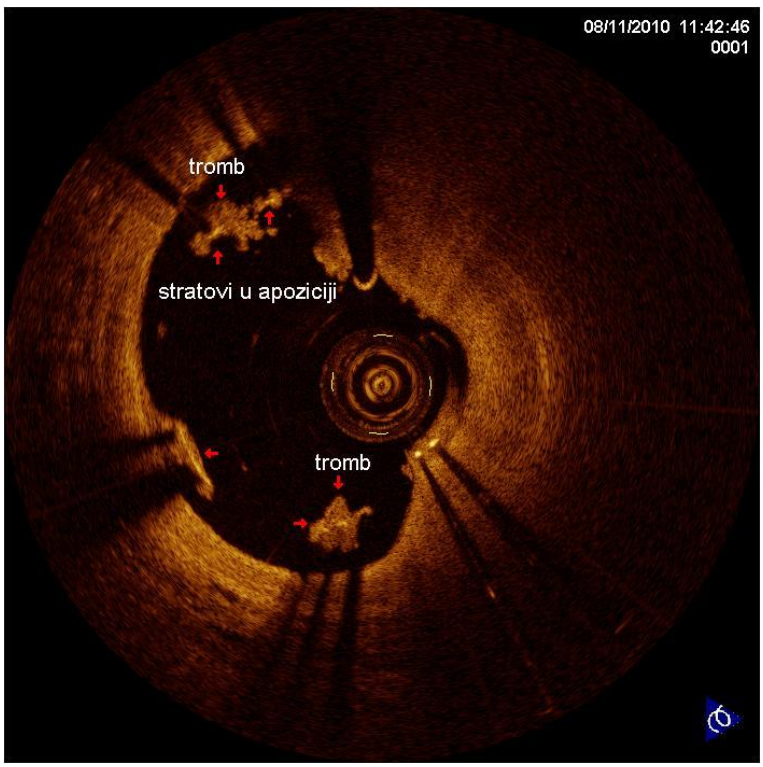


Figure 4. OCT image with few struts in apposition

We decided to postdilate implanted stents. Noncompliant balloon Sprinter NC 3.5x15 mm (Medtronic, USA) was placed over the coronary wire and insufflated at 14 atmospheres through the length of both stents. No thrombotic masses after post-dilatation could be seen on angiography and TIMI 3 flow was present (Figure 5). The artery was examined by OCT once again. All the struts were adherent to endothelium with very little thrombosis over the arterial wall which did not affect the blood flow. There were no more struts that were in apposition (Figure 6). After this, PCI was done without another stent implantation. Enoxaparin was continued for another 24 hours and the patient was discharged two day after in good general condition and symptoms free. Six month after the procedure, the patient is symptoms free.

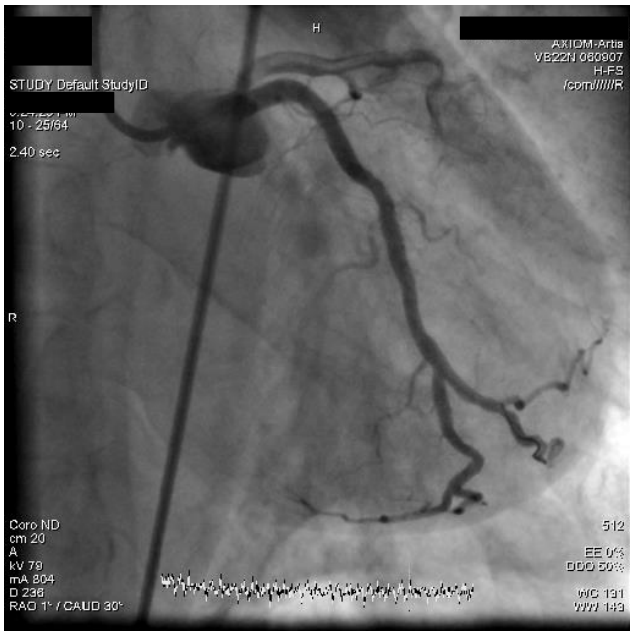


Figure 5. The circumflex artery after optimization of PCI and using of OCT (TIMI 3 flow)

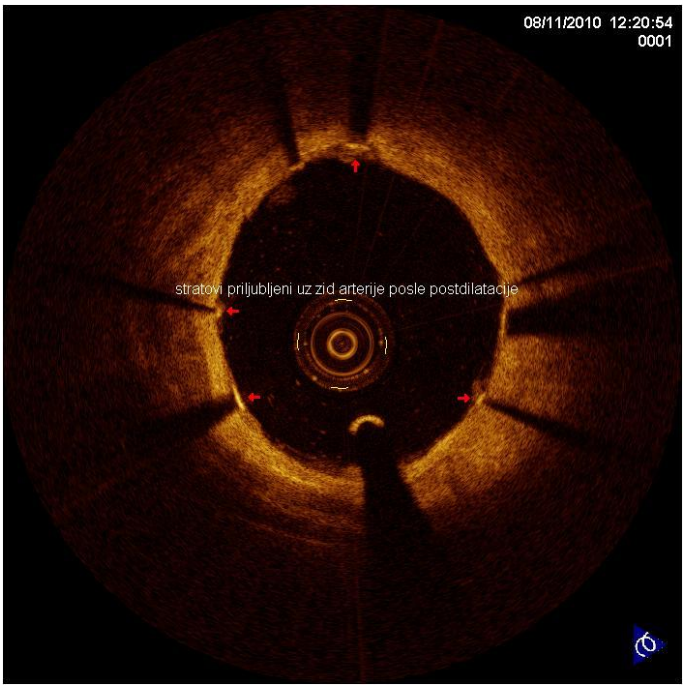


Figure 6. OCT image at the end of the procedure: struts adhering to the wall of the coronary artery after post-dilatation

Discussion and conclusion

In the last couple of interventional cardiology congresses, optical coherence tomography has become optimal or at least suboptimal diagnostic method for assessment of coronary morphology. OCT enables enormous possibilities for inspection of inner structures and around coronary arteries up to 10 microns. Compared to IVUS which was a golden standard until now, OCT has many advantages but it also has limitations. The use of OCT system provides us to see fine morphological details in arteries, it is much faster and has more flexibility. On the other hand IVUS is better for inspection of bigger arteries and the presence of blood in the blood vessels does not affect examination. Table 1 shows comparison of physical characteristics of OCT and IVUS (6).

Table 1. View of the parallel characteristics of OCT and IVUS

	OCT	**IVUS**
Frames per second	100	30
Pullback speed	20 mm/sec	0.5 or 1 mm/sec
Lines per frame	500	100
Axial resolution	12 um	225 um
Scan diameter	7-10 mm	12-15 mm

Stent thrombosis is present as long as there is PCI and it has always been Damocles' Sword above the head of the patient as well as the interventional cardiologist. Stent thrombosis refers to the presence of thrombotic formation in already implanted stent in the coronary artery. Drug coated stents, PCI in acute myocardial infarction and bifurcation lesions are thought to be risk factors for stent thrombosis and as for main mechanical predictor stands stent malapposition or overestimating of the coronary artery and the use of smaller stent (7, 8). Until now, IVUS was used as the gold standard for estimating stent apposition and newer imaging methods have been described in order to make identification of stent apposition easier (9). For the last couple of years, OCT has been described in the literature as a very successful method in detection of stent apposition mainly because of its possibility of detail visualization to size up to the one hundredth part of a millimeter. Compared to other OCT systems in which a complete closure of the artery was needed, the new LightLab C7 XR Imaging system requires only the presence of contrast in the blood vessels during one time application. The simplicity in use of OCT system and high quality of acquired information enables easy and simple identification of even small details as it is the distance of free struts of the stent from the coronary artery wall. All authors agree that this is the main mechanical reason for stent thrombosis. Once identified, stent malaposition can be easily solved by using post-dilatation with noncompliant balloons of adequate size and OCT(or IVUS) can determine the size of the balloon, that is, in which size the stent should be expanded (10). After the procedure is completed like this one, anticoagulation therapy can be stopped and double antiaggregation therapy can be continued. The number of all the complications (death, lesion revascularization or vessel revascularization, stent thrombosis) was significantly smaller in all major studies in which PCI optimization was guided and performed by IVUS compared to those in which IVUS was not used. There are not many major studies where OCT was performed, but individual studies reveal that OCT guided PCI enables significantly greater survival of patients and less complications after PCI (11, 12, 13).

References

1. Tearney GJ, Brezinski ME, Bouma BE, et al. *In vivo* endoscopic optical biopsy with optical coherence tomography. Science 1997;276(5321):2037-9. [CrossRef] [PubMed]
2. Boppart SA, Bourna BE, Pitris C, Southern JF, Brezinski ME, Fujimoto JG. *In vivo* cellular optical coherence tomography imaging. Nat Med 1998;4: 861.65. [CrossRef] [PubMed]
3. Yabushita H, Bouma BE, Houser SL, Thomas AH, Jang I-K, Schlendorf KH, et al. Characterization of human atherosclerosis by optical coherence tomography. Circulation 2002;106:1640-5. [CrossRef] [PubMed]
4. Kubo T, Imanishi T, TakaradaS, Kuroi A, Ueno S, Yamano T, et al. Assessment of culprit lesion morphology in acute myocardical infranction: ability of optical coherence tomography compared with intravascular ultrasound and coronary angioscopy. J Am Coll Cardiol 2007;50: 933-9. [CrossRef] [PubMed]
5. Perišić Z. Optička koherentna tomografija – nova metoda u interventnoj kardiologiji. Srce i krvni sudovi. 2011;30:13-7. [CrossRef]
6. Gonzalo N, Ligthart J, Regar E. Tips & trics of optical coherence tomography. In: Manuel Sabate. Percutaneous cardiac interventions – tips and trics of new techniques beyond stenting. PCR Publishing, Europa Edition. 2010;49-55.
7. Gonzalo N, Escaned J, Alfonso F, Jiménez-Quevedo P, Zakhem B, Bañuelos C, et al. Is refined OCT guidance of stent implantation needed? EuroIntervention. 2010; 6 Suppl 145-53.
8. Ozaki Y, Okumura M, Ismail TF, Naruse H, Hattori K, Kan S, et al. The fate of incomplete stent apposition with drug-eluting stents: an optical coherence tomography-based natural history study. Eur Heart J 2010; 31(12):1470-6. [CrossRef] [PubMed]
9. Rogers RK, Michaels AD. Enhanced x-ray visualization of coronary stents: clinical aspects. Cardiol Clin 2009; 27(3):467-75. [CrossRef] [PubMed]
10. Gonzalo N, Serruys P, Macaya C, Regar E. Optical coherence tomography in the study of coronary stenosis. In: Escaned Javier, Serruys Patrick. Coronary stenosis: imaging, structure and physiology. PCR Publishing, Europa Edition. 2010;219-32. [PubMed]
11. Tenekecioglu E, Albuquerque FN, Sotomi Y, Zeng Y, Suwannasom P, Tateishi H et al. Intracoronary optical coherence tomography: Clinical and research applications and intravascular imaging software overview. Catheter Cardiovasc Interv 2017;89(4):679-89. [CrossRef] [PubMed]
12. Kurogi K, Ishii M, Yamamoto N, Yamanaga K, Tsujita K. Optical coherence tomography-guided percutaneous coronary intervention: a review of current clinical applications. Cardiovasc Interv Ther. 2021; [CrossRef] [PubMed]
13. Iannaccone M, D'Ascenzo F, Templin C, Omedè P, Montefusco A, Guagliumi G, et al. Optical coherence tomography evaluation of intermediate-term healing of different stent types: systemic review and meta-analysis. Eur Heart J Cardiovasc Imaging 2017; 18(2): 159-66. [CrossRef] [PubMed]

Prikaz bolesnika **UDC: 616.12-089.819.5-073.7**
doi:10.5633/amm.2021.0209

OPTIMIZACIJA PERKUTANE KORONARNE INTERVENCIJE UZ POMOĆ OPTIČKE KOHERENTNE TOMOGRAFIJE

Zoran Perišić[1], Nenad Božinović[1], Mihajlo Lazarević[1]

[1]Univerzitetski klinički centar Niš, Kilinika za kardiovaskularne bolesti, Niš, Srbija

Kontakt: Zoran Perišić
Sterijina 12A, 18000 Niš, Srbija
E-mail: perisic.zoran@me.com

Optička koherentna tomografija (OCT) je metoda koja omogućava precizno sagledavanje koronarne morfologije. Upotrebom OCT može se procenjivati aterosklerotski plak, proceniti debljina fibrozne kape, a može se dobiti i precizna slika pozicije stenta u koronarnoj arteriji.

Žena stara 72 godine primljena je sa slikom akutnog infarkta sa elevacijom ST segmenta lateralne lokalizacije i odmah je upućena na primarnu perkutanu koronarnu intervenciju (PCI). Tokom procedure, pronađena je okludirana cirkumfleksna arterija, koja se otvara predilatacijom, nakon čega se postavljaju 2 stenta. Protok kroz stentove veoma je slab i registruju se znaci tromboze unutar stentova. Protok se ne popravlja ni posle postdilatacije i aspiracije tromba. Zbog naglog pogoršanja stanja bolesnika, prekida se procedura, a nastavlja se terapija tirofibanom, uz antikoagulantnu, antiagregacionu i drugu simptomatsku terapiju. Posle 7 dana, ponavlja se angiografija i upotrebljava se OCT sistem. Nađena je značajna apozicija stenta, gde su pojedini stratovi stenta i do 0,54 mm odmaknuti od zidova koronarne arterije. Urađena je postdilatacija većim balonom prema izmerenom dijametru arterije, nakon čega tromboza nestaje, a stratovi se pri ponovljenom OCT snimku nalaze uz zid koronarne arterije. Bolesnik se otpušta sa dvojnom antiagregacionom terapijom nakon par dana u dobrom opštem stanju.

OCT je relativno nova dijagnostička metoda, koja omogućava izuzetno lako i precizno identifikovanje nedovoljno ekspandiranih stentova u koronarnoj arteriji, što predstavlja glavni mehanički razlog tromboze posle PCI.

Acta Medica Medianae 2021;60(2):69-76.

Ključne reči: *optička koherentna tomografija, perkutana koronarna intervencija, apozicija stenta, koronarna tromboza*

Review article

UDC: 616.857:616-073.7
doi:10.5633/amm.2021.0210

BRAIN MAGNETIC RESONANCE SPECTROSCOPY IN MIGRAINE

Filip Petrović[1,2], Dragan Stojanov[1,2], Aleksandra Aracki Trenkić[1,2], Jovana Petrović[2,3], Marta Petrović[2], Sonja Janković[1,2]

Migraine is a common neurological disorder that is characterized by episodes of moderate to severe headache. Magnetic resonance spectroscopy (MRS) is a noninvasive method that enables *in vivo* studying of tissue metabolism by utilizing the magnetic properties of certain atomic nuclei, mainly hydrogen (1H) and phosphorous (31P).

1H-MRS is most commonly used to measure the concentration of gamma aminobutyric acid (GABA), glutamate, phosphocreatine (PCr), creatine, choline, N-acetylaspartate (NAA), myo-inositol, aspartate and lactate.

31P-MRS enables noninvasive *in vivo* measuring of concentration of compounds containing phosphorus nuclei. This allows the measurement of metabolites involved in brain energy metabolism including concentrations of phosphocreatine (PCr), inorganic phosphate, creatine, adenosine diphosphate (ADP) and adenosine triphosphate (ATP).

1H-MRS studies reported significant differences in levels of GABA, glutamate, lactate and NAA between migraine patients and controls, measured in various brain regions, while most of the studies found no significant differences in levels of myo-inositol, choline and total creatine.

The main consistent findings using 31P-MRS are concomitantly decreased PCr and increased inorganic phosphate, that is, a decreased PCr/inorganic phosphate ratio, as well as decreased magnesium measured in cortical regions of migraine patients.

For identifying a biomarker in migraine it is necessary for future MRS studies to obtain additional information of the ictal state in migraine as well as before and after interventions. Severity of the disease (disease duration and migraine attack frequency) has to be taken into account to detect possible correlation with MRS findings which also needs further research.

Acta Medica Medianae 2021;60(2):77-87.

Key words: *migraine, headache, magnetic resonance spectroscopy*

[1]Radiology Center, University Clinical Center Niš, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia
[3]"Gornja Toponica" Special Hospital for Psychiatric Diseases, Niš, Serbia

Contact: Filip Petrović
2/30 Dragiše Cvetkovića St., 18000 Niš, Serbia
E-mail: drfilippetrovic@gmail.com

Introduction

Migraine is a common neurological disorder that is characterized by episodes of moderate to severe headache. It is associated with autonomic symptoms and sometimes is preceded by aura in the form of transient neurological symptoms (1). It is considered that migraine affects subjects with an inherited alteration of brain excitability and that it is a neurovascular disorder with recurrent sensitization and activation of the trigemino-vascular pathways, as well as diencephalic nuclei and brain stem (1-3).

Imaging and neurophysiological studies indicate that chronic migraine can be correlated with functional and structural alterations in some brain regions, especially brainstem dysfunction and cortical hyperexcitability (4-6). Technological advances in neuroimaging have enabled the exploration of different aspects of cerebral metabolism in migraine patients, and complementary animal research indicates that there are possible links between trigeminovascular activation and metabolic factors in migraine pathophysiology (7).

The implementation of advanced magnetic resonance imaging (MRI), including magnetic resonance spectroscopy (MRS), represents a significant step forward in the understanding of the underlying mechanisms in migraine, giving insight into structural and functional brain alterations in migraine patients (1). Magnetic resonance spectroscopy (MRS) is a noninvasive method that enables *in vivo* studying of tissue metabolism by utilizing the magnetic properties of certain atomic nuclei, mainly hydrogen (1H) and phosphorous (31P). This technique

has been used for the past three decades to study brain metabolism in a variety of diseases, including migraine (8).

1H-MRS

1H-MRS is a neuroimaging technique that allows the separation of neurometabolites according to their chemical structure. Differentiation of spectra is possible through observation of the radiofrequency signal detected from hydrogen nuclei spins and their chemical environment when placed in a magnetic field (9). Neurometabolites can be differentiated along an x-axis depending on their radiofrequency that is chemical-specific, also termed chemical shift. Signal strength represents the level of neurometabolite (10).

1H-MRS is most commonly used to measure the concentration of gamma aminobutyric acid (GABA), glutamate, phosphocreatine (PCr), creatine, choline, N-acetylaspartate (NAA), myo-inositol, aspartate and lactate, depending on field strength and the exact sequence (8). The concentration of the measured metabolite can be reported as absolute concentration, quantified from measured water peak and assumptions of water concentration or as the relative ratio to the measured total creatine concentration in the spectrum (11, 12).

1H-MRS studies reported significant differences in levels of GABA, glutamate, lactate and NAA between migraine patients and controls, measured in various brain regions, while most of the studies found no significant differences in levels of myo-inositol, choline and total creatine.

Gamma aminobutyric acid (GABA)

Gamma aminobutyric acid (GABA) is a predominant inhibitory neurotransmitter in the central nervous system (13) and can serve as a potential biomarker for migraine (14). GABA has been implicated in neuronal disorders, such as pain, and the temporal modulation of neuronal excitability and it is widely distributed in the brain (15). Changes in GABA levels in the brain could result in pathophysiological events leading to migraine (16) as it is a crucial regulator of excitation and inhibition (17). In order to gain an understanding of migraine pathogenesis, it is essential to study migraine GABA levels (14).

