УНИВЕРЗИТЕТ У БЕОГРАДУ
МЕДИЦИНСКИ ФАКУЛТЕТ
UNIVERSITY OF BELGRADE
FACULTY OF MEDICINE

MEDICINSKA ISTRAŽIVANJA | MEDICAL RESEARCH

Časopis Medicinskog fakulteta Univerziteta u Beogradu | *The Journal of the Faculty of Medicine University of Belgrade*

Vol 57 | Sv. 3 | 2024

Skeniranjem QR koda otvoriće Vam se uputstvo autorima koje je na adresi: http://med.bg.ac.rs/wp-content/uploads/2018/01/Instructions-for-authors-26_04_2022.pdf?script=lat

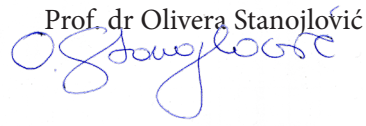
Reč Urednika

„Medicinska istraživanja“ je recenzirani naučni časopis Medicinskog fakulteta Univerziteta u Beogradu, koji je prvi put objavljen 1971 godine, dakle, pre tačno pola veka. Časopis „Medicinska istraživanja“ je prvenstveno posvećen publikovanju rezultata naučno-istraživačkog rada nastavnika i saradnika Medicinskog fakulteta, kao i drugih naučnika u oblasti biomedicinskih istraživanja. Časopis „Medicinska istraživanja“ objavljuje naučne radove na engleskom jeziku uz sažetak na srpskom jeziku i ima usvojenu politiku otvorenog pristupa (open access). Izlazi tri puta godišnje, dok se u četvrtoj svesci objavljuju sažeci izlaganja na simpozijumu „Stremljenja i novine u medicini“. U časopisu „Medicinska istraživanja“ se objavljuju originalni i prethodno neobjavljeni radovi: a. originalni radovi u kojima se prvi put objavljuju rezultati sopstvenih istraživanja; b. pregledni radovi (Review), kritički i originalno prikazani (sa autocitatima); c. prikazi bolesnika i slučajeva (Case report).
Radovi koji se objavljuju u časopisu „Medicinska istraživanja“ pripadaju oblastima: bazičnih biomedicinskih istraživanja, kliničkih istraživanja i preventivne medicine. U cilju stručne recenzije prispelih radova, Uređivački odbor časopisa je podeljen u tri sekcije: bazične, kliničke i preventivne nauke, a njihovim radom koordiniraju Urednici sekcija. Sekcija za statističku analizu podataka će biti uključena u evaluaciju statističkih apsekata prispelih radova.

Sa velikim entuzijazmom želimo da unapredimo naš medicinski časopis i da u godinama koje slede bibliometrijski status „Medicinskih istraživanja“ bude podignut sa kategorije nacionalnog časopisa (M53) na kategoriju međunarodnog časopisa (M23). Ovaj naš zajednički put koji podrazumeva indeksiranje časopisa u Journal Citation Reports i dobijanje impakt faktora neće biti ni jednostavan, ni brz, ali je nezaobilazan.
Ostvarivanje ove vizije časopisa podrazumeva i zajedničke napore. Svi članovi uredništva časopisa su posvećeni osiguranju kvaliteta, integriteta i promociji inovativnih izvora informacija zasnovanih na dokazima u cilju dalje promocije časopisa.

Uređivački odbor prima radove pripemljene u skladu sa Uputstvom autorima.

GLAVNI I ODGOVORNI UREDNIK
Prof. dr Olivera Stanojlović



CONTENTS

Medicinska istaživanja 2024; 57(3):1-7 | DOI: 10.5937/medi57-45107

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Morpho-dynamic and functional breast MRI features in the assessment of ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) in postmenopausal female patients

Marko Mihajlović[1], Anđela Đajić[1], Iva Krušac[1], Dejan Dimitrijević[1], Luka Raspopović[1], Mirjan Nadrljanski[1, 2]

[1]Institute of Oncology and Radiology of Serbia, Department of Radiology, Belgrade, Serbia
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Recived: 20 June 2023
Revised: 30 April 2024
Accepted: 30 April 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Mirjan Nadrljanski
Institute of Oncology and Radiology of Serbia, Department of Radiology
14 Pasterova Street, 11000 Belgrade, Serbia
E-mail: mirjan.nadrljanski@med.bg.ac.rs

Summary

Introduction: Most frequent histologic types of breast cancer include invasive ductal carcinoma (IDC) and its most common precursor: ductal carcinoma in situ (DCIS). Based on morpho-dynamic and functional parameters: lesion size, initial signal intensity enhancement (wash-in), time-intensity curve (TIC) type, apparent diffusion coefficient (ADC), and positive enhancement integral (PEI), breast dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) allows the differentiation between IDC and *in situ* lesions in postmenopausal female patients.

Methods: A single-center retrospective study included 40 postmenopausal female patients with histopathologically confirmed diagnosis (DCIS n_1=20; IDC n_2=20), examined with full diagnostic protocol (FDP) on 1.5T and 3T MRI scanners. The SPSS 21.0 software package was used for the statistical analysis of the defined parameters in two predefined subgroups.

Results: Tumor size was significantly larger ($p<0.001$) in patients with DCIS. The IDC group showed significantly higher wash-in and PEI values ($p<0.001$). ADC was significantly higher ($p<0.001$) in DCIS. There was a statistically significant difference ($p<0.05$) in the TIC type distribution: TIC type 2 was predominant in patients with DCIS, while the TIC type 3 was predominant in patients with IDC.

Conclusion: Based on predefined morpho-dynamic and functional parameters, breast MRI may allow the differentiation between the two types of breast ductal carcinoma: IDC and DCIS. However, histopathological confirmation remains the "golden standard" in differentiation, taking the nature of the disease into account.

Keywords: DCE-MRI, ductal carcinoma in situ, invasive ductal carcinoma, morpho-dynamic parameters, functional parameters

Cite this article as: Mihajlović M, Đajić A, Krušac I, Dimitrijević D, Raspopović L, Nadrljanski M. Morpho-dynamic and functional breast MRI features in the assessment of ductal carcinoma in situ (dcis) and invasive ductal carcinoma (idc) in postmenopausal female patients; Medicinska istaživanja 2024; 57(3):1-7 DOI: 10.5937/medi57-45107

INTRODUCTION

According to the World Health Organization (WHO), breast carcinoma is the most frequent type of cancers in the female population and the most common cause of death by cancer types in female population globally (1). Breast carcinoma predominantly occurs in the female population after the age of 50 (2, 3).

Pathohistologically, breast carcinoma is