Some studies of migraine with and without aura showed decreased GABA levels in the occipital lobe measured interictally (18, 19) which could be explained as increased susceptibility to excitatory inputs and/or reduction in the inhibitory mechanisms (8). However, one meta-analysis showed that the level of GABA in migraine was significantly increased compared with controls (10) which is more difficult to explain (19, 20). It is hypothesized that increased GABA levels reflect a homeostatic response to the increased glutamate through the GABA metabolic pathway (21) or that GABA has a protective role in suppressing headaches (19). Increased GABA levels may represent a pathophysiological migraine mechanism that has yet to be fully explained (10). For example, GABA may have a role in neurogenic inflammation in migraine (22) or control of vasodilation (23). GABA is generally thought to reflect "inhibitory tone" (24) but increased GABA may be a response to increased excitation (10). Studies have implicated polymorphisms in genes encoding for GABA receptor subunits in migraine (25). Reduced GABA-receptor activity may lead to hyperexcitability of both inhibitory and excitatory neurons and hence increased levels of neurotransmitters (10). Some studies indicate that drugs targeting GABAA or GABAB-receptor activity (26) could be used as a treatment for pain disorders, including migraine.

Glutamate

There is no consensus on the best way to test glutamate levels. Glutamate is present at higher concentrations (27) than GABA, but difficulties in distinguishing it from glutamine and glutathione (24) have been highlighted. Although some studies assess glutamate alone (28), others choose to estimate Glx, the combined measure of glutamate and glutamine, even though the signal also contains glutathione (10).

1H-MRS studies in migraine with and without aura reported increased levels of glutamate in the anterior paracingulate (29) and visual cortex (30), and during visual stimulation in migraine with aura in the visual cortex (18). Glutamate is the main excitatory neurotransmitter in the brain and is thought to be a central factor in the migraine brain hyperexcitability theory, which entails an imbalance in excitatory and/or inhibitory activity (16). This possibly enhances the excitability of the migraine brain, both by leading to the mechanism of cortical spreading depression in the migraine aura and the activation of trigeminovascular pain pathways (16, 31-33).

Cortical spreading depression is a process that is uniquely associated with transient neurological conditions such as epilepsy and migraine (34). Cortical spreading depression is characterized as a wave of excitation accompanied by inhibition that spreads through the brain. High levels of glutamate have been thought to trigger this process (34, 35). As the recorded increases in glutamate are measured interictally, migraine patients may exert persistently altered brain excitability and increased sensitivity to excitatory stimulation (8). The glutamate abnormalities are consistent with previous genetic findings of glutamate regulation and homeostasis abnormalities, likely involving the glutamate transporter-1 receptor (36, 37). Also, some studies reported increased glutamate levels in plasma and cerebrospinal fluid interictally (38).

Lactate

The inconsistency in patient selection criteria and methodologies in brain lactate level studies in migraine patients means that a firm conclusion cannot be drawn (39, 40). Brain lactate levels were elevated in patients with migraine with aura (41, 42) but not in those with migraine without aura (43-45). Occipital baseline lactate levels were increased in patients with a purely visual aura relative to healthy

controls but not in those with complex neurological auras (41). Lactate levels increased significantly during photic stimulation in patients with complex neurological auras but not in patients with a purely visual aura (41).

A significant consideration is that stimulus-induced rises in cortical lactate levels are physiological (46) and are explained by the astrocyte-to-neuron lactate shuttle (47), the process by which astrocytes supply neurons with energy when they become activated. The lack of a stimulus-induced increase in lactate levels in migraine patients may therefore be considered pathological, as it could make them vulnerable to an energy crisis, particularly because neuronal activation is likely to have a higher energy demand in migraine patients than in healthy individuals because their sensory information processing is abnormal (48). Research incorporating the quantification of lactate in the cortex and the electrophysiological monitoring of brain-evoked responses would be able to explain this relationship between function and metabolism (7).

N-acetylaspartate

N-acetylaspartate (NAA) is widely distributed in the central nervous system in both neurons and glia (49). It has a variety of functions, and it can be a potential marker for neuronal health as measured using MRS techniques (50). In the healthy brain, NAA is one of the highest peaks of the acquired MRS spectrum (51). The ratio between NAA and total creatine (NAA/Cre) was found to be clinically useful, as total creatine usually remains constant (52).

Studies reported decreased levels of NAA in the occipital lobe (53) and thalamus (45, 54) in migraine with and without aura and in the cerebellum of sporadic and familiar hemiplegic migraine (55, 56). Low NAA levels have been reported in the serum of migraine patients (57). No studies reported ictal findings using 1H-MRS.

Decreased NAA level is generally believed to indicate a neuronal loss (58) and impairments of energy metabolism decrease NAA levels in the brain (51). Migraine brain has been suggested to be hyperexcitable or to have alterations in migraine energetics due to possible mitochondrial dysfunction (51) and it has been proposed that the NAA decrease might indicate a subsided mitochondrial dysfunction if accepted that the synthesis is mitochondrial, thus contributing to the abnormal energy metabolism (58). In addition, one genetic study documented a higher prevalence of mitochondrial DNA mutations in migraine patients relative to controls, indicating a link between mitochondrial dysfunction and susceptibility to migraine (59).

31P-MRS

31P-MRS enables noninvasive *in vivo* measuring of concentration of compounds containing phosphorus nuclei. This allows for measurement of metabolites involved in brain energy metabolism including concentrations of phosphocreatine (PCr), inorganic phosphate, creatine, adenosine diphosphate (ADP) and adenosine triphosphate (ATP). Energy in the form of ATP is formed by oxidative phosphorylation under aerobic conditions. ATP is also generated with a higher synthesis rate by glycolysis under anaerobic conditions, resulting in concomitant lactate production and decreased intracellular pH. Intracellular pH is estimated from the chemical shift between PCr and inorganic phosphate in the 31P-MRS spectrum (60). Transfer of inorganic phosphate from PCr to ADP, by the creatine kinase, produces ATP and creatine (8).

The main consistent findings using 31P-MRS are concomitantly decreased PCr and increased inorganic phosphate, that is, a decreased PCr/inorganic phosphate ratio, measured in cortical regions of migraine patients with and without aura in both ictal and interictal conditions (61-64). In addition, four studies recorded decreased phosphorylation potential (61, 62, 65, 66), three of which additionally reported increased ADP and V/Vmax in migraine with (61, 62) and without aura (65). Overall, the results suggest that there is insufficient availability of free energy in the cell (67-69).

The use of 31P-MRS has shown that mitochondrial oxidative phosphorylation is impaired in the brain of migraine patients between (39, 61-63, 65, 70, 71) and during migraine attacks (64). This impairment is seen as decreased levels of organic phosphate, decreased phosphorylation potential and increased levels of ADP (7). Modified 31P-MRS technique was used to specifically measure the brain ATP, which was found to be decreased by 16% between attacks in patients with migraine without aura compared with healthy controls (66). Most severely affected patients had the lowest ATP concentrations, a result that coincides with other studies showing moderate associations between brain hypometabolism and attack frequency (66, 71, 72).

Consistently reported finding was also decreased magnesium in the ictal and interictal state in cortical regions in migraine with and without aura in cortical regions (62, 71, 73, 74). 31P-MRS studies of neural metabolism often measure magnesium because it is an essential cofactor for ATP production (7). These studies have shown that cytosolic free magnesium is reduced in the occipital lobes of patients with migraine, consistent with alterations in oxidative phosphorylation (62, 71, 74). Decreased serum magnesium levels have been shown to raise the chances of a migraine attack (75). Since magnesium is a cofactor in oxidative phosphorylation and stabilizes the mitochondrial membrane, magnesium level abnormalities can suggest a mitochondrial factor in migraine pathophysiology (76). These results, therefore, indicate reduced availability of neuronal energy and mitochondrial dysfunction in the migraine brain (8).

The possible mitochondrial dysfunction in migraine can be explained by a decrease in the number or efficiency of the mitochondria (66) or a decrease of mitochondrial enzymes (77). Q10 (78) and riboflavin (79) have shown efficacy as preventive migraine treatment, possibly by increasing the mitochondrial activity (8).

Table 1. 31P-magnetic resonance spectroscopy and 1H-magnetic resonance spectroscopy studies in migraine

Study	Participants	Type of MRS	Brain region	Scanner Strength (model, brand)	TR/TE (ms)	Results
Barbiroli et al. (82)	MpA (4) MS (4) C (15)	31P-MRS	Occipital	1.5 T (Signa, GE)	5000/NS	+ PCr/Pi + PCr/ATP = PME/ATP = PDE/ATP = pH
Barbiroli et al. (61)	MA (12) C (12)	31P-MRS	Occipital	1.5 T (Signa, GE)	5000/NS	- PCr + Pi - PP + ADP + V/Vmax - pHi = Magnesium
Lodi et al. (62)	MA (7) MpA (3) MbA (5) C (12)	31P-MRS	Occipital	1.5 T (Signa, GE)	5000/NS	- PCr + Pi - PP + ADP + V/Vmax - Magnesium + pHi
Lodi et al. (71)	MO (21) MA (37) MpA (13) C (36)	31P-MRS	Occipital	1.5 T (Signa, GE)	5000/NS	- Magnesium - DGATPhyd
Montagna et al. (65)	MO (22) C (18)	31P-MRS	Occipital	1.5 T (Signa, GE)	5000/NS	- PCr - PP + ADP + V/Vmax = Pi
Reyngoudt et al. (66)	MO (19) C (25)	31P-MRS	Occipital	3.0 T (Siemens)	4000/2.3	- PCr - PP - ATP = Pi = Magnesium = pHi
Uncini et al. (83)	FHM (2) MO (1) Family members (2) C (30)	31P-MRS	Occipital	1.5 T (Signa, GE)	5000/NS	- PCr + Pi - PP + ADP + V/Vmax = pH
Arngrim et al. (84)	MA (15) C (14)	1H-MRS	Occipital	3.0 T (Achieva, Philips) PRESS	5000/36.5	= Glutamate = Lactate = NAA = tCr
Bigal et al. (19)	MO (10) MA (9) C (9)	NS	Occipital	4.0 T (Inova, Varian)	NS	- GABA
Bridge et al. (18)	MA (13) C (13)	-MRS (MRS type not specified in paper, assumed 1H-MRS)	Occipital	3.0 T (Verio, Siemens)	4000/8.5	No visual stimulation: - GABA = Glutamate —————————— Visual stimulation: = GABA + Glutamate
Reyngoudt et al. (85)	MO (25) C (25)	1H-MRS	Occipital	3.0 T (TIM Trio, Siemens) PRESS	2000/30	= NAA = tCr = Choline = Myo-inositol
Reyngoudt et al. (43)	MO (20) C (20)	1H-MRS	Occipital	3.0 T (TIM Trio, Siemens)	2000/288	= Lactate/ tCr = Lactate/NAA = NAA/ tCr = Choline/ tCr
Sarchielli et al., 2005 (53)	MA (22) MO (22) C (10)	1H-MRS	Occipital	1.5 T (GEMS LX system)	2000/144	No visual stimulation: - NAA/Choline - NAA/ tCr = Choline = tCr —————————— Visual stimulation: - NAA/Choline - NAA/ tCr = Choline = tCr

Siniatchkin et al. (30)	MA (10) C (10)	1H-MRS	Occipital	3.0 T (Achieva, Philips) PRESS	2000/37	+ Glx = NAA
Watanabe et al. (42)	MA (3) MbA (1) MI (1) MpA/MI (1) C (6)	1H-MRS	Occipital	1.5 T (Signa, GE)	1500/270	+ Lactate/NAA = NAA/Choline = NAA/(Choline+ tCr)
Ramadan et al. (74)	MO (11) MA (8) C (25)	31P-MRS	Frontal Temporal Occipital	1.89 T (Bruker)	NS	- Magnesium = pHi
Welch et al. (86)	MO (12) MA (8) C (27)	31P-MRS	Frontal Occipital	1.89 T (Bruker)	NS	= pHi
Welch et al. (64)	MO (12) MA (8) C (27)	31P-MRS	Frontal Occipital	1.89 T (Bruker)	NS	Ictal: - PCr/Pi - PCr/TP + Pi/ TP = pHi ———————— Interictal: +Pi/TP = PCr/Pi = PCr/ TP = Pi/ TP = pH
Dichgans et al. (40, 55)	FHM1 (15) C (17)	1H-MRS	Parietal Occipital Cerebellum	1.5 T (Signa, GE)	2000/35	- Glutamate - NAA + Myo-inositol = tCr = Choline
González de la Aleja et al. (87)	MO (19) MA (8) C (19)	1H-MRS	Anterior paracingulate cortex Occipital	3.0 T (Signa, GE)	2000/28	Anterior paracingulate cortex: + Glutamate = Glutamine = Glutamate/Glutamine = tNAA = Choline ———————— Occipital lobe: + Glutamate/Glutamine = Glutamate = Glutamine = tNAA = Choline
Grimaldi et al. (88)	FHM2 (4) C (10)	1H-MRS	Parietooccipital Ventricles	1.5 T (Signa, GE)	4000/35 1500/288	= Lactate = NAA = Choline = Myo-inositol
Sándor et al. (41)	MA (5) FHM/SHM (5) C (11)	1H-MRS	Occipital + Tempoparietal	1.5 T (Gyroscan ACS-NT, Philips)	1500/288	+ Lactate = tCr = Choline
Zielman et al. (56)	SHM (10) FHM1 (5) FHM2 (3) C (19)	1H-MRS	Cerebellum Pons Occipital Hypothalamus	7.0 T (Achieva, Philips)	2000/21	- tNAA/ tCr = Glx/ tCr = Myo-inositol/ tCr = Choline/tCr
Becerra et al. (89)	MO (17) MA (15) C (33)	1H-MRS	Anterior cingulate cortex	3.0 T (TIM Trio, Siemens)	2000/31-229	= Glutamine = Glutamate = GABA = NAA = Aspartate = NAAG = Lactate = Myo-inositol
Prescot et al. (90)	MX (10) C (8)	1H-MRS	Anterior cingulate cortex Insula	4.0 T (Inova, Varian)	2000/30-260	= Glutamate = NAAG = Glutamine = Lactate = NAA = Choline
Aguila et al. (20)	MX (19) C (19)	1H-MRS	Posterior cingulate cortex	3.0 T (Discovery MR750, GE)	1800/68	+ GABAþ = Glx
Fayed et al. (91)	MX (33) C (183)	1H-MRS	Posterior cingulate gyrus	1.5 T (Signa, GE)	2000/35	= NAA = Glutamate = Glx = Myo-inositol = Choline

Boska et al. (92)	MO (19) MA (19) SHM (4) FHM (4) C (40)	31P-MRS	Anterior Posterior	3.0 T (Magnex)	1000/NS	Posterior region: - Magnesium (FHM+SHM) + PDE (MO) ———————— Anterior and posterior regions: = PCr = Magnesium = Pi = PME = pH
Schulz et al. (63)	MA (10) SHM+FHM (11) C (16)	31P-MRS	Temporoparietal	2.0 T (Bruker)	2500/NS	- PCr/P + Pi/ATP = PCr/ATP = pH
Lirng et al. (93)	MX (14) MX with depression (16)	1H-MRS	Dorsolateral prefrontal cortex	1.5 T (Signa, GE)	1500/35	+ Myo-inositol = NAA = Choline
Gu et al. (54)	MO (20) C (14)	1H-MRS	Thalamus, bilaterally	3.0 T (Signa, GE)	1000/144	- NAA/Choline = NAA/ tCr = Choline/ tCr
Mohamed et al. (45)	MO (22) C (10)	1H-MRS	Thalamus, bilaterally	1.5 T (Signa, GE)	1000/144 1000/35	- NAA/Choline - NAA/Cr + Myo-inositol /NAA + Lactate/NAA = Choline/Cr
Wang et al. (94)	CM (16) C (21)	1H-MRS	Hypothalamus, bilaterally	1.5 T (Signa, GE)	1500/144	= NAA = Choline
Schulz et al. (63)	MA (10) SHM+FHM (11) C (16)	1H-MRS	Basal ganglia	2.0 T (Bruker)	1500/135	= Lactate = NAA = Choline/Cr
Lai et al. (95)	EM (19) CM (53) C (16)	1H-MRS	Pons, dorsal rostral bilaterally PAG	1.5 T (Signa, GE)	1000/144	+ NAA = Choline
Macrì et al. (96)	MA (8) C (7)	1H-MRS	Cerebellum	1.5 T (Signa, GE)	1500/30	- Choline/NAA - Choline/ tCr = Choline/ tCr = tCr/NAA = Myo-inositol/NAA = Myo-inositol/ tCr = Myo-inositol/Choline
Stærmose et al. (97)	MA (14) C (16)	1H-MRS	Occipital, Somatosensory cortex	3.0 T (Magnetom Trio System, Siemens)	4000/8.50	= GABA = GABA/Cr+PCr (Total Creatinine) = GABA/NAA +NAAG(N-acetylaspartate + N-acetylaspartylglutamate)
Bell et al. (98)	PM (29) C (27)	1H-MRS	Thalamus, Sensorimotor cortex, Visual cortex	3.0 T (GE)	1800/80; 1800/35	= Glx = Glu = GABA
Bathel et al. (99)	M (15) C (15)	1H-MRS	Thalamus, Occipital	3.0 T (Achieva, Philips)	2000/30; 2000/68	+ Glx = GABA
Niddam et al. (100)	CM (25) EM (24) C (25)	1H-MRS	Anterior cingulate cortex, Occipital cortex, Thalamus	3.0 T (Trio, Siemens)	NS	- NAA

+: Significant increase when compared to controls; -: Significant decrease when compared to controls;
=: No significant difference when compared to controls; ADP, adenosine diphosphate; ATP, adenosine triphosphate;
C, controls; CM, chronic migraine; Cr, Creatine;
EM, episodic migraine; FHM, familiar hemiplegic migraine; FHM1, familiar hemiplegic migraine Type 1;
FHM2, familiar hemiplegic migraine Type 2;
GABA, g-aminobutyric acid; Glx, glutamate and glutamine;
MA, migraine with aura patients; MbA, basilar type migraine; MI, migraineous infarction; MO, Migraine without aura;
MpA, migraine with prolonged aura; MS, migraineous stroke;
PM pediatric migraine; MX, migraine type not reported; N, number; NAA, N-acetylaspartarte;
NAAG, N-acetyl aspartarylglutamate A; NS, not specified; PCr, phosphocreatine; PDE, phosphosdiesterase;
pHi, intracellular pH; Pi, inorganic phosphate; PME, phosphomonoesterase; PP, phosphorylation potential;
SHM, sporadic hemiplegic; tCr, creatine and phosphocreatine; TE, echo time;
tNAA, N-acetylaspartarte and N-acetyl aspartarylglutamate A;
TP, total phosphorous signal; TR, repetition time; V/Vmax, ATP-synthesis rate.

In a state of reduced available energy and mitochondrial dysfunction, it is expected that ATP would be synthesized at an increased rate under anaerobic conditions to meet the increased energy needs (8). This process is followed by an increase in lactate concentration and a decrease in intracellular pH (68, 80). Lactate increase may be caused by glutamate increase to protect against glutamate excitotoxicity (81). However, these findings were not consistently reproducible in either the 1H-MRS or the 31P-MRS studies (8). It remains to be determined if the mitochondrial migraine deficiency is primary or secondary. (1). The defect of oxidative energy metabolism represents the rationale for the use of metabolic enhancers (riboflavin, coenzyme Q10, magnesium and ketogenic diet) in migraine prevention (40).

Conclusion

The limited reproducible findings are partly explained by the different techniques used in the studies, often conducted below the magnetic field strength of 3.0 T, inhomogeneity of migraine cohorts and variation in studied brain areas. Despite of the variation between the MRS migraine studies over time, some results were reproducible and consistent. 1H-MRS studies reported significant differences in levels of GABA, glutamate, lactate and NAA between migraine patients and controls measured in various brain regions. The main consistent findings using 31P-MRS are concomitantly decreased PCr and increased inorganic phosphate as well as decreased magnesium measured in cortical regions of migraine patients. Most of the MRS studies investigated the interictal state of migraine patients. For identifying a biomarker in migraine it is necessary for future MRS studies to obtain additional information of the ictal state in migraine as well as before and after interventions. Also, there are no studies that have taken the severity of the disease (disease duration and migraine attack frequency) into account to detect possible correlation with MRS findings which also needs further research (8).

References

1. Ferroni P, Barbanti P, Spila A, Fratangeli F, Aurilia C, Fofi L, et al. Circulating Biomarkers in Migraine: New Opportunities for Precision Medicine. Curr Med Chem. 2018;26(34):6191-206. [CrossRef] [PubMed]
2. Bernstein C, Burstein R. Sensitization of the Trigeminovascular Pathway: Perspective and Implications to Migraine Pathophysiology Vascular Theory of Migraine- Extracranial Origin Vascular Theory of Migraine-Intracranial Origin. J Clin Neurol. 2012;8:89-99. [CrossRef] [PubMed]
3. Akerman S, Holland PR, Goadsby PJ. Diencephalic and brainstem mechanisms in migraine. Nat Rev Neurosci. 2011;12(10):570-84. [CrossRef] [PubMed]
4. Aurora SK, Barrodale PM, Tipton RL, Khodavirdi A. Brainstem dysfunction in chronic migraine as evidenced by neurophysiological and positron emission tomography studies. Headache. 2007;47(7):996-7. [CrossRef] [PubMed]
5. Lai T-H, Chou K-H, Fuh J-L, Lee P-L, Kung Y-C, Lin C-P, et al. Gray matter changes related to medication overuse in patients with chronic migraine. Cephalalgia. 2016 Dec;36(14):1324-33. [CrossRef] [PubMed]
6. Mathew NT. Pathophysiology of chronic migraine and mode of action of preventive medications. Headache. 2011;51 Suppl 2:84-92. [CrossRef] [PubMed]
7. Gross EC, Lisicki M, Fischer D, Sándor PS, Schoenen J. The metabolic face of migraine — from pathophysiology to treatment. Nat Rev Neurol. 2019;15(11): 627-43. [CrossRef] [PubMed]
8. Younis S, Hougaard A, Vestergaard MB, Larsson HBW, Ashina M. Migraine and magnetic resonance spectroscopy: A systematic review. Curr Opin Neurol. 2017; 30(3):246-62. [CrossRef] [PubMed]
9. Puts NAJ, Edden RAE. In vivo magnetic resonance spectroscopy of GABA: A methodological review. Prog Nucl Magn Reson Spectrosc. 2012;60:29-41. [CrossRef] [PubMed]
10. Peek AL, Rebbeck T, Puts NA, Watson J, Aguila MER, Leaver AM. Brain GABA and glutamate levels across pain conditions: A systematic literature review and meta-analysis of 1H-MRS studies using the MRS-Q

quality assessment tool. Neuroimage. 2020; 210 (January):116532. [CrossRef] [PubMed]
11. Jansen JFA, Backes WH, Nicolay K, Kooi ME. 1H MR spectroscopy of the brain: absolute quantification of metabolites. Radiology. 2006 Aug;240(2):318-32. [CrossRef] [PubMed]
12. Christiansen P, Henriksen O, Stubgaard M, Gideon P, Larsson HB. In vivo quantification of brain metabolites by 1H-MRS using water as an internal standard. Magn Reson Imaging. 1993;11(1):107-18. [CrossRef] [PubMed]
13. Enna SJ, McCarson KE. The role of GABA in the mediation and perception of pain. Adv Pharmacol. 2006;54:1-27. [CrossRef] [PubMed]
14. Li Q, Chen C, Gong T. High-field MRS study of GABA+ in patients with migraine: Response to levetiracetam treatment. Neuroreport. 2018;29(12):1007-10. [CrossRef] [PubMed]
15. Kupers R, Danielsen ER, Kehlet H, Christensen R, Thomsen C. Painful tonic heat stimulation induces GABA accumulation in the prefrontal cortex in man. Pain. 2009 Mar;142(1-2):89-93. [CrossRef] [PubMed]
16. Vecchia D, Pietrobon D. Migraine: a disorder of brain excitatory-inhibitory balance? Trends Neurosci. 2012 Aug;35(8):507-20. [CrossRef] [PubMed]
17. Mann EO, Paulsen O. Role of GABAergic inhibition in hippocampal network oscillations. Trends Neurosci. 2007 Jul;30(7):343-9. [CrossRef] [PubMed]
18. Bridge H, Stagg CJ, Near J, Lau C, Zisner A, Cader MZ. Altered neurochemical coupling in the occipital cortex in migraine with visual aura. Cephalalgia. 2015 Oct; 35(11):1025-30. [CrossRef] [PubMed]
19. Bigal ME, Hetherington H, Pan J, Tsang A, Grosberg B, Avdievich N, et al. Occipital levels of GABA are related to severe headaches in migraine. Neurology. 2008 May;70(22):2078-80. [CrossRef] [PubMed]
20. Aguila M-ER, Lagopoulos J, Leaver AM, Rebbeck T, Hübscher M, Brennan PC, et al. Elevated levels of GABA+ in migraine detected using (1) H-MRS. NMR Biomed. 2015 Jul;28(7):890-7. [CrossRef] [PubMed]
21. Pearl PL, Hartka TR, Cabalza JL, Taylor J, Gibson MK. Inherited disorders of GABA metabolism. Future Neurol. 2006 Sep;1(5):631-6. [CrossRef] [PubMed]
22. Palmer AM, Marion DW, Botscheller ML, Bowen DM, DeKosky ST. Increased transmitter amino acid concentration in human ventricular CSF after brain trauma. Neuroreport. 1994 Dec;6(1):153-6. [CrossRef] [PubMed]
23. Kocharyan A, Fernandes P, Tong X-K, Vaucher E, Hamel E. Specific subtypes of cortical GABA interneurons contribute to the neurovascular coupling response to basal forebrain stimulation. J Cereb blood flow Metab Off J Int Soc Cereb Blood Flow Metab. 2008 Feb;28(2):221-31. [CrossRef] [PubMed]
24. Rae CD. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. Neurochem Res. 2014 Jan;39(1):1-36. [CrossRef] [PubMed]
25. García-Martín E, Ramos MI, Cornejo-García JA, Galván S, Perkins JR, Rodríguez-Santos L, et al. Missense Gamma-Aminobutyric Acid Receptor Polymorphisms Are Associated with Reaction Time, Motor Time, and Ethanol Effects in Vivo. Vol. 12, Frontiers in Cellular Neuroscience. 2018. p. 10. [CrossRef] [PubMed]
26. Diener H-C, Charles A, Goadsby PJ, Holle D. New therapeutic approaches for the prevention and treatment of migraine. Lancet Neurol. 2015 Oct;14(10): 1010-22. [CrossRef] [PubMed]
27. Choi C, Coupland NJ, Bhardwaj PP, Kalra S, Casault CA, Reid K, et al. T2 measurement and quantification of glutamate in human brain *in vivo*. Magn Reson Med. 2006;56(5):971-7. [CrossRef] [PubMed]
28. Schubert F, Gallinat J, Seifert F, Rinneberg H. Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 Tesla. Neuroimage. 2004 Apr;21(4):1762-71. [CrossRef] [PubMed]
29. González De La Aleja J, Ramos A, Mato-Abad V, Martínez-Salio A, Hernández-Tamames JA, Molina JA, et al. Higher glutamate to glutamine ratios in occipital regions in women with migraine during the interictal state. Headache. 2013;53(2):365-75. [CrossRef] [PubMed]
30. Siniatchkin M, Sendacki M, Moeller F, Wolff S, Jansen O, Siebner H, et al. Abnormal changes of synaptic excitability in migraine with aura. Cereb Cortex. 2012 Oct;22(10):2207-16. [CrossRef] [PubMed]
31. Noseda R, Burstein R. Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neurological symptoms, cortical spreading depression, sensitization, and modulation of pain. Pain. 2013 Dec; 154 Suppl:S44-53. [CrossRef] [PubMed]
32. Lauritzen M, Hansen AJ. The effect of glutamate receptor blockade on anoxic depolarization and cortical spreading depression. J Cereb blood flow Metab Off J Int Soc Cereb Blood Flow Metab. 1992 Mar;12(2): 223-9. [CrossRef] [PubMed]
33. Oshinsky ML, Sanghvi MM, Maxwell CR, Gonzalez D, Spangenberg RJ, Cooper M, et al. Spontaneous trigeminal allodynia in rats: a model of primary headache. Headache. 2012 Oct;52(9):1336-49. [CrossRef] [PubMed]
34. Cozzolino O, Marchese M, Trovato F, Pracucci E, Ratto GM, Buzzi MG, et al. Understanding spreading depression from headache to sudden unexpected death. Front Neurol. 2018;9(FEB):1-13. [CrossRef] [PubMed]
35. Charles AC, Baca SM. Cortical spreading depression and migraine. Nat Rev Neurol. 2013 Nov;9(11):637-44. [CrossRef] [PubMed]
36. Chasman DI, Schürks M, Anttila V, de Vries B, Schminke U, Launer LJ, et al. Genome-wide association study reveals three susceptibility loci for common migraine in the general population. Nat Genet. 2011 Jun;43(7):695-8. [CrossRef] [PubMed]
37. Anttila V, Stefansson H, Kallela M, Todt U, Terwindt GM, Calafato MS, et al. Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. Nat Genet. 2010 Oct;42(10):869-73. [CrossRef] [PubMed]
38. van Dongen RM, Zielman R, Noga M, Dekkers OM, Hankemeier T, van den Maagdenberg AM, et al. Migraine biomarkers in cerebrospinal fluid: A systematic review and meta-analysis. Cephalalgia. 2017 Jan; 37(1):49-63. [CrossRef] [PubMed]
39. Reyngoudt H, Achten E, Paemeleire K. Magnetic resonance spectroscopy in migraine: what have we learned so far? Cephalalgia. 2012 Aug;32(11):845-59. [CrossRef] [PubMed]
40. Cevoli S, Favoni V, Cortelli P. Energy Metabolism Impairment in Migraine. Curr Med Chem. 2019; 26(34):6253-60. [CrossRef] [PubMed]
41. Sándor PS, Dydak U, Schoenen J, Kollias SS, Hess K, Boesiger P, et al. MR-spectroscopic imaging during visual stimulation in subgroups of migraine with aura. Cephalalgia. 2005 Jul;25(7):507-18. [CrossRef] [PubMed]
42. Watanabe H, Kuwabara T, Ohkubo M, Tsuji S, Yuasa T. Elevation of cerebral lactate detected by localized 1H-magnetic resonance spectroscopy in migraine during the interictal period. Neurology. 1996 Oct; 47(4):1093-5. [CrossRef] [PubMed]
43. Reyngoudt H, Paemeleire K, Dierickx A, Descamps B, Vandemaele P, De Deene Y, et al. Does visual cortex lactate increase following photic stimulation in mig-

raine without aura patients? A functional 1H-MRS study. J Headache Pain. 2011;12(3):295-302. [CrossRef] [PubMed]
44. Prescot A, Becerra L, Pendse G, Tully S, Jensen E, Hargreaves R, et al. Excitatory neurotransmitters in brain regions in interictal migraine patients. Mol Pain. 2009;5:1-11. [CrossRef] [PubMed]
45. Mohamed RE, Aboelsafa AA, Al-Malt AM. Interictal alterations of thalamic metabolic concentration ratios in migraine without aura detected by proton magnetic resonance spectroscopy. Egypt J Radiol Nucl Med. 2013;44(4):859-70. [CrossRef]
46. Sappey-Marinier D, Calabrese G, Fein G, Hugg JW, Biggins C, Weiner MW. Effect of photic stimulation on human visual cortex lactate and phosphates using 1H and 31P magnetic resonance spectroscopy. J Cereb blood flow Metab Off J Int Soc Cereb Blood Flow Metab. 1992 Jul;12(4):584-92. [CrossRef] [PubMed]
47. Magistretti PJ, Pellerin L. Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. Philos Trans R Soc London Ser B, Biol Sci. 1999 Jul;354(1387):1155-63. [CrossRef] [PubMed]
48. Gantenbein AR, Sandor PS, Fritschy J, Turner R, Goadsby PJ, Kaube H. Sensory information processing may be neuroenergetically more demanding in migraine patients. Neuroreport. 2013;24(4). [CrossRef] [PubMed]
49. Baslow MH. N-acetylaspartate in the vertebrate brain: metabolism and function. Neurochem Res. 2003 Jun; 28(6):941-53. [CrossRef] [PubMed]
50. Luyten PR, den Hollander JA. Observation of metabolites in the human brain by MR spectroscopy. Radiology. 1986 Dec;161(3):795-8. [CrossRef] [PubMed]
51. Becerra L, Veggeberg R, Prescot A, Jensen JE, Renshaw P, Scrivani S, et al. A "complex" of brain metabolites distinguish altered chemistry in the cingulate cortex of episodic migraine patients. NeuroImage Clin. 2016;11:588-94. [CrossRef] [PubMed]
52. Miller BL. A review of chemical issues in 1H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. NMR Biomed. 1991 Apr;4(2):47-52. [CrossRef] [PubMed]
53. Sarchielli P, Tarducci R, Presciutti O, Gobbi G, Pelliccioli GP, Stipa G, et al. Functional 1H-MRS findings in migraine patients with and without aura assessed interictally. Neuroimage. 2005 Feb;24(4):1025-31. [CrossRef] [PubMed]
54. Gu T, Ma X-X, Xu Y-H, Xiu J-J, Li C-F. Metabolite concentration ratios in thalami of patients with migraine and trigeminal neuralgia measured with 1H-MRS. Neurol Res. 2008 Apr;30(3):229-33. [CrossRef] [PubMed]
55. Dichgans M, Herzog J, Freilinger T, Wilke M, Auer DP. 1H-MRS alterations in the cerebellum of patients with familial hemiplegic migraine type 1. Neurology. 2005 Feb;64(4):608-13. [CrossRef] [PubMed]
56. Zielman R, Teeuwisse WM, Bakels F, Van der Grond J, Webb A, van Buchem MA, et al. Biochemical changes in the brain of hemiplegic migraine patients measured with 7 tesla 1H-MRS. Cephalalgia. 2014 Oct;34(12): 959-67. [CrossRef] [PubMed]
57. de Tommaso M, Ceci E, Pica C, Trojano M, Delussi M, Franco G, et al. Serum levels of N-acetyl-aspartate in migraine and tension-type headache. J Headache Pain. 2012 Jul;13(5):389-94. [CrossRef] [PubMed]
58. Clark JB. N-acetyl aspartate: a marker for neuronal loss or mitochondrial dysfunction. Dev Neurosci. 1998; 20(4-5):271-6. [CrossRef] [PubMed]
59. Mancuso M, Orsucci D, Angelini C, Bertini E, Carelli V, Comi G Pietro, et al. The m.3243A>G mitochondrial DNA mutation and related phenotypes. A matter of gender? J Neurol. 2014 Mar;261(3):504-10. [CrossRef] [PubMed]
60. Petroff OA, Prichard JW, Behar KL, Alger JR, den Hollander JA, Shulman RG. Cerebral intracellular pH by 31P nuclear magnetic resonance spectroscopy. Neurology. 1985 Jun;35(6):781-8. [CrossRef] [PubMed]
61. Barbiroli B, Montagna P, Cortelli P, Funicello R, Iotti S, Monari L, et al. Abnormal brain and muscle energy metabolism shown by 31P magnetic resonance spectroscopy in patients affected by migraine with aura. Neurology. 1992 Jun;42(6):1209-14. [CrossRef] [PubMed]
62. Lodi R, Montagna P, Soriani S, Iotti S, Arnaldi C, Cortelli P, et al. Deficit of brain and skeletal muscle bioenergetics and low brain magnesium in juvenile migraine: an *in vivo* 31P magnetic resonance spectroscopy interictal study. Pediatr Res. 1997 Dec;42(6): 866-71. [CrossRef] [PubMed]
63. Schulz UG, Blamire AM, Corkill RG, Davies P, Styles P, Rothwell PM. Association between cortical metabolite levels and clinical manifestations of migrainous aura: an MR-spectroscopy study. Brain. 2007 Dec; 130(Pt 12):3102-10. [CrossRef] [PubMed]
64. Welch KM, Levine SR, D'Andrea G, Schultz LR, Helpern JA. Preliminary observations on brain energy metabolism in migraine studied by *in vivo* phosphorus 31 NMR spectroscopy. Neurology. 1989 Apr;39(4): 538-41. [CrossRef] [PubMed]
65. Montagna P, Cortelli P, Monari L, Pierangeli G, Parchi P, Lodi R, et al. 31P-magnetic resonance spectroscopy in migraine without aura. Neurology. 1994 Apr;44(4): 666-9. [CrossRef] [PubMed]
66. Reyngoudt H, Paemeleire K, Descamps B, De Deene Y, Achten E. 31P-MRS demonstrates a reduction in high-energy phosphates in the occipital lobe of migraine without aura patients. Cephalalgia. 2011 Sep; 31(12):1243-53. [CrossRef] [PubMed]
67. Veech RL, Lawson JW, Cornell NW, Krebs HA. Cytosolic phosphorylation potential. J Biol Chem. 1979 Jul; 254(14):6538-47. [CrossRef] [PubMed]
68. Montagna P, Cortelli P, Barbiroli B. Magnetic resonance spectroscopy studies in migraine. Cephalalgia. 1994 Jun;14(3):184-93. [CrossRef] [PubMed]
69. Nioka S, Chance B, Hilberman M, Subramanian H V, Leigh JSJ, Veech RL, et al. Relationship between intracellular pH and energy metabolism in dog brain as measured by 31P-NMR. J Appl Physiol. 1987 May; 62(5):2094-102. [CrossRef] [PubMed]
70. Kim JH, Kim S, Suh S-I, Koh S-B, Park K-W, Oh K. Interictal metabolic changes in episodic migraine: a voxel-based FDG-PET study. Cephalalgia. 2010 Jan; 30(1):53-61. [CrossRef] [PubMed]
71. Lodi R, Iotti S, Cortelli P, Pierangeli G, Cevoli S, Clementi V, et al. Deficient energy metabolism is associated with low free magnesium in the brains of patients with migraine and cluster headache. Brain Res Bull. 2001 Mar;54(4):437-41. [CrossRef] [PubMed]
72. Lodi R, Kemp GJ, Montagna P, Pierangeli G, Cortelli P, Iotti S, et al. Quantitative analysis of skeletal muscle bioenergetics and proton efflux in migraine and cluster headache. J Neurol Sci. 1997 Feb;146(1):73-80. [CrossRef] [PubMed]
73. Boska MD, Welch KM, Barker PB, Nelson JA, Schultz L. Contrasts in cortical magnesium, phospholipid and energy metabolism between migraine syndromes. Headache J Head Face Pain. 2003 Apr 1;43(4):425. [CrossRef] [PubMed]

74. Ramadan NM, Halvorson H, Vande-Linde A, Levine SR, Helpern JA, Welch KM. Low brain magnesium in migraine. Headache. 1989 Oct;29(9):590-3. [CrossRef] [PubMed]
75. Assarzadegan F, Asgarzadeh S, Hatamabadi HR, Shahrami A, Tabatabaey A, Asgarzadeh M. Serum concentration of magnesium as an independent risk factor in migraine attacks: a matched case-control study and review of the literature. Int Clin Psychopharmacol. 2016 Sep;31(5):287-92. [CrossRef] [PubMed]
76. Welch KM, Ramadan NM. Mitochondria, magnesium and migraine. J Neurol Sci. 1995 Dec;134(1-2):9-14. [CrossRef] [PubMed]
77. Sangiorgi S, Mochi M, Riva R, Cortelli P, Monari L, Pierangeli G, et al. Abnormal platelet mitochondrial function in patients affected by migraine with and without aura. Cephalalgia. 1994 Feb;14(1):21-3. [CrossRef] [PubMed]
78. Sándor PS, Di Clemente L, Coppola G, Saenger U, Fumal A, Magis D, et al. Efficacy of coenzyme Q10 in migraine prophylaxis: a randomized controlled trial. Neurology. 2005 Feb;64(4):713-5. [CrossRef] [PubMed]
79. Schoenen J, Jacquy J, Lenaerts M. Effectiveness of high-dose riboflavin in migraine prophylaxis. A randomized controlled trial. Neurology. 1998 Feb;50(2): 466-70. [CrossRef] [PubMed]
80. Biermans W, Bakker A, Jacob W. Contact sites between inner and outer mitochondrial membrane: A dynamic microcompartment for creatine kinase activity. Biochim Biophys Acta - Bioenerg. 1990;1018(2): 225-8. [CrossRef] [PubMed]
81. Jourdain P, Allaman I, Rothenfusser K, Fiumelli H, Marquet P, Magistretti PJ. L-Lactate protects neurons against excitotoxicity: implication of an ATP-mediated signaling cascade. Sci Rep. 2016;6(1):21250. [CrossRef] [PubMed]
82. Barbiroli B, Montagna P, Cortelli P, Martinelli P, Sacquegna T, Zaniol P, et al. Complicated migraine studied by phosphorus magnetic resonance spectroscopy. Cephalalgia. 1990 Oct;10(5):263-72. [CrossRef] [PubMed]
83. Uncini A, Lodi R, Di Muzio A, Silvestri G, Servidei S, Lugaresi A, et al. Abnormal brain and muscle energy metabolism shown by 31P-MRS in familial hemiplegic migraine. J Neurol Sci. 1995 Apr;129(2):214-22. [CrossRef] [PubMed]
84. Arngrim N, Schytz HW, Britze J, Amin FM, Vestergaard MB, Hougaard A, et al. Migraine induced by hypoxia: an MRI spectroscopy and angiography study. Brain. 2016 Mar;139(Pt 3):723-37. [CrossRef] [PubMed]
85. Reyngoudt H, De Deene Y, Descamps B, Paemeleire K, Achten E. (1)H-MRS of brain metabolites in migraine without aura: absolute quantification using the phantom replacement technique. MAGMA. 2010 Sep; 23(4):227-41. [CrossRef] [PubMed]
86. Welch KM, Levine SR, D'Andrea G, Helpern JA. Brain pH in migraine: an *in vivo* phosphorus-31 magnetic resonance spectroscopy study. Cephalalgia. 1988 Dec; 8(4):273-7. [CrossRef] [PubMed]
87. González de la Aleja J, Ramos A, Mato-Abad V, Martínez-Salio A, Hernández-Tamames JA, Molina JA, et al. Higher glutamate to glutamine ratios in occipital regions in women with migraine during the interictal state. Headache. 2013 Feb;53(2):365-75. [CrossRef] [PubMed]
88. Grimaldi D, Tonon C, Cevoli S, Pierangeli G, Malucelli E, Rizzo G, et al. Clinical and neuroimaging evidence of interictal cerebellar dysfunction in FHM2. Cephalalgia. 2010 May;30(5):552-9. [CrossRef] [PubMed]
89. Becerra L, Veggeberg R, Prescot A, Jensen JE, Renshaw P, Scrivani S, et al. A "complex" of brain metabolites distinguish altered chemistry in the cingulate cortex of episodic migraine patients. NeuroImage Clin. 2016;11:588-94. [CrossRef] [PubMed]
90. Prescot A, Becerra L, Pendse G, Tully S, Jensen E, Hargreaves R, et al. Excitatory neurotransmitters in brain regions in interictal migraine patients. Mol Pain. 2009 Jun;5:34. [CrossRef] [PubMed]
91. Fayed N, Andrés E, Viguera L, Modrego PJ, Garcia-Campayo J. Higher glutamate+glutamine and reduction of N-acetylaspartate in posterior cingulate according to age range in patients with cognitive impairment and/or pain. Acad Radiol. 2014 Sep;21(9): 1211-7. [CrossRef] [PubMed]
92. Boska MD, Welch KMA, Barker PB, Nelson JA, Schultz L. Contrasts in cortical magnesium, phospholipid and energy metabolism between migraine syndromes. Neurology. 2002 Apr;58(8):1227-33. [CrossRef] [PubMed]
93. Lirng J-F, Chen H-C, Fuh J-L, Tsai C-F, Liang J-F, Wang S-J. Increased myo-inositol level in dorsolateral prefrontal cortex in migraine patients with major depression. Cephalalgia. 2015 Jul;35(8):702-9. [CrossRef] [PubMed]
94. Wang S-J, Lirng J-F, Fuh J-L, Chen J-J. Reduction in hypothalamic 1H-MRS metabolite ratios in patients with cluster headache. J Neurol Neurosurg Psychiatry. 2006 May;77(5):622-5. [CrossRef] [PubMed]
95. Lai T-H, Fuh J-L, Lirng J-F, Lin C-P, Wang S-J. Brainstem 1H-MR spectroscopy in episodic and chronic migraine. J Headache Pain. 2012 Nov;13(8):645-51. [CrossRef] [PubMed]
96. Macrì MA, Garreffa G, Giove F, Ambrosini A, Guardati M, Pierelli F, et al. Cerebellar metabolite alterations detected *in vivo* by proton MR spectroscopy. Magn Reson Imaging. 2003 Dec;21(10):1201-6. [CrossRef] [PubMed]
97. Stærmose TG, Knudsen MK, Kasch H, Blicher JU. Cortical GABA in migraine with aura -an ultrashort echo magnetic resonance spectroscopy study. J Headache Pain. 2019;20(1). [CrossRef] [PubMed]
98. Bell T, Stokoe M, Khaira A, Webb M, Noel M, Amoozegar F, et al. GABA and glutamate in pediatric migraine. Pain. 2021;162(1):300-8. [CrossRef] [PubMed]
99. Bathel A, Schweizer L, Stude P, Glaubitz B, Wulms N, Delice S, et al. Increased thalamic glutamate/glutamine levels in migraineurs. J Headache Pain. 2018;19(1). [CrossRef] [PubMed]
100. Niddam DM, Lai KL, Tsai SY, Lin YR, Chen WT, Fuh JL, et al. Neurochemical changes in the medial wall of the brain in chronic migraine. Brain. 2018;141(2): 377-90. [CrossRef] [PubMed]

Pregledni rad **UDC: 616.857:616-073.7**
doi:10.5633/amm.2021.0210

MAGNETNO REZONATNA SPEKTROSKOPIJA MOZGA U MIGRENI

Filip Petrović[1,2], Dragan Stojanov[1,2], Aleksandra Aracki Trenkić[1,2], Jovana Petrović[2,3], Marta Petrović[2], Sonja Janković[1,2]

[1]Centar za radiologiju, Univerzitetski klinički centar Niš, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[3]Specijalna bolnica za psihijatrijske bolesti „Gornja Toponica", Niš, Srbija

Kontakt: Filip Petrović
Dragiše Cvetkovića 2/30, 18000 Niš, Srbija
E-mail: drfilippetrovic@gmail.com

Migrena je čest neurološki poremećaj, koji se karakteriše epizodama umerene do teške glavobolje. Magnetno rezonatna spektroskopija (MRS) je neinvazivna metoda, koja omogućuva *in vivo* proučavanje metabolizma tkiva korišćenjem magnetnih karakteristika određenih anatomskih jezgara, pre svega vodonika (1H) i fosfora (31P).

1H-MRS najčešće se koristi za merenje koncentracije gama aminobuterne kiseline (GABA), glutamata, fosfokreatina (PCr), kreatina, holina, N-acetilaspartata (NAA), mioinozitola, aspartata i laktata.

31P-MRS omogućava neinvazivno *in-vivo* merenje koncentracije jedinjenja koja sadrže jezgra fosfora. Ovo omogućava merenje metabolita uključenih u moždani energetski metabolizam, uključujući koncentracije fosfokreatina (PCr), neorganskog fosfata, kreatina, adenozin-difosfata (ADP) i adenozin-trifosfata (ATP).

1H-MRS studije pokazale su signifikantne razlike u nivoima GABA, glutamata, laktata i NAA između bolesnika sa migrenom i bolesnika iz kontrolnih grupa, merenih u različitim regionima mozga, dok u većini studija nije pronađena signifikantna razlika u nivoima mioinozitola, holina i ukupnog kreatina.

Glavni konzistentni nalaz u 31P-MRS studijama je konkomitantno smanjenje PCr i povećanje nivoa neorganskog fosfata, odnosno povećanje PCr / neorganski fosfat odnosa, kao i smanjenje nivoa magnezijuma merenih u kortikalnim regionima mozga bolesnika sa migrenom.

Za identifikaciju biomarkera u migreni nephodno je da u budućim studijama budu pribavljene dodatne informacije o iktalnom stanju u migreni, kao i o stanju pre i posle terapije. Težina bolesti (trajanje bolesti i frekvencija migrenoznih napada) mora biti uzeta u obzir da bi se detektovala moguća korelacija sa MRS nalazima, što takođe zahteva dalje istraživanje.

Acta Medica Medianae 2021;60(2):77-87.

Ključne reči: *migrena, glavobolja, magnetno rezonatna spektroskopija*

Review article

UDC: 616.727.3-089
doi:10.5633/amm.2021.0211

ARTHROSCOPIC MANAGEMENT OF STIFF ELBOW

Ivan Micić[1,2], In Ho Jeon[3], Dong Ho Kang[4], Han Pyo Hong[5], Abdullah Baqays[6], Predrag Stojiljković[1,2], Hong Jun Jung[7]

Stiff elbow is a common clinical consequence in the elbow joint. Impairment in active daily living is noted in flexion-extension stiffness as well as rotational stiffness.

Elbow stiffness can be divided into 3 different types: intrinsic, extrinsic and mixed type. The intrinsic type is referred to as articular deficit and loss of conformity resulting from trauma, infection or arthritic changes of intra-articular origin. The extrinsic stiffness is caused by fibrotic changes or scar formation of surrounding muscles, ligaments or skin. Ectopic ossification is a typical example of the extrinsic type of stiff elbow. The mixed type is defined when both intrinsic and extrinsic causes are combined, and is most common among post-traumatic elbow stiffness.

Arthroscopic release of the anterior capsule for flexion contracture can be done in a safe way. In cases of severe joint contracture, however, it is safe to release and isolate the ulnar nerve with minimal incision before the arthroscopic capsular release; otherwise sudden gain of flexion after extensive release of the capsule may tether the ulnar nerve around posterior aspect of the medial epicondyle.

A recent trend of arthroscopy is to draw equal clinical outcomes compared to existing open surgery by incising minimally and starting early rehabilitation to lessen patients' pain and achieve better range of motion. But a thoughtful understanding of the elbow anatomy and skillful arthroscopic technique is essential to minimize neurovascular insult or other surgical complication caused by inexperience.

Acta Medica Medianae 2021;60(2):88-95.

Key words: *arthroscopy, stiff elbow*

[1]University of Niš, Faculty of Medicine, Niš, Serbia
[2]Clinic for Orthopaedic Surgery and Traumatology, University Clinical Center Niš, Niš, Serbia
[3]Asan Medical Center, Department of Orthopaedic Surgery, Seoul, Republic of Korea
[4]W Hospital, Institute for Hand and Microreconstructive surgery, Daegu, Republic of Korea
[5]Linker Hospital, Seoul, Republic of Korea
[6]King Saud Medical City, Riyadh, Saudi Arabia
[7]Sahmyook Medical Center, Department of Orthopedic Surgery, Seoul, Republic of Korea

Contact: Hong Jun Jung
82 Mangu-ro, Dongdaemun-gu, 02500 Seoul, Republic of Korea
E-mail: amcos.shoulder@gmail.com

Introduction

Stiff elbow is a common clinical consequence in the elbow joint. Impairment in active daily living is noted in flexion-extension stiffness as well as rotational stiffness. There are many potential causes, such as simple dislocation or fracture of the radial head or intra-articular distal humerus fracture. Elbow contracture can happen as the result of a complex fracture caused by high energy and usually associated with the surrounding muscles, collateral ligaments and joint capsule, and this has been indicated as the cause of post-traumatic joint stiffness or systemic diseases like hemophilia, osteoarthritis and rheumatoid arthritis (1).

The treatment of stiff elbow was classically done in open procedures, yet worsening of residual pain and stiffness, as well as infection are possible complications of open surgery. Arthroscopic procedures have advantages in minimizing damage to the surrounding soft tissues and relieving post-operative pain allowing the patients to start early mobilization and easy rehabilitation (2). Significant improvement of pain and elbow motion in patients who underwent arthroscopic treatment was observed in different studies (2-4). The trans-articular portal devised by Kim et al. is frequently used in elbow arthroscopy because it has a safer approach to the elbow joint, compared to others.

Diagnosis

There are different opinions about normal range of motion in elbow joint. Morrey from Mayo

clinic defined it as possible performance of more than 90% of daily activities with flexion-extension range of motion from 30 to 130 degrees and supination-pronation range of motion of 60 degrees (5).

Precise investigation regarding the cause of stiffness is a key to successful treatment. Elbow stiffness can be divided into 3 different types: intrinsic, extrinsic and mixed type. The intrinsic type is referred to as articular deficit and loss of conformity resulting from trauma, infection or arthritic changes of intra-articular origin. The extrinsic stiffness is caused by fibrotic changes or scar formation of the surrounding muscles, ligaments or skin. Ectopic ossification is a typical example of extrinsic type of stiff elbow. The mixed type is defined when both intrinsic and extrinsic causes are combined, and is most common among post-traumatic elbow stiffness.

Treatment

Conservative treatment

Most of the patients with a mild limitation of elbow motion showed good response to conservative treatment and satisfying clinical results were reported with aggressive rehabilitation in patients with mild flexion contracture, especially caused by long-term immobilization due to simple dislocation of the elbow. NSAIDS and intra-articular steroid injection are good in controlling patients' pain and improve their daily activities.

Arthroscopic surgery

Many authors have reported techniques of arthroscopic elbow surgery since the first introduction of arthroscopic capsular release of elbow in 1992 (6, 7). Anterior and posterior capsular release is applicable to post-traumatic patients with external type of stiff elbow. Arthroscopic release of anterior capsule for flexion contracture can be done in a safe way. In cases of severe joint contracture, however, it is safer to release and isolate the ulnar nerve with minimal incision before arthroscopic capsular release; otherwise sudden gain of flexion after extensive release of the capsule may tether the ulnar nerve around the posterior aspect of the medial epicondyle (8).

Although we generally use a 4.5 mm arthroscopic device, a 2.7 mm arthroscopic device for small joints can be used. The cannula is rarely used during elbow arthroscopy for its disadvantages in limiting the mobility of instruments. Prone position is the preferred position as it allows better access to the anterior and posterior capsular structures. However, other positions may be used such as the supine or lateral decubitus (1). Approach procedures for capsular release differ depending on the operator and the lesions affected, but the author prefer releasing anterior capsule first since neurological complications can occur more often in situation of visual disturbance in severe joint swelling. On the contrary, it is better to start with posterior capsular release when the ulnar nerve decompression is necessary. As contractures may alter the normal anatomy of the elbow, and the neurovascular structures may be displaced during insufflation (5). Moreover, it is important to precisely mark the anatomical structures on the skin in stiff elbow surgery, thus the medial/lateral epicondyle, radial head, ulnar nerve, intermuscular septum should be marked before joint influation (Figure 1).

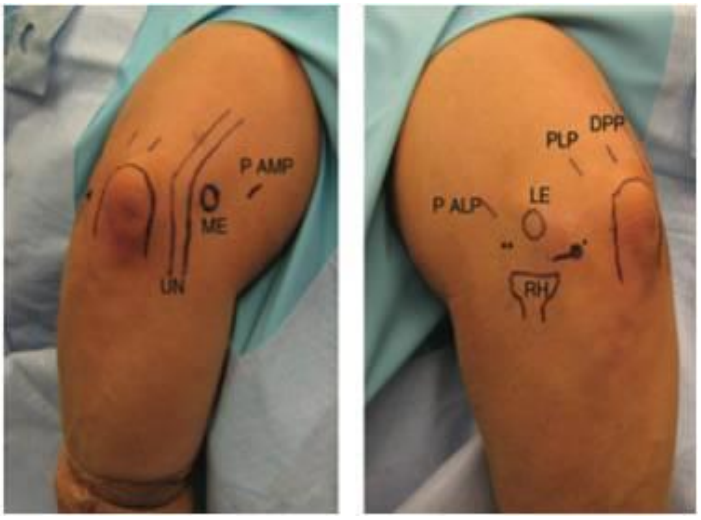


Figure 1. Exact skin marking is crucial in arthroscopic stiff elbow surgery.
The lateral epicondyle, medial epicondyle, radial head and ulnar nerve are drawn.
The arthroscopic portals are marked around the elbow joint.

Sometimes it is hard to insert instruments in to the joint, especially in severe stiff elbow. In such circumstances, the trans-articular portal devised by Kim et al. (9) is useful in inserting the device safely into the elbow joint. The entry point for the trans-articular approach is the intersecting point of horizontal line drawn from the capitellum to the olecranon and the sagittal line of the lateral margin of the olecranon. After the entry portal is made, a trocar is inserted and the space between radius-ulna-capitellum is carefully widen (Figure 2). Using this widened space, we can easily access to the anterior compartment.

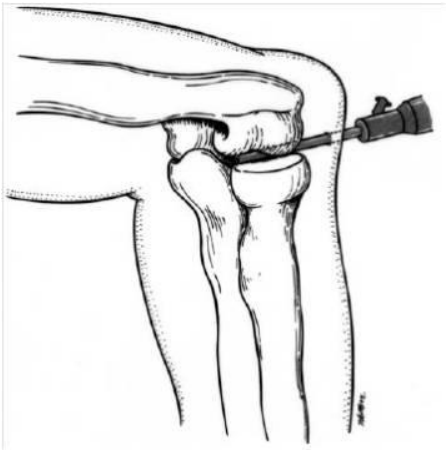


Figure 2. Approach to the anterior compartment of the elbow by using the trans-articular portal, devised by Kim et al.

The extra-articular approach is another method to reach in to the space between the fibrous joint capsule and the brachioradialis muscle, but there are more chances of nerve damage (10).

The proximal anterolateral portal (P-ALP) and anteromedial portal (P-AMP) are frequently used in procedures. The P-ALP is located 2 cm proximal, 1 cm anterior to lateral epicondyle, and is a safer portal in terms of ulnar nerve damage. It is used as an extra portal to insert traction device into the joint. An intra-articular traction device is especially helpful when scar formation is very severe or extensive bone resection is required to gain operating space and secure good visual field in the anterior compartment (Figure 3).

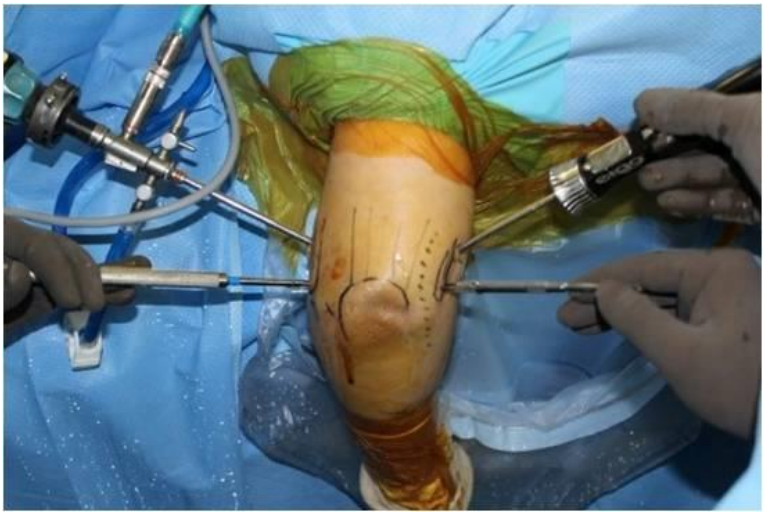


Figure 3. The anterolateral portal (ALP) can serve as an extra portal for intra-articular traction device. Traction device is put to use in extending the operating room in the stiff elbow surgery and maintaining good arthroscopic vision during surgery.

First, we insert the shaver to remove the loose body and synovium, then use burr to remove the radial head fossa and bony spurs. It is favorable to deal with a lesion around the anterior compartment before releasing the anterior capsule. The capsule should be released from the lateral side and proximal attachment of the distal humerus (Figure 4). Since the radial nerve runs between the brachialis and brachioradialis muscle, just 2-3 cm anterior to the radial head, scar formation and adhesion derived from the capsular contracture can deteriorate normal anatomical structure. Thus, extreme caution is required during the soft tissue dissection and release in order to avoid radial nerve damage.

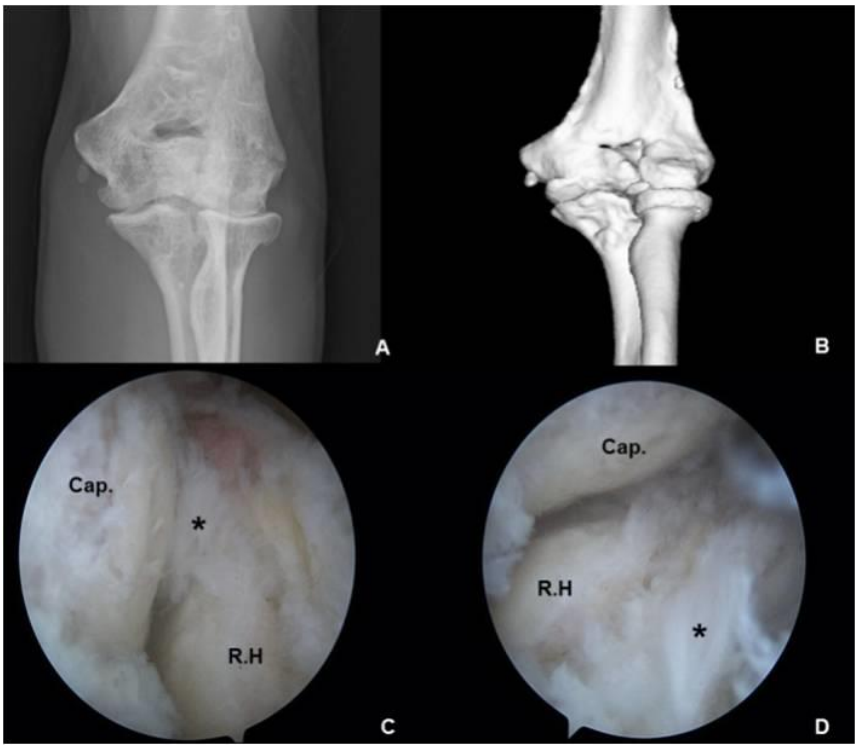


Figure 4. Radiologic and arthroscopic images of stiff elbow.
A. AP view X-ray image of stiff elbow.
B. 3D reconstruction image of affected elbow.
C-D. Arthroscopic image of the radiocapitellar joint.
The forearm should be supinated and pronated during operation to visually identify fibrotic scars (*) around radial head and severe fibrosis should be removed around the radiocapitellar joint, ulno-humeral joint, proximal radioulnar joint space. (Cap: capitellum, R.H: Radial head)

The posterior compartment is relatively safe from neurovascular insult compared to the anterior compartment. The posterior, posterolateral, and soft spot portals are commonly used in operation. Primarily, the posterolateral portal should be made at the lateral border of the triceps residing directly superior to the olecranon. Then, make the posterior portal by using spinal needle to insert the shaver. The posterior capsular release is done after obtaining clear vision, by removing fibrotic tissues and fat tissues with the shaver. Special attention should be paid on ulnar nerve damage during posteromedial capsular release and possibility of bony impingement during elbow flexion and extension as well (Figure 5).

The next step is to move the scope posteriorly to visualize the radiocapitellar joint and radio-ulnar joint and look for any rotational disability. If rotational abnormality is mainly due to abnormal articular surface of the proximal radius, radial head resection and removal of fibrotic scars can help to restore rotational stiffness.

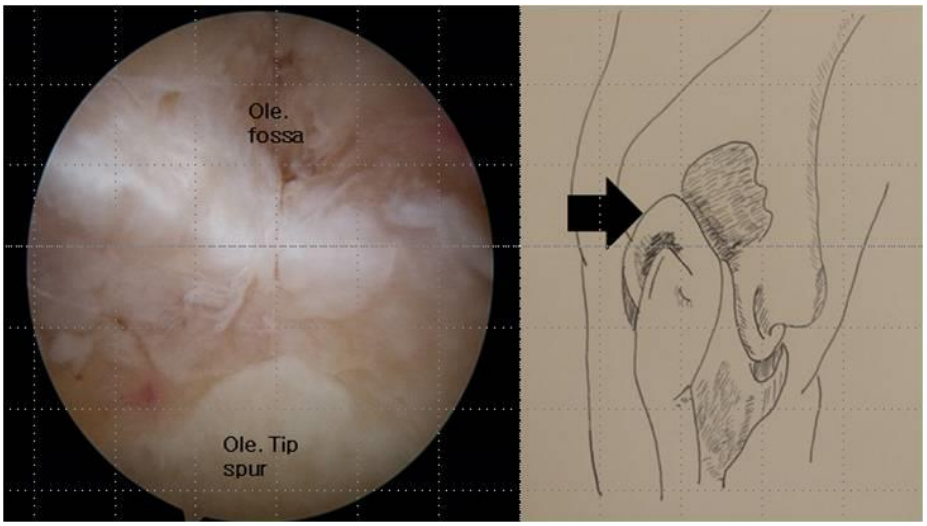


Figure 5. The posterior compartment of stiff elbow.
A. Arthroscopic image of the elbow joint.
Posterior bony impingement can be visualized by passive flexion and extension during surgery.
B. Schematic drawing of bony spurs at the tip of the olecranon.

Complication

Complication rate of arthroscopic stiff elbow surgery is relatively high among others. Infection rate is reported to be about 0.8-2%. Since the elbow joint is located just beneath the skin tissue, infection through the portals or fistula formation is more common and for this reason post-operative wound management is very crucial in elbow surgery. Furthermore, the nerve damage rate of around 2.5% is reported. Temporary nerve palsy was reported the most frequently (11). The risk factors are rheumatoid arthritis and contracture. However, permanent neurovascular injuries, hematomas or compartment syndrome were low or even neglected.

Rehabilitation

Post-operative immobilization is unnecessary in most cases. Early rehabilitation is started as early as within 3-4 postoperative days. In fact, the primary target of early rehabilitation is to lessen the pain of patients and to reduce swelling of the affected joint in order to start active exercise. We recommend repeating active assisted elbow ROM exercise 4-5 times a day, 5-10 minutes each (Figure 6).

Figure 6. Post-operative elbow rehabilitation.
Repeat active assisted elbow ROM exercise 4-5 times a day, 5-10 minutes each.

Clinical Results

Overall, clinical outcome after arthroscopic surgery of the elbow is favorable. Many of the reports included both the intrinsic and extrinsic type of stiff elbow showed good results in mild and moderate grade of stiff elbow without articular defect (Table 1). Savoie et al. (12) have reported 388 cases of arthroscopic capsular release of the stiff elbow. Clinical results showed the increase in the range of average elbow extension from -40 degrees to -5 degrees, while elbow ROM increased by 65 degrees. Overall 93% of the patients expressed satisfaction about the result of surgery.

Table 1. Outcomes of elbow arthroscopy in the treatment of elbow stiffness

Study	No. of patient	Surgery	Mean Follow-up in months (Range)
Nguyen et al.	22 (11 arthritic, 8 trauma related)	Capsulectomy/capsulotomy. Removal of loose body and osteophytes when needed	25 (12-47)
Lapner et al.	12 (stiffness following radial head fracture)	Debridement (n = 12) and capsular release	54 (12-120)
Ball et al.	14 (all post trauma)	Debridement and capsular release	(12-29)
Savoie et al. (12)	24 (all arthritic)	Debridement of osteophytes, capsular release, removal of radial head	32 (24-60)
Phillips and Strasburger	25 (10 arthritic)	Debridement	18 (6-34)
Kim et al.	25 (12 post-trauma)	Loose body removal, anterior capsule release, osteophyte removal, partial radial head resection	25 (12-46)
Timmerman and Andrews	19 (all post-trauma, 4 with moderate arthritic)	Debridement, capsular release and manipulation	29 (12-51)
Byrd	5 (type 1 radial head fracture)	Arthroscopic debridement	24 (12-41)
Jones and Savoie	12	Arthroscopic release	22 (15-32)

Conclusion

Stiff elbow is hard to treat effectively and most of the intrinsic and extrinsic causes bring about limitation of motion. A recent trend of arthroscopy is to draw equal clinical outcomes compared to existing open surgery by incising minimally and starting early rehabilitation to lessen patients' pain and achieve better range of motion (13). But a thoughtful understanding of the elbow anatomy and skillful arthroscopic technique is essential to minimize neurovascular insult or other surgical complication caused by inexperience.

Acknowledgements

This paper is related to research project "Virtual human osteoarticular system and Its application in preclinical and clinical practice", number III41017, Ministry of Education, Science and Technological Development of Republic of Serbia and Convergence technology development program for bionic arm through the National Research Foundation of Korea (NRF) funded by the Ministry of Science & ICT (No.2014M3C1B2048422).

References

1. Keener JD, Galatz LM. Arthroscopic management of the stiff elbow. J Am Acad Orthop Surg 2011;19:265-74. [CrossRef] [PubMed]
2. Tang HC, Xiang M, Chen H, Hu XC, Yang GY. Arthroscopic surgery for the treatment of stiff elbow. Zhongguo Gu Shang 2014;27:943-7. [PubMed]
3. Meluzinová P, Kopp L, Edelmann K, Obruba P, Avenarius J. Elbow arthroscopy in the surgical treatment of post-traumatic changes of the elbow joint. Acta Chir Orthop Traumatol Cech 2014;81:399-406. [PubMed]
4. Pederzini LA, Nicoletta F, Tosi M, Prandini M, Tripoli E, Cossio A. Elbow arthroscopy in stiff elbow. Knee Surg Sports Traumatol Arthrosc 2014;22:467-73. [CrossRef] [PubMed]
5. Morrey BF, Askew LJ, Chao EY. A biomechanical study of normal functional elbow motion. J Bone Joint Surg Am 1981;63:872-7. [CrossRef] [PubMed]
6. Jones GS, Savoie FH 3rd. Arthroscopic capsular release of flexion contractures (arthrofibrosis) of the elbow. Arthroscopy 1993;9:277-83. [CrossRef] [PubMed]
7. Van Zeeland NL, Yamaguchi K. Arthroscopic capsular release of the elbow. J Shoulder Elbow Surg 2010; 19:13-9. [CrossRef] [PubMed]
8. Jeon IH, Lee SM, Kim PT. Acute ulnar nerve palsy after outerbridge-kashiwagi procedure. J Hand Surg Eur 2007;32:596. [CrossRef] [PubMed]
9. Kim SJ, Jeong JH. Transarticular approach for elbow arthroscopy. Arthroscopy 2003;19:E37. [CrossRef] [PubMed]
10. Brooks-Hill AL, Regan WD. Extra-articular arthroscopic lateral elbow release. Arthroscopy 2008;24:483-5. [CrossRef] [PubMed]
11. Kelly EW, Morrey BF, O'Driscoll SW. Complications of elbow arthroscopy. J Bone Joint Surg Am 2001;83-A:25-34. [CrossRef] [PubMed]
12. Savoie FH 3rd, Nunley PD, Field LD. Arthroscopic management of the arthritic elbow: indications, technique, and results. J Shoulder Elbow Surg 1999;8: 214-9. [CrossRef] [PubMed]
13. Cohen AP, Redden JF, Stanley D. Treatment of osteoarthritis of the elbow: a comparison of open and arthroscopic debridement. Arthroscopy 2000;16:701-6. [CrossRef] [PubMed]

Pregledni rad **UDC: 616.727.3-089**
doi:10.5633/amm.2021.0211

ARTROSKOPSKO LEČENJE UKOČENOG LAKTA

Ivan Micić[1,2], In Ho Jeon[3], Dong Ho Kang[4], Han Pyo Hong[5], Abdullah Baqays[6], Predrag Stojiljković[1,2], Hong Jun Jung[7]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Klinika za ortopedsku hirurgiju i traumatologiju, Univerzitetski klinički centar Niš, Niš, Srbija
[3]Asan medicinski centar, Odeljenje za ortopedsku hirurgiju, Seul, Republika Koreja
[4]Bolnica W, Institut za ruku i mikrorekonstruktivnu hirurgiju, Daegu, Republika Koreja
[5]Bolnica Linker, Seul, Republika Koreja
[6]Medicinski grad Kralj Saud, Rijad, Saudijska Arabija
[7]Sahmiok medicinski centar, Odeljenje za ortopedsku hirurgiju, Seul, Republika Koreja

Kontakt: Hong Jun Jung
82 Mangu-ro, Dongdaemun-gu, 02500 Seul, Republika Koreja
E-mail: amcos.shoulder@gmail.com

Ukočen lakat je česta klinička posledica u zglobu lakta. Ukočenost u vidu savijanja i opružanja u laktu, kao i ukočenost rotacionih pokreta u laktu, u mnogome utiče na normalno obavljanje svakodnevnih aktivnosti.

Ukočenost lakta deli se na tri tipa: unutrašnji, spoljašnji i kombinovani tip.

Unutrašnji tip ukočenosti u laktu podrazumeva zglobni deficit, koji je rezultat traume, infekcije ili artrotičnih promena unutar zgloba.

Spoljašnji tip ukočenosti u laktu uzrokuju fibrozne ili ožiljne promene na okolnim ligamentima, mišićima ili koži oko lakta. Ektopični kalcifikati su tipičan primer spoljašnjeg tipa ukočenog lakta.

Kombinovani tip ukočenog lakta posledica je kombinacije unutrašnjih i spoljašnjih uzroka i najčešći je uzrok posttraumatske ukočenosti.

Artroskopsko osolobađanje prednje kapsule zgloba lakta za fleksionu kontrakturu bezbedana je hirurška tehnika. U slučajevima teške kontrakture, potrebno je osloboditi i izolovati ulnarni živac minimalnom kožnom incizijom pre artroskopske operacije, budući da bi dobijeni pokret fleksije u laktu, nakon opsežnog oslobađanja kapsule, mogao da dovede do natezanja ulnarnog živca oko zadnjeg dela medijalnog epikondila.

Najnoviji rezultati u lečenju ukočenosti lakta metodom artroskopije ukazuju na postizanje jednakih kliničkih rezultata u poređenju sa tradicionalnom otvorenom hirurgijom i omogućavanje rane rehabilitacije, kako bi se postigao što bolji obim pokreta. Za uspeh artroskopskog lečenja ukočenog lakta neophodni su odlično poznavanje anatomije i vešta artroskopska tehnika, radi minimalizovanja neurovaskularnih povreda ili drugih hirurških komplikacija uzrokovanih neiskustvom.

Acta Medica Medianae 2021;60(2):88-95.

Ključne reči: *artroskopija, ukočen lakat*

Review article

UDC: 616.155-07-08
doi:10.5633/amm.2021.0212

CONTEMPORARY APPROACH IN THE DIAGNOSIS AND MANAGEMENT OF PRIMARY MYELOFIBROSIS

Irena Ćojbašić[1,2]

Primary myelofibrosis (PMF) is an infrequent chronic myeloproliferative neoplasm. PMF is a result of clonal expansion of myeloid cells and is distinguished by the variable presence of mutations, morphologically by increased proliferation of megakaryocytes, progressive bone marrow fibrosis, hepatosplenomegaly, anemia, leukoerythroblastosis, with constitutional symptoms and shortened survival. World Health Organization defined the current diagnostic criteria for PMF in 2016, which involve a combined assessment of clinical, histological, mutational and laboratory features of diseases. Recently, a several new PMF prognostic scoring systems have started being used in the clinical practice, which are based solely on genetic markers or include clinical variables in addition to mutations and karyotype. In the treatment of myelofibrosis, risk adapted therapy has been applied, which implies the selection of the type of therapy according to the risk category obtained by calculating the valid prognostic scores. Allogenic stem cell transplant remained the only potentially curative therapy for PMF treatment but is suitable only for a small number of high risk patients who have a matching donor. In the last decade, the development and approval of ruxolitinib for the treatment of PMF has been of the greatest importance in the treatment of this disease, although it is a palliative therapy. Ruxolitinib is a potent JAK1/JAK2 inhibitor that leads to decreases in splenomegaly and symptoms and has prolonged overall survival in patients with this disease.

Acta Medica Medianae 2021;60(2):96-105.

Key words: *primary myelofibrosis, prognostic modeling, treatment*

[1]University of Niš, Faculty of Medicine, Niš, Serbia
[2]Clinic of Hematology and Clinical Immunology, University Clinical Centre Niš, Niš, Serbia

Contact: Irena Ćojbašić
48 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: icojbasic@gmail.com

Introduction

Myeloproliferative neoplasms (MPNs) are clonal diseases of hematopoietic stem cells distinguished by excessive production of terminally differentiated myeloid lineage cells. MPN are associated with clinical conditions that significantly shorten the overall survival and reduce the patient's quality of life (1). Myelofibrosis (MF) belongs to the group of BCR-ABL1 negative clonal myeloproliferative disorders. Myelofibrosis encompasses primary myelofibrosis (PMF), prefibrotic-myelofibrosis (PF-MF), and post-polycythaemia vera myelofibrosis (post PV-MF) or post-essential thrombocythaemia myelofibrosis (post ET-MF) that occur after PV and ET (2, 3). PMF is a heterogeneous disease, not only in terms of clinical and hematological manifestations, but also in terms of prognosis. It is characterized by megakaryocytes proliferation, reactive bone marrow fibrosis, peripheral blood leukoerythroblastosis, anemia, hepatosplenomegaly, and constitutional symptoms (4). MF belongs to a group of rare diseases that usually occur in elderly people, with an average survival of 6 years, which can vary from 1 year to more than 2 decades (5, 6).

The true cause of myelofibrosis is still unknown, but multiple pathogenetic mechanisms are considered responsible for the main features of the disease: genetic mutations, cytokine overproduction and stem cell-derived clonal myeloproliferation (7). In primary myelofibrosis somatic mutations are categorized into two groups: "driver" mutations that are associated with JAK-STAT hyperactivation, JAK2, MPL, and CALR in addition "other" mutations connected to epigenetic dysregulation in some. The existence of mutations in the JAK2 and MPL genes causes constitutive activation of the JAK2/STAT signaling pathway leading to increased production of myeloid and megakaryocyte progenitors. CALR gene encodes calreticulin, a significant role-playing protein in intracellular signaling, gene expression regulation, Ca2+ storage, apoptosis, cell adhesion and autoim-

mune response (8). Research has shown that in PMF, 45%-68% of patients are carriers of the JAK2 V617F mutation, MPL mutations occur in 5%-10% and CALR mutations hold 25%-35% of patients. It is assessed that around 9% of patients with PMF have no "driver" mutations, when the disease is referred to as "triple-negative" PMF, which is considered to be an indicator of a poor prognosis. Superior overall survival of CALR-mutated MF compared to JAK2-mutated or "triple-negative" patients has been reported in several studies (9, 10, 11). In a large Italian study by Rumi et al. (12), the median overall survival was longest in patients with a CALR mutation of 17.7 years, while the shortest survival of 3.2 years was found in triple-negative patients.

"Driver" mutations may be associated with "other" mutations whose effect on pathogenesis has not yet been fully elucidated like ASXL1, SRSF2, IDH1/2, EZH2, TET2, DNMT3A and CBL. It is widely accepted that "other" mutations have affected disease progression and leukemic transformation while "driver" mutations are all-important for the MPN phenotype. The ASX1, EZH2 and SRSF2 mutations have been related with shorter survival, and AXL1, SRSF2 and IDH1/2 mutations with increased risk of leukemic transformation compared with patients without mutations (9, 13). The previously mentioned mutations are included in the group of so-called High Molecular Risk (HMR) mutations, and it was found that 24%-35% of patients carry one mutation and 7%-9% carry at least 2 mutations (14). The presence of U2AF1Q157 mutation was shown to be correlated with shorter overall survival and anemia, but not with poor leukemia-free survival, in contrast to other high molecular risk mutations (15).

This article will address the impact of genetic mutations on the diagnosis and development of new prognostic models in patients with myelofibrosis. The application of risk adapted therapy and different current treatment options will also be analyzed.

Diagnosis

The latest classification of myeloid malignant diseases by the World Health Organization of 2016 recognizes two categories: acute myeloid leukemia and linked neoplasms and chronic myeloid neoplasms, with the latter category including MPN to which PMF belongs (16). World Health Organization defined the current criteria for PMF diagnosis in 2016 (17) which present a complex evaluation of clinical, histological, mutational and laboratory features as represented in Table 1.

Table 1. World health organization (WHO) 2016 revised diagnostic criteria for primary myelofibrosis

Primary myelofibrosis (prefibrotic)	Primary myelofibrosis (overtly fibrotic)
Major criteria 1. Typical megakaryocyte changes, an accompanied by ≤ grade 1 reticulin/collagen fibrosis 2. Not meeting the WHO criteria for other myeloid neoplasms 3. Presence of JAK2, CALR or MPL mutations, or presence of other clonal markers, or absence of evidence for reactive bone marrow fibrosis	**Major criteria** 1. Typical megakaryocyte changes, an accompanied by ≥ grade 2 reticulin/collagen fibrosis 2. Not meeting WHO criteria for other myeloid neoplasms 3. Presence of JAK2, CALR or MPL mutations, or presence of other clonal markers, or absence of evidence for reactive bone marrow fibrosis
Minor criteria a. Anemia not attributed to a comorbid condition b. Leukocytosis ≥ $11x10^9$/L c. Palpable splenomegaly d. Increased serum lactate dehydrogenase	**Minor criteria** a. Anemia not attributed to a comorbid condition b. Leukocytosis ≥ $11x10^9$/L c. Palpable splenomegaly d. Increased serum lactate dehydrogenase e. Leukoerythroblastosis
Diagnosis requires meeting all 3 major criteria and one minor criterion	Diagnosis requires meeting all 3 major criteria and one minor criterion

The difference from the previous WHO diagnostic criteria of 2008 is the definition of prefibrotic myelofibrosis as a new entity of the disease. Prefibrotic MF and ET remain entities that are frequently difficult to distinguish but this can be achieved using histomorphological findings and occurrence of minor clinical criteria (18). In addition, it is necessary to make a difference between prefibrotic and overtly

fibrotic PMF on the basis of clinical data of prodromal stages of PMF which are distinguished by mild anemia, minimal splenomegaly, absence of leukoerythroblastosis but with the presence of thrombocytosis and a morphological presence of fibrosis grade 0-1 (17, 19).

Prognostication in myelofibrosis

PMF prognostic models have been devised and introduced into clinical practice to suggest the most suitable therapy for each patient individually (20). The International Prognostic Scoring System (IPSS) has been in use since 2009 (5) and developed with the purpose of assessing the prognosis at the initial diagnosis time. IPSS uses five predictors for shortened survival: age > 65 years, hemoglobin < 10 g/dL, leukocyte count > 25×10^9/L, circulating blasts ≥ 1% and the presence of constitutional symptoms. Depending on the presence of unfavorable factors, four risk categories of low, intermediate-1, intermediate-2 and high were defined, which correlated with median survivals from 11.3 years to 2.3 years.

In the year 2010, the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) (6) established a dynamic prognostic model (DIPSS) based on the same five variables as IPSS, that may be used whenever during the disease and be helpful for treatment decision-making. In DIPSS for hemoglobin < 10 g/dL two negative points were awarded. In low risk patients the respective median of survival was not attained, while in high risk patients it was 1.5 years. Next year, the DIPSS-plus system additionally included an unfavorable karyotype presence that contained +8, -7/7q, i(17q), inv(3), -5/5q-, 12p- or an 11q23 rearrangement, transfusion dependency and thrombocytopenia. There are also four risk categories for DIPSS-plus, with appropriate median survivals from 15.4 years to 1.3 years (21).

During the year 2018, MIPSS70 (mutation-enhanced international prognostic scoring system for transplant-age patients), a newer prognostic model, was being utilized. It encompassed clinical features plus mutations and karyotype to be pertinent for transplant decision making in patients with PMF. Table 2 summarizes a few of the latest prognostic models in PMF.

Table 2. Novel prognostic models in myelofibrosis

Prognostic models	MIPSS70	MIPSS70+ version2.0	GIPSS	MPN personalized risk calculator
Criteria	Hb < 100 g/L (1 point) WCC > 25×10^9/L (2points) PB blasts ≥ 2% (1point) Constitutional Sx (1point) Plt < 100×10^9/L (2points) BM fibrosis Gr ≥ 2 (1point) Absence of *CALR* Type 1/like mutations (1point) HMR category (1point) ≥ 2HMRmutations (2points)	Severe anemia (2points) Moderate anemia (1point) PB blasts ≥ 2% (1point) Constitutional Sx (2points) VHR karyotype (4points) Unfavorable karyotype (3points) ≥ 2 HMR mutations (3points) One HMR mutation (2points) Type 1/like CALR absent (2 points)	VHR karyotype (2points) Unfavorable karyotype (1point) Type1/like CALAR absent (1point) ASXL1 mutation (1point) SRS2 mutation (1point) U2AF1Q157 mutation (1point)	Age at diagnosis Hb WCC Platelet count Gender Prior Thrombosis Splenomegaly *JAK2 V617F* *MPL* *CALR* *JAK2 Exon 12* Other mutation [a]
Risk groups (median survival)				
Very low Low Intermediate-1 Intermediate Intermediate-2 High Very high	0-1 point (27.7y) 2-4 points (7.1y) ≥ 5 points (2.3y)	0 point(not reached) 1-2 points (16.4y) 3-4 points (7.7y) 5-8 points (4.1y) ≥ 9 points (1.8y)	0 point (26.4y) 1 point (8.0y) 2 points (4.2y) ≥ 3 points (2y)	N/A as risk personalized and not grouped

MIPSS-Mutation enhanced international prognostic scoring system;
MIPSS70+ version 2.0: mutation and karyotype enhanced international prognostic system;
GIPSS-Genetic inspired prognostic scoring system; Hb-Hemoglobin; WCC-white cell count; PB-peripheral blood;
Sx-symptoms; HMR: high molecular risk mutations include ASXL1, SRSF2, EZH2, IDH1, IDH2 and, in addition, for GIPSS and MIPSS70+ version 2.0, U2AF1Q157;
VHR: very high risk karyotype. Severe anemia: Hemoglobin < 8 g/dL in women and < 9 g/dL in men. Moderate anemia: Hemoglobin 8-9.9 g/dL in women and 9-10.9 g/dL in men.

MIPSS70 includes nine variables, tree genetic and six clinical risk factors. MIPSS70 prognostic model has tree risk categories with appropriate median survival rates from 2.3 years to 27.7 years (22). MIPSS70+ prognostic model includes 7 independent variables, of which four aregenetic (CALR type 1/like mutation absence; HMR presence; more than 2 HMR presence; and "unfavorable" karyotype) and three are clinical risk factors (hemoglobin < 10 g/dl; circulating blasts ≥ 2%; and constitutional symptoms). There are four levels of risk categories in MIPSS70+, low (0-2 points), intermediate (3 points), high (4-6 points), and very high risk (≥ 7 points) with an approximated average survival of 20 years, 6.3 years, 3.9 years, and 1.7 years (23). A few months later, the same authors presented revised MIPSS70+ version 2.0 because they had recognized U2AF1Q157 as an added HMR mutation (23) and defined new hemoglobin thresholds accommodated for sex and severity (24), so this score includes 5 genetic and 4 clinical factors. MIPSS70+ version 2.0 considered five risk patient groups with significantly different median survival of 1.8 years to 16.4 years and "median not reached" (25).

GIPSS prognostic scoring system is based solely on genetic markers. GIPSS encompasses the following variables: "Very high risk" (VHR) karyotype (-7, i(17q), inv(3)/3q21, 12p-/12p11.2, 11q-/11q23,+21, or other autosomal trisomy's, not including +8/+9), "unfavorable" karyotype, absence of type 1/like CALR mutation and presence of ASXL1, SRSF2, or U2AF1Q157 mutation, as interindependent predictors for poor survival. GIPSS recognizes four categories of risk with different survival lengths from 26.4 years to 2 years (26).

Latterly, Grinfeld et al. (27) developed the "MPN personalized risk calculator" which predicts the clinical outcome for each individual patient based on the analysis of available clinical, laboratory and genomic characteristics of patients with myeloproliferative neoplasms. The authors linked disease characteristics and 69 myeloid cancer genes and created prognostic models that allow personal prediction of clinical outcome. This prognostic model showed superior performance compared to the prognostic models used until then. The combination of genetic and clinical characteristics enabled personalized prediction of clinical outcomes and may be helpful in choosing the type and intensity of therapy.

Risk-adapted therapy

During several years, risk adapted therapy was applied in the treatment of myelofibrosis, which implies that the selection of the type of therapy was made according to the risk category obtained by calculating the valid prognostic scores. In patients with determined genetic markers, the use of myelofibrosis treatment algorithm based on the revised MIPSS70+ version 2.0 prognostic scoring system and treatment algorithm based on GIPSS risk stratification is recommended (25, 26). Patients belonging to the high-risk group according to GIPSS correlate with the group of high-risk and very high-risk patients according to MIPSS70 + version 2.0. and involves the use of allogenic stem cell transplant (ASCT) in transplant eligible patients as the only ones potentially curative therapy and therapy that can prolong significant survival of patients with MF (28, 29, 30). A patient who is not a suitable candidate for ASCT due to advanced age and comorbidity or does not have a matching donor should be treated with conventional drugs, study drugs, radiotherapy, or splenectomy. Also, a parallel can be drawn between a group of low-risk patients according to GIPSS and low and very low risk according to MIPSS70+ version 2.0. For patients with MF who are asymptomatic, only regular monitoring of the disease is recommended. Patients belonging to the intermediate risk group according to MIPSS70+ version 2.0 are treated depending on whether they have symptoms that require management. These patients are treated by using conventional therapy based on treatment indications such as anemia, splenomegaly, constitutional symptoms, bone pain or extramedullary hematopoiesis. The prognosis of patients with GIPSS intermediate-1 and intermediate-2 is very diverse, so it requires additional evaluation of the risks by MIPSS70+ version 2.0 application and the treatment algorithm for intermediate risk patients (25, 28, 29).

JAK inhibitor therapies for myelofibrosis

Ruxolitinib

Discovery of the crucial function of dysregulation JAK-STAT signaling in pathophysiology of MF enabled the detection and development of new inhibitors for its treatment. Ruxolitinib directly acts on the basic mechanism of the disease, JAK2 dysregulation, blocks excessive stimulation of the JAK/STAT pathway leading to a decrease in STAT-3/5 activity and Akt/ERK phosphorylation which then causes a reduction of cell expansion and initiation of apoptosis (31). Since 2011, MF patients with intermediate-2 and high-risk disease have been able to be treated with ruxolitinib. It was the first approved JAK1/JAK2 inhibitor causing a reduction in the enlarged spleen and constitutional symptoms and prolonging overall survival in patients with MF. Furthermore, ruxolitinib can reduce hepatomegaly in splenectomized patients, relieves cachexia-related weight loss, and what is particularly significant, reduces the level of cytokines that lead to systemic inflammation in the MF. Although the response rate to ruxolitinib may vary significantly, most patients have benefited from its use. (32).

The COMFORT studies, which compared the effectiveness and toxicity of ruxolitinib versus placebo or best available therapy (BAT), were the most significant studies that demonstrated that ruxolitinib reduced spleen volume and disease-related symptoms, in addition to the prolonging of the overall survival of MF patients (33, 34). In the COMFORT-1 study that made comparison of the drug with placebo, it was shown that after 24 weeks of therapy, a decrease in splenomegaly of ≥ 35% was

achieved in 41.9% of patients treated with ruxolitinib vs < 1% for patients treated with placebo. In addition, a reduction in constitutional symptoms of at least 50% with regard to the baseline was demonstrated in 45.9% of patients in group with ruxolitinib compared to 5.3% in group with placebo, and what was particularly significant, this occurred regardless of risk group (33). The COMFORT-2 trial compared ruxolitinib with the best available therapy, showing that after a period of 48 weeks, splenomegaly was reduced by more than 35% in 28.5% of patients in the ruxolitinib group in comparison to 0% in the BAT group. It was also shown that the reduction in constitutional symptoms after 48 weeks was significantly higher in the ruxolitinib treated group of patients. (34). In both studies, the most common hematologic adverse events were ruxolitinib-related moderate to severe anemia and thrombocytopenia, which was corrected by dose adjustment, discontinuation of therapy and substitution therapy. With median follow-ups of approximately three years, the overall survival rate was significantly higher in the ruxolitinib group compared to placebo (35). Correspondingly, after three years of treatment, the estimated probability of survival was higher in the ruxolitinib group in comparison to the therapy considered as the best available, 81% versus 61% (36).

Other JAK inhibitors

Currently, three new JAK inhibitors are examined in phase III clinical trials in terms of their efficacy and safety compared to ruxolitinib. The JAKARTA-2 study (37) examined fedratinib, a JAK2-selective inhibitor, in patients with myelofibrosis who have shown intolerance or resistance to ruxolitinib. In the study group of patients with intermediate or high risk disease, 55% of patients achieved a reduction in spleen volume of more than 35%, while 26% achieved a reduction in disease-related symptoms by more than 50% after 6 months of therapy. In the analyzed group of patients, anemia and thrombocytopenia were the most reported side effects. The PERSIST-2 study (38) examined pacritinib, a JAK2 and Fms-like tyrosine kinase 3 inhibitor, comparing it with the best available therapy for myelofibrosis. In a patient with myelofibrosis and thrombocytopenia, pacritinib has been shown to be more effective in reducing spleen volume and constitutional symptoms compared to the best available therapy. The SIMPIFY-1 study (39) examined momelotinib, a potent and selective JAK1/2 inhibitor compared with ruxolitinib, in patients not previously treated with JAK1/JAK2 inhibitors. After 6 months of follow-up, momelotinib was not inferior to ruxolitinib in decreasing splenomegaly, however, the same did not apply for reduction of symptoms. In this study, patients treated with momelotinib were less transfusion dependent.

Non-JAK inhibitor therapies for myelofibrosis

Allogenic stem cell transplant

Up to the present moment, allogeneic stem cell transplant remained the only therapy that could potentially lead to cure in patients with myelofibrosis. This therapeutic option is applicable to a relatively small number of patients due to their advanced age, poor performance status, comorbidity, and donor availability. All available prognostic information should be used by calculating new prognostic models, such as MIPSS70 and MIPSS70+ version 2.0 which include mutation analysis and assess the clinical outcome and risk-benefit ratio for each patient individually. According to the valid consensus of European Society for Blood and Marrow Transplantation/European LeukemiaNet international working group (40) patients with intermediate-2 or high risk disease and age of less than 70 years are potential candidates to be treated with ASCT. To the contrary, patients with myelofibrosis who have intermediate-1 risk disease and age of less than 65 years may be candidates for treatment with ASCT if there is anemia requiring transfusion, or the presence of peripheral blasts > 2%, or unfavorable cytogenetics.

The study by Ballen K et al. (41) was one of the largest studies examining long-term outcome after the application of ASCT as a possible therapeutic line in PMF. The researchers showed that after 5 years of follow-up in matched related transplants, the progression-free survival rates and overall survival rates were 33% and 37%, while in unrelated transplants these rates were 27% and 30%. A recently published study by Tefferi A et al. (42) showed that after ASCT administration in patients with MF the median survival was almost 10 years, while the 5-year overall survival rate was 62%. This study proved that the very high risk mutations or unfavorable karyotype presence was not affecting survival. In order to predict the post-transplant outcome using multivariate analysis, it was determined that each risk variable that is an integral part of the DIPPS plus model has significance in predicting overall mortality, relapse-free survival, and non-relapse mortality rates (43). JAK inhibitors are now included in pre-ASCT therapy for many patients and their application has been shown to be safe, with no side effects on engrafment and long-term outcome (44).

Hydroxycarbamide

Hydroxycarbamide is a non-alkylating antiproliferative drug that has its application in the treatment of various hematological, oncological and infectious diseases. Before 2011, hydroxycarbamide

was often used in the treatment of myelofibrosis, but data on its efficacy and safety have been limited. Studies have shown that hydroxycarbamide was effective in reducing constitutional symptoms in 80% of patients and splenomegaly in 40% of patients. The average response duration to hydroxycarbamide is slightly longer than one year, although there may be large differences in the length of response (45). The most common side effects after this therapy were worsening of the anemia, the onset of severe pancytopenia and cutaneous complications such as oral or leg ulcers. Its toxicity is largely dose related, while its potential for leukemic transformation as a single agent is still a matter of controversy (46). According to the recommendations of European LeukemiaNet, resistance and intolerance to hydroxycarbamide in myelofibrosis is precisely defined as not achieving the desired reduction of splenomegaly, uncontrolled myeloproliferation, existence of cytopenias or appearance of signs of non-hematological toxicities (47).

Interferon-alpha

Interferon-alpha (IFN-α) has been shown to have potential to curb clonal myeloproliferation, may inhibit fibrogenic cytokines and angiogenesis in myelofibrosis, with the best results achieved at the onset of the disease. To date, this is the only treatment option in myelofibrosis that is used safely in pregnancy (48). Pegylated interferon therapy use in myelofibrosis leads to a satisfactory therapeutic response and a moderate toxic profile. Constitutional symptoms have been reported to disappear in 82% of patients while spleen size decreases in 46.5% of patients (49). Estimates of overall survival rates of patients having intermediate and high risk myelofibrosis treated with pegylated interferon were significantly higher compared with historical cohorts. Furthermore, the overall survival rate was found to be significantly connected with the pegylated interferon therapy duration (50). Recent studies have shown that new goal in the management of myelofibrosis is achieving minimal residual disease and potentially curing patients using a drug combinations, in which IFN-α predominantly and directly targets the malignant cells while anti-inflammatory agent such as JAK1/2 inhibitors that affect clonal expansion and disease progression (51).

Splenectomy

During disease, most patients with symptomatic splenomegaly become refractory to drugs and may require splenectomy. The results of one large study showed durable remissions after splenectomy with reduction of disease-related symptoms achieved in 67% of patients, transfusion-dependent anemia in 23% and portal hypertension in 50% of patients. In the same study, there was an acceptable operative mortality rate of 9% while the morbidity rate was 31% (52). Recently published Mayo Clinic's results confirmed that the median post splenectomy survival was 18 months and negative prognostic factors for survival were identified: age > 65 years, transfusion dependence, leukocytes > $25x10^9$/L and peripheral blasts ≥ 5% (53). Current guidelines suggest that splenectomy remains an acceptable palliative treatment option for patients having symptomatic splenomegaly that does not respond to therapy, development of splenic infarction, portal hypertension with complications, or severe hypercatabolic syndrome (54, 55).

Splenic irradiation

If patients with myelofibrosis are not acceptable candidates for splenectomy but need further treatment, splenic irradiation is an alternative option when there is massive symptomatic splenomegaly and an appropriate platelet count of more than $50x10^9$/l. The optimal dose and frequency of radiation has not been determined yet, but based on the results of different studies, the use of low-dose intermittent radiation is suggested. In most patients, a mild to moderate reduction in spleen volume is achieved after 6 months. It is considered that radiotherapy should not be used as a substitute for splenectomy (54).

Immunomodulatory drugs

Thalidomide, lenalidomide, and pomalidomide have anti-angiogenic, and immunomodulatory effects on several hematologic diseases including myelofibrosis (56). Examinations have confirmed that low-dose thalidomide or lenalidomide represents a productive treatment for myelofibrosis, because it enables the absence of transfusion dependence, increases the number of platelets and reduces the volume of the spleen in a certain number of patients (57, 58). Overall response rates to thalidomide and lenalidomide were 20% vs. 22% for anemia, 21% vs. 50% for thrombocytopenia, and 31% vs. 33% for splenomegaly, respectively (59, 60). However, their value is diminished by their capacity to cause peripheral neuropathy and myelosuppression. According to a study by Tefferi et al. (61), pomalidomide shows fewer toxic effects in contrast to thalidomide and lenalidomide and its therapeutic activity leads to delicate advancement between pomalidomide therapy with or without steroids and placebo in the treatment of myelofibrosis associated anemia.

Androgens

Severe anemia has been an important problem in patients with myelofibrosis. Danazol, a synthetic attenuated drug has proved to be useful in the cure of anemia in myelofibrosis. In patients with myelofibrosis, the mode of action of androgens is not fully clarified but is thought to lead to stimulation of bone marrow function. When danazol therapy was conducted at a dose of 600-800 mg per day for 3-6 months, it led to an overall response rate of 40% - 55% of patients and was generally well tolerated. In patients on danazol therapy, monitoring of liver function and periodic imaging of the liver are needed

for early detection of liver tumors in both sexes, while in men screening for prostate cancer should be done (62, 63).

Erythroid-stimulating agents

Human recombinant erythropoietin (EPO) represents an exogenous form of the kidney produced hormone that stimulates erythropoiesis. It has been proved successful in the treatment of MF-associated anemia. If EPO levels are < 500 IU, then EPO replacement therapy can be considered. The starting dose of EPO is 10,000 IU once a week, the dose can be escalated up to 40,000 IU once a week. Analysis of study Cervantes et al. (64) suggested that the overall response rate to human recombinant erythropoietin was 45%-55%, while serum erythropoietin levels < 125U/l, higher hemoglobin concentration, and transfusion independence were associated with a favorable response to human recombinant erythropoietin.

Conclusion

A state-of-the-art approach to patients with primary myelofibrosis is the determination of genetic markers, which play a significant role in diagnosis, prognostic modeling, and treatment decision. Genetic markers have become an integral part of WHO diagnostic criteria and because of their ability to predict survival rates somewhat accurately they have entered new prognostic scoring systems. As of recently, risk adapted therapy has been applied as well as genetic prediction of treatment response. Since most patients with primary myelofibrosis die from this disease, one should strive for more personalized treatments based on genetic markers with the development of more efficient therapies or combinations of therapies that will lead to molecular remission and prolonged overall survival.

References

1. Levine RL, Gilliland DG. Myeloproliferative disorders. Blood 2008;112(6):2190-8. [CrossRef] [PubMed]
2. Tefferi A, Cervantes F, Mesa R, Passamonti F, Verstovsek S, Vennucchi A, et al. Revised response criteria for myelofibrosis: International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) and European LeukemiaNet (ELN) consensus report. Blood 2013;122:1395-8. [CrossRef] [PubMed]
3. Mudireddy M, Shah S, Lasho T, Baracco D, Hanson C, Ketterling R, et al. Prefibrotic versus overtly fibrotic primary myelofibrosis: clinical, cytogenetic, molecular, and prognostic comparisons. Br J Haematol 2017; 182(4):594-7. [CrossRef] [PubMed]
4. Tefferi A. Myelofibrosis with myeloid metaplasia. N Engl J Med 2000;342:1255-65. [CrossRef] [PubMed]
5. Cervantes F, Dupriez B, Pereira A, Passamonti F, Reilly JT, Morra E, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. Blood 2009;113:2895-901. [CrossRef] [PubMed]
6. Passamonti F, Cervantes F, Vannucchi AM, Morra E, Rumi E, Pereira A, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). Blood 2010;115:1703-8. [CrossRef] [PubMed]

7. Tefferi A. Primary myelofibrosis: 2013 update on diagnosis, risk-stratification, and management. Am J Hematol 2013;88:141-50. [CrossRef] [PubMed]
8. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. J Clin Oncol 2005;23:8520-30. [CrossRef] [PubMed]
9. Vannucchi AM, Lasho TL, Guglielmelli P, Biamonte F, Pardanani A, Pereiraet A, et al. Mutations and prognosis in primary myelofibrosis. Leukemia 2013;27: 1861-9. [CrossRef] [PubMed]
10. Tefferi A, Lasho TL, Finke CM, Knudson RA, Ketterling R, Hanson CH, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic, and molecular comparisons. Leukemia 2014;28:1472-7. [CrossRef] [PubMed]
11. Tefferi A, Guglielmelli P, Larson DR, Finke C, Wassie EA, Pieri L, et al. Long-term survival, and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. Blood 2014;124:2507-13. [CrossRef] [PubMed]
12. Rumi E, Pietra D, Pascutto C, Guglielmelli P, Martinez-Trillos A, Casetti I, et al. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. Blood 2014;124:1062-9. [CrossRef] [PubMed]
13. Brecqueville M, Rey J, Bertucci F, Coppin E, Finetti P, Carbuccia N, et al. Mutation analysis of ASXL1, CBL, DNMT3A, IDH1, IDH2, JAK2, MPL, NF1, SF3B1, SUZ12, and TET2 in myeloproliferative neoplasms. Genes Chromosomes Cancer 2012;51:743-55. [CrossRef] [PubMed]
14. Guglielmelli P, Lasho TL, Rotunno G, Score J, Mannarelli C, Pancrazzi A, et al. The number of prognostically detrimental mutations and prognosis in primary myelofibrosis: an international study of 797 patients. Leukemia 2014;28:1804-10. [CrossRef] [PubMed]
15. Tefferi A, Finke CM, Lasho TL, Wassie EA, Knudson R, Ketterling RP, et al. U2AF1 mutations in primary myelofibrosis are strongly associated with anemia and thrombocytopenia despite clustering with JAK2V617F and normal karyotype. Leukemia 2014;28:431-3. [CrossRef] [PubMed]
16. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz M, Le Beau M, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391-405. [CrossRef] [PubMed]
17. Barbui T, Thiele J, Gisslinger H, Kvasnicka H, Vannucchi A, Guglielmelli P, et al. The 2016 WHO classification and diagnosis criteria for myeloproliferative neoplasms document summery and depth discussion. Blood Cancer J 2018;15:1-11. [CrossRef] [PubMed]
18. Jeryczynski G, Thiele J, Gisslinger B, Wolfler A, Schalling M, Gleis A, et al. Prefibrotic/early primary myelofibrosis vs. WHO-defined essential thrombocythemia: the impact of minor clinical diagnostic criteria on the outcome of the disease. Am J Hematol 2017; 92:885-91. [CrossRef] [PubMed]
19. Kvasnicka HM, Thiele J. Prodromal myeloproliferative neoplasms: the 2008 WHO classification. Am J Hematol 2010;85:62-96. [CrossRef] [PubMed]
20. Grinfeld J. Prognostic models in the myeloproliferative neoplasms. Blood Reviews 2020;42. [CrossRef] [PubMed]
21. Gangat N, Caramazza D, Vaidya R, George G, Begna K, Schwager S, et al. DIPSS plus: a refined dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. J Clin Oncol 2011;29:392-7. [CrossRef] [PubMed]
22. Guglielmelli P, Lasho TL, Rotunno G, Mudireddy M, Mannarelli C, Nicolosi M, et al. MIPSS70: mutation-enhanced international prognostic score system for transplantation-age patients with primary myelofibrosis. J Clin Oncol 2018;36:310-8. [CrossRef] [PubMed]
23. Tefferi A, Finke CM, Lasho TL, Hanson CA, Ketterling RP, Gangat N, et al. U2AF1 mutation types in primary myelofibrosis: phenotypic and prognostic distinctions. Leukemia 2018;32:2274-8. [CrossRef] [PubMed]
24. Nicolosi M, Mudireddy M, Lasho TL, Hanson C, Ketterling R, Gangatet N, et al. Sex and degree of severity influence the prognostic impact of anemia in primary myelofibrosis: analysis based on 1109 consecutive patients. Leukemia 2018;32:1254-8. [CrossRef] [PubMed]
25. Tefferi A, Guglielmelli P, Lasho TL, Gangat N, Ketterling RP, Pardanani A, et al. MIPSS70+ version 2.0: mutation and karyotype-enhanced international prognostic scoring system for primary myelofibrosis. J Clin Oncol 2018;36:1769-70. [CrossRef] [PubMed]
26. Tefferi A, Guglielmelli P, Nicolosi M, Mannelli F, Mudireddy M, Bartalucci N, et al. GIPSS: genetically inspired prognostic scoring system for primary myelofibrosis. Leukemia 2018;32:1631-42. [CrossRef] [PubMed]
27. Grinfeld J, Nangalia J, Baxter J, Wedge D, Angelopoulos N, Cantrill R, et al. Classification and personalized prognosis in myeloproliferative neoplasms. NEJM 2018;379:1416-30. [CrossRef] [PubMed]
28. Tefferi A, Guglielmelli P, Pardanani A, Vannucchi A. Myelofibrosis treatment algoritham 2018. Blood Cancer Journal 2018; 72:1-7. [CrossRef] [PubMed]
29. Tefferi A. Primary myelofibrosis: 2019 update on diagnosis, risk-stratification, and management. Am J Hematol 2018;93(12):1551-60. [CrossRef] [PubMed]
30. Tefferi A, Partain DK, Palmer JM, Slack JL, Roy V, Hogan WJ, et al. Allogeneic hematopoietic stem cell transplant overcomes the adverse survival effect of very high risk and unfavorable karyotype in myelofibrosis. Am J Hematol 2018;93:649-54. [CrossRef] [PubMed]
31. Tefferi A. JAK inhibitors for myeloproliferative neoplasms: clarifying facts from myths. Blood 2012;119: 2721-30. [CrossRef] [PubMed]
32. Verstovsek S, Kantarjian H, Mesa RA, Pardanani A, Cortes-Franco J, Thomas D, et al. Safety and efficacy of INCB018424, a JAK1 and JAk2 inhibitor, in myelofibrosis. N Engl J Med 2010;363(12):1117-27. [CrossRef] [PubMed]
33. Verstovsek S, Mesa RA, Gotlib J, Levy R, Gupta V, DiPersio J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med 2012;366:799-807. [CrossRef] [PubMed]
34. Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med 2012;366:787-98. [CrossRef] [PubMed]
35. Verstovsek S, Mesa R, Gotlib J, Levy R, Gupta V, DiPersio J, et al. Efficacy, safety, and survival with ruxolitinib in patients with myelofibrosis: results of a median 3-year follow-up of COMFORT-I. Haematologica 2015;100:479-88. [CrossRef] [PubMed]
36. Cervantes F, Vannucchi AM, Kiladjian JJ, Al-Ali HK, Sirulnik A, Stalbovskaya V, et al. Three-year efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for myelofibrosis. Blood 2013;122:4047-53. [CrossRef] [PubMed]
37. Harrison CN, Schaap N, Vannucchi AM, Kiladjian JJ, Tiu RV, Zachee P, et al. Janus kinase-2 inhibitor

fedratinib in patients with myelofibrosis previously treated with ruxolitinib (JAKARTA-2): a single-arm, open-label, non-randomised, phase 2, multicentre study. Lancet Haematol 2017;4:317-24. [CrossRef] [PubMed]
38. Mascarenhas J, Hoffman R, Talpaz M, Gerds AT, Stein B, Gupta V, et al. Pacritinib vs best available therapy, including ruxolitinib, in patients with myelofibrosis. JAMA Oncol 2018;e175818. [CrossRef] [PubMed]
39. Mesa RA, Kiladjian JJ, Catalano JV, Devos T, Egyed M, Hellmann A, et al. SIMPLIFY-1: a phase III randomized trial of momelotinib versus ruxolitinib in janus kinase inhibitor-naive patients with myelofibro-sis. J Clin Oncol 2017;35:3844-50. [CrossRef] [PubMed]
40. Kroger NM, Deeg JH, Olavarria E, Niederwieser D, Bacigalupo A, Barbui T, et al. Indication and management of allogeneic stem cell transplantation in primary myelofibrosis: a consensus process by an EBMT/ELN international working group. Leukemia 2015;29:2126-33. [CrossRef] [PubMed]
41. Ballen KK, Shrestha S, Sobocinski KA, Zhang MJ, Bashey A, Bolwell BJ, et al. Outcome of transplantation for myelofibrosis. Biol Blood Marrow Transplant 2010;16:358-67. [CrossRef] [PubMed]
42. Tefferi A, Partain DK, Palmer JM, Slack J, Roy V, Hogan W, et al. Allogeneic hematopoietic stem cell transplant overcomes the adverse survival effect of very high risk and unfavorable karyotype in myelofibrosis. Am J Hematol 2018;93:649-54. [CrossRef] [PubMed]
43. Samuelson BT, Salit RB, Storer BE, Stevens E, Wu D, Yeunget C, et al. Hematopoietic cell transplantation for myelofibrosis: the dynamic international prognostic scoring system plus risk predicts post-transplant outcomes. Biol Blood Marrow Transplant 2018;24:386-92. [CrossRef] [PubMed]
44. McLornan DP, Yakoub-Agha I, Robin M, Chalandon Y, Harrison CN, Kroger N. State-of-the-art review: allogeneic stem cell transplantation for myelofibrosis in 2019. Haematologica 2019;104:659-68. [CrossRef] [PubMed]
45. Martinez-Trillos A, Gaya A, Maffioli M, Arellano-Rodrigo E, Calvo X, Diaz-Beya M, et al. Efficacy and tolerability of hydroxyurea in the treatment of the hyperproliferative manifestations of myelofibrosis: results in 40 patients. Ann Hematol 2010;89:1233-7. [CrossRef] [PubMed]
46. Spivak J, Hasselbach H. Hydroxycarbamide: a user's guide for chronic myeloproliferative disorders. Expert Rev Anticancer Ther 2011;11:403-14. [CrossRef] [PubMed]
47. Barosi G, Birgegard G, Finazzi G, Griesshammer M, Harrison C, Hasselbalch H, et al. A unified definition of clinical resistance and intolerance to hydroxycarbamide in polycythaemia vera and primary myelofibrosis: results of a European LeukaemiaNet (ELN) consensus process. Br J Haematol 2010;148:961-3. [CrossRef] [PubMed]
48. Tefferi A, Elliot MA, Yoon SY, Li CY, Mesa RA, Call TG, et al. Clinical and bone marrow effects interferon alfa therapy in myelofibrosis with myeloid metaplasia. Blood 2001;97:1896-7. [CrossRef] [PubMed]
49. Ianotto JC, Boyer-Perrard F, Gyan E, Laribi K, Cony-Makhoul P, Demory JL, et al. Efficacy and safety of pegylated-interferon α-2a in myelofibrosis: a study by the FIM and GEM French cooperative groups. Br J Haematol 2013;162:783-91. [CrossRef] [PubMed]
50. Ianotto JC, Chauveau A, Boyer-Perrard F, Gyan E, Laribi K, Cony-Makhoul P, et al. Benefits and pitfalls of pegylated interferon-α2a therapy in patients with myeloproliferative neoplasm-associated myelofibrosis: a French Intergroup of Myeloproliferative neoplasms (FIM) study. Haematologica 2018;103:438-46. [CrossRef] [PubMed]
51. Hasselbalch HC, Holmstrom MO. Perspectives on interferon-alpha in the treatment of polycythemia vera and related myeloproliferative neoplasms: minimal residual disease and cure? Semin Immunopathol 2019;41:5-19. [CrossRef] [PubMed]
52. Tefferi A, Mesa R, Nagorney D, Schroeder G, Silverstein M. Splenectomy in myelofibrosis with myeloid metaplasia: a single-institution experience with 223 patients. Blood 2000;95:2226-33. [CrossRef] [PubMed]
53. Tefferi A, Mudireddy M, Gangat N, Hanson C, Ketterling R, Pardananiet A, et al. Risk factors and a prognostic model for postsplenectomy survival in myelofibrosis. Am J Hematol 2017;92:1187-92. [CrossRef] [PubMed]
54. Reilly JT, McMullin MF, Beer PA, Butt N, Conneally E, Duncombe A, et al. Guideline for the diagnosis and management of myelofibrosis. Br J Haematol 2012; 158:453-71. [CrossRef] [PubMed]
55. Barbui T, Tefferi A, Vannucchi A, Passamonti F, Silver R, Hoffman R. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European Leukemia Net. Leukemia 2018;32:1057-69. [CrossRef] [PubMed]
56. Thomas D, Giles F, Albitar M, Cortes J, Verstovsek S, Faderl S, et al. Thalidomide therapy for myelofibrosis with myeloid metaplasia. Cancer 2006;106:1974-84. [CrossRef] [PubMed]
57. Mesa R, Steensma D, Pardanani A, Li CY, Elliott M, Kaufmann S, et al. A phase 2 trial of combination low-dose thalidomide and prednisone for the treatment of myelofibrosis with myeloid metaplasia. Blood 2003; 101:2534-41. [CrossRef] [PubMed]
58. Quintas-Cardama A, Kantarjian HM, Manshouri T, Thomas D, Cortes J, Ravandi F, et al. Lenalidomide plus prednisone results in durable clinical, histopathologic, and molecular responses in patients with myelofibrosis. J Clin Oncol 2009;27:4760-6. [CrossRef] [PubMed]
59. Thomas D, Giles F, Albitar M, Cortes J, Verstovsek S, Faderl S, et al. Thalidomide therapy for myelofibrosis with myeloid metaplasia. Cancer 2006;106:1974-84. [CrossRef] [PubMed]
60. Tefferi A, Cortes J, Verstovsek S, Mesa R, Thomas D, Lasho T, et al. Lenalidomide therapy in myelofibrosis with myeloid metaplasia. Blood 2006;108:1158-64. [CrossRef] [PubMed]
61. Tefferi A, Verstovsek S, Barosi G, Passamonti F, Roboz GJ, Gisslinger H, et al. Pomalidomide is active in the treatment of anemia associated with myelofibrosis. J Clin Oncol 2009;27:4563-9. [CrossRef] [PubMed]
62. Cervantes F, Alvarez-Larran A, Domingo A, Arellano-Rodrigo E, Montserrat E. Efficacy and tolerability of danazol as a treatment for the anaemia of myelofibrosis with myeloid metaplasia: long-term results in 30 patients. Br J Haematol 2005;129:771-5. [CrossRef] [PubMed]
63. Cervantes F, Hernandez-Boluda JC, Alvarez A, Nadal E, Montserrat E. Danazol treatment of idiopathic myelofibrosis with severe anaemia. Haematologica 2000; 85:595-9. [PubMed]
64. Cervantes F, Alvarez-Larran A, Hernandez-Boluda J, Sureda A, Torrebadell M, Montserrat E. Erythropoietin treatment of the anaemia of myelofibrosis with myeloid metaplasia: results in 20 patients and review of the literature. Br J Haematol 2004;127:399-403. [CrossRef] [PubMed]

Pregledni rad **UDC: 616.155-07-08**
doi:10.5633/amm.2021.0212

SAVREMENI PRISTUP U DIJAGNOZI I LEČENJU PRIMARNE MIJELOFIBROZE

Irena Ćojbašić[1,2]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Klinika za hematologiju i kliničku imunologiju, Univerzitetski klinički centar Niš, Niš, Srbija

Kontakt: Irena Ćojbašićš
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: icojbasic@gmail.com

Primarna mijelofibroza (PMF) je retka hronična mijeloproliferativna neoplazma (MPN). PMF je rezultat klonske ekspanzije mijeloidnih ćelija i odlikuje se varijabilnim prisustvom mutacija, morfološki povećanom proliferacijom megakariocita, progresivnom fibrozom koštane srži, hepatosplenomegalijom, anemijom, leukoeritroblastozom, konstitucionalnim simptomima i kraćim vremenom preživljavanjem. Svetska zdravstvena organizacija je 2016. godine definisala trenutne dijagnostičke kriterijume za PMF, koji uključuju kombinovanu procenu kliničkih, histoloških, mutacionih i laboratorijskih karakteristika bolesti. Nedavno, nekoliko novih prognostičkih skoring sistema za PMF počeli su da se koriste, koji se zasnivaju isključivo na genetskim markerima ili uključuju kliničke promenljive pored mutacija i kariotipa. U lečenju mijelofibroze primenjuje se terapija prilagođena riziku, što podrazumeva izbor vrste terapije prema kategoriji rizika dobijenoj izračunavanjem važećih prognostičkih skoring sistema. Alogena transplantacija matičnih ćelija ostala je jedina potencijalno kurativna terapija za lečenje PMF, ali je pogodna za mali broj visoko rizičnih bolesnika, koji imaju podudarnog davaoca. U proteklih deset godina, razvoj i odobravanje ruksolitiniba za lečenje PMF bilo je od najveće važnosti u tretmanu ove bolesti, iako je to palijativna terapija. Ruksolitinib je snažan JAK1/JAK2 inhibitor, koji dovodi do smanjenja splenomegalije i simptoma i produžava ukupno vreme preživljavanje kod bolesnika sa ovom bolešću.

Acta Medica Medianae 2021;60(2):96-105.

Ključne reči: *primarna mijelofibroza, prognostičko modeliranje, lečenje*

JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

Ideja o postavljanju jedinstvenih kriterijuma za objavljivanje radova u časopisima za biomedicinske nauke iskristalisana je 1978. godine u Vankuveru. Ovi kriterijumi za rukopise, uključujući pravila za pisanje bibliografije, prvi put su objavljeni 1979. godine. Vankuverska grupa je vremenom prerasla u Međunarodni komitet urednika medicinskih časopisa — International Committee of Medical Journal Editors (ICMJE). Trenutno je na snazi peta revizija kriterijuma za objavljivanje radova u biomedicinskim časopisima, doneta 1997. godine.

Kriterijumi za citiranje i navođenje referenci

Reference se obeležavaju arapskim brojevima u zagradama, pri čemu se reference obele-žavaju brojevima onim redosledom kojim se pojavljuju u tekstu. Reference citirane jedino u tabelama ili legen-di moraju se obeležiti brojem u skladu sa redosledom pojavljivanja u tekstu.

Naslove medicinskih časopisa treba pisati u skraćenom obliku onako kako su navedeni u poglavlju **List of Journals Indexed in Index Medicus**. Lista skraćenih naziva medicinskih časopisa objavljuje se svake godine u januarskom broju ***Index Medicusa***. Ova lista se takođe može naći na adresi **www.nlm.nih.gov**

Izbegavati upotrebu apstrakata kao referenci, već koristiti samo izvorne tekstove (*in extenso* članci). Reference koje se odnose na radove koji su prihvaćeni, ali još nisu odštampani, treba označiti sa "u štampi", pri čemu autor mora imati pismeno odobrenje da citira takve radove i da priloži pismeni dokaz da je citirani rad prihvaćen za štampu. Informacije iz rukopisa koji nisu prihvaćeni za štampanje mogu se citirati u tekstu kao "neobjavljeni rezultati", ali sa pismenom dozvolom autora.

Izbegavati citiranje prethodnih saopštenja (personal communication) ukoliko ona ne obezbeđuju esencijalne rezultate koji još nigde nisu objavljeni. U ovom slučaju, neophodno je u zagradi navesti ime osobe i datum usmenog saopštenja rezultata. Za objavljivanje ovih podataka neophodno je pismeno odobrenje autora.

Kriterijumi za pisanje referenci korišćenih u radu

U ovom pregledu biće obrađena pravila za pi-sanje literaturnih referenci samo za najčešće korišćene tipove publikacija.

Članci u časopisima

1. Standardni članak u časopisu

Navesti prvih šest autora, ukoliko ih je više iza šestog dodati **et al.** ukoliko je referenca na engleskom jeziku ili **i sar.** ukoliko je referenca na srpskom jeziku.

Vega KJ, Pina I, Krevsky B. Heart transplantation is associated with an increased risk for pancreatobiliary disease. Ann Intern Med 1996; 124(11):980-3.

Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood–leukaemia in Europe after Chernobyl: 5 year follow-up. Br J Cancer 1996;73:1006-12.

2. Organizacija kao autor

The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. Med J Aust 1996;164:282-4.

3. Članak bez poznatih autora

Cancer in South Africa (editorial). S Afr Med J 1994;84:15.

4. Volumen sa suplementom

Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.

5. Broj sa suplementom

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1 Suppl 2):89-97.

6. Volumen sa više delova

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem 1995;32(Pt 3):303-6.

7. Broj sa više delova

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. N Z Med J 1994;107(986 Pt 1):377-8.

8. Časopisi sa brojem bez volumena

Turan I, Wredmark T, Fellander-Tsai L. Arthroscopic ankle arthrodesis in rheumatoid arthritis. Clin Orthop 1995;(320):110-4.

9. Časopisi bez volumena i broja

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. Curr Opin Gen Surg 1993:325-33.

10. Reference u obliku apstrakta ili prethodnih saopštenja

Enzensberger W, Fischer PA. Metronome in Parkinson's disease (letter) Lancet 1996;347:1337.

Clement J, De Bock R. Hematological complica–tions of hantavirus nephropathy (HVN) (abstract). Kidney Int 1992; 42:1285.

Udžbenici i monografije

11. Monografija

Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

12. Autori kao urednici

Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

13. Organizacija kao autor i izdavač

Institute of Medicine (US). Looking at the future of the Medicaid program. Washington: The Institute; 1992.

14. Poglavlje u knjizi

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

15. Conference proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

16. Conference paper

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors.

MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-5.

17. Istraživački ili tehnički izveštaji

Službeni izveštaji (Issued by funding / sponsoring agency):

Smith P, Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX): Dept. of Health and Human Services (US), Office of Evaluation and Inspections; 1994 Oct. Report No.: HHSIGOEI69200860.

Sponzorisani izveštaji (Issued by performing agency)

Field MJ, Tranquada RE, Feasley JC, editors. Health services research: work force and educational issues. Washington: National Academy Press; 1995. Contract No.: AHCPR282942008. Sponsored by the Agency for Health Care Policy and Research.

18. Magistarske i doktorske disertacije

Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

Druge vrste publikovanog materijala

Neobjavljeni materijal

19. U štampi (In press)

Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med. In press 1996.

Elektronski zapisi

20. Internet članak u elektronskom formatu

Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar "cited 1996 Jun 5"; 1(1)(24 screens). Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm

21. Monografija u elektronskom formatu

CDI, clinical dermatology illustrated (monograph on CD-ROM). Reeves JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego: CMEA; 1995.

22. Kompjuterski podaci

Hemodynamics III: the ups and downs of hemodynamics (computer program). Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

PROPOZICIJE ZA PISANJE RADOVA U ACTA MEDICA MEDIANAE

Acta Medica Medianae (AMM) je tematski časopis iz oblasti medicinskih nauka. Časopis objavljuje originalne radove koji nisu prethodno publikovani.

U AMM se objavljuju: uvodnici, naučni i stručni članci, prethodna ili kratka saopštenja, revijski radovi tipa opšteg pregleda, aktuelne teme, meta-analize, prikazi slučajeva, prikazi knjiga i drugi prilozi. Radovi se štampaju na srpskom ili engleskom jeziku sa apstrak-tom na srpskom i engleskom jeziku. Radovi na engles-kom jeziku se prezentuju u elektronskom formatu na sajtu Medicinskog fakulteta u Nišu, kao i na među-narodnim sajtovima iz oblasti medicinskih nauka. Acta Medica Medianae izlazi četiri puta godišnje, od 1962 godine.

Svi radovi koji se objavljuju u AMM podležu anonimnoj recenziji, a Uređivački odbor određuje redosled njihovog štampanja. Primedbe i sugestije urednika i recenzenata dostavljaju se autoru radi konačnog oblikovanja. Radovi se predaju u pisanom ili elektronskom obliku na srpskom i engleskom jeziku. Rukopisi radova prihvaćenih za štampu ne vraćaju se autoru.

Rukopis treba predati sa jednostrukim proredom, formata A4, sa levom marginom od 3 cm.

Prva strana rada treba da sadrži: a) naslov rada b) puna imena i prezimena autora c) puni nazivi ustanova i organizacijskih jedinica u kojima je rad realizovan i mesta u kojima se ustanove nalaze, d) znacima *, **, ***, #, ##, ###,…označavaju se redom autori i njihove institucije e) puna adresa, broj telefona i e-mail osobe zadužene za korespondenciju u vezi predatog rukopisa.

Druga strana treba da sadrži samo naslov rada, rezime i ključne reči, bez imena autora i institucija. Veličina rezimea za naučne i stručne članke, revijske radove tipa opšteg pregleda i meta–analize može da bude do 250 reči. Ispod rezimea sa podnaslovom "Klučne reči" navesti 3-5 ključnih reči ili izraza. Poželjno je da autori za ključne reči koriste odgovarajuće deskriptore, tj. definisane termine iz *Medical Subject Heading* (MeSH) liste *Index Medicus-a*. Prva i druga strana se predaju na srpskom i engleskom jeziku i ne obeležavaju se brojevima.

Tekst članka: Naučni i stručni članci, kao i opšti pregledi i meta-analize ne smeju prelaziti 11 stranica sa prilozima; aktuelne teme– 6 stranica; kazuistika 6–stranica; prethodna saopštenja– 5 stranica, a izveštaji sa skupova i prikazi knjiga 2 stranice. Naučni i stručni članci obavezno treba da sadrže poglavlja: uvod, cilj, materijal i metode, rezultati, diskusija i zaključak. Zahvalnost ili komentar povodom sponzorstva rada dati na kraju teksta članka iza poglavlja "zaključak".

U tekstu naznačiti mesta priloga i obeležiti ih onako kako su obeleženi u prilogu.

Literatura se daje u posebnom poglavlju, pri čemu se navodi onim redosledom kojim se citati pojavljuju u tekstu. Broj literaturne reference se u tekstu označava arapskim brojem u zagradi. Za navođenje literature koristiti pravila Vankuverske konvencije. Strane se numerišu arapskim brojevima u donjem desnom uglu

Priloge u vidu teksta, tabela i ilustracija (grafikoni, crteži i dr.) ne unositi u tekst članka, već na kraju teksta, na posebnim stranicama obeleženim u gor-njem levom uglu sa "Tabela, Grafikon, Ilustracija" i arapskim brojem redosledom pojavljivanja u tekstu (npr. Tabela 1, Grafikon 1 i dr.) i svakoj se daje kratak naslov. Kratka objašnjenja i skraćenice daju se u fusnoti. Za fusnotu koristiti sledeće simbole: *, **, ***, #, ##, ###, …itd. Tabele, grafikone i ilustracije treba praviti korišćenjem nekog od programa iz Microsoft Office paketa. Izbegavati upotrebu boja kod izrade grafika.

Za izradu grafičkih priloga može se koristiti bilo koji grafički program, pri čemu slike moraju biti snimljene u jpg formatu rezolucije 300 dpi (u originalnoj veličini). Grafički prilozi se ne unose u Word dokument već se predaju kao posebni JPG fajlovi.

Ukoliko je tabela ili ilustracija već negde objavljena, citirati izvor i priložiti pismeno odo-brenje, ukoliko se radi o zaštićenom materijalu. Ukoliko je na fotografiji prikazan bolesnik tako da se može prepoznati, potrebno je njegovo pismeno odobrenje, u suprotnom, delovi fotografije se moraju izbrisati da bolesnik ne može biti identifikovan.

Uz rad, na posebnom listu, treba dostaviti: a) izjavu da rad do sada nije objavljivan, b) potpise svih autora, c) ime, prezime, tačnu adresu i broj telefona prvog autora.

Rad je preporučljivo predati u elektronskom obliku na e-mail adresu uredništva: acta@medfak.ni.ac.rs ili poslati poštom na CD ili DVD disku sa materijalom u celini na srpskom i engleskom jeziku.

Rad treba otkucati u programu ms office Word verzija 2003. ili novija. Za verziju na engleskom jeziku koristiti font Verdana, veličine 9 pt, kodna stranica (English). Za verziju na srpskom jeziku koristiti font Verdana, veličine 9 pt, kodna stranica (Serbian lat ili Croatian).

U radu je obavezno korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina.

AMM zadržava pravo dalje distribucije i štampanja radova.

SECRETARIAT

GUIDELINES FOR PAPER SUBMISSION TO ACTA MEDICA MEDIANAE

Acta Medica Medianae (AMM) is a thematic journal for medical sciences. The Journal publishes original reasearch articles that have not been published before.

AMM also publishes editorials, observational and experimental articles, procedings or short communications, review articles, meta-analyses, case reports, current topics, articles from the history of medicine as well as other contributions related to medical sciences. All articles are printed in Serbian or English with short abstracts in both Serbian and English. Articles in English are published in electronic form on the University of Nis Medical Faculty website as well as international sites related to medical sciences. Acta Medica Medianae is published four times a year. The first issue appeared as early as 1962.

General Guidelines

Paper Submission

All research articles published in this journal undergo rigorous peer review, based on initial editor screening and anonymized refereeing by at least two anonymous referees. Remarks and suggestions made by the editors and reviewers are sent to the author for final revision. The papers in English are to be submitted by e-mail: acta@medfak.ni.ac.rs. Manuscripts accepted for publication are not returned to authors.

The first page of a research article must contain: a) article title b) full name of author(s) c) full name(s) of institutions and/or address(es) of department(s) where either reasearch was conducted or research article written d) following signs *, **, ***, #, ##, ### signifying author(s) and institutions e) full address, phone number and e-mail of a corresponding author.

The second page should contain only research article title, abstract and key words without names of author(s) and institution(s). Abstract for research and professional articles, review articles and meta-analyses should have up to 350 words while abstract for all other types of publications should consist of 250 words. Key Words section should have up to 5 key words or phrases related to a submitted article. It is desirable that authors use corresponding descriptors from Medical Subject Heading (MeSH) that can be found on Index Medicus list for key words. The first and the second page should not be numbered.

Body of a Research Article – Research and professional articles, as well as general surveys and meta-analyses should not exceed 11 pages altogether; current topics - 6 pages, casuistics - 6 pages and proceding statements - 5 pages, history of medicine articles - 3 pages while conference reports and book reviews - 2 pages. Research and professional articles should comprise the following mandatory chapters: introduction, aim(s), material and method(s), result(s), discussion and conclusion(s). Result(s) and discussion can be comprized into one chapter. Acknowledgments of any kind in a submitted article should be written at the end of the paper after "Conclusion(s)". It is necessary to clearly mark a place for additions in the text.

References should be written in a separate chapter in the same order of appearance as in a research article. Reference numbers that appear in the text should be written in Arabic numerals and put in brackets. All authors should be listed. If there are more than six authors this should be indicated with *et al*. Use the rules of the Vancouver convention when quoting literature. Pages should be enumerated in Arabic numerals in the bottom right corner.

Additions in form of texts, tables and illustrations (photos, drawings, diagrams, etc) should not be inserted in the reasearch article body but at the end of the text on separate pages which should be marked in the upper left corner as "Table(s), Graphic(s), Illustration(s) etc) with Arabic numeral in the same order of appearance as in the text (for instance, Table 1, Graph 1, etc) with a short title. Short explanations and abbreviations should be stated in footnotes where the following symbols should be used: *, **, ***, #, ##, ###, etc. Table(s), graph(s) and ilustration(s) should be drawn in a Microsoft Office Program. Color should be avoided.

Any graphic program can be used for making graphic addition(s) while picture(s) should be saved in .jpg format with 300 dpi resolution (original size). Graphic addition(s) should be sent as separate jpg file(s), and not inserted in the body of a research or any other article submitted to AMM.

If some additions, included in a submitted reasearch article, have already been published, source of publication should be clearly stated, alongside written approval in case the material is copyright protected. Patients on photographs have privacy rights that should not be infringed without their consent. Namely, if a photograph shows a patient who can be recognized, his/her written approval should also be submitted; otherwise, visible and recognisable facial or bodily parts should be blackened so that the patient cannot be identified by readership.

On a separate sheet the author should also enclose: a) his/her statement that a submitted article has not been published before, b) signatures of all authors, c) full name, address, e-mail and phone number of the first author.

Submitted article should be typed in Word Version 2003 for Windows (or more recent ones), font Verdana 9 pt size; code page (English) should be used.

The authors are required to use international measurement standards (SI) and internationally accepted standard terms.

Acta Medica Medianae reserves the right for further distribution and printing of published reasearch articles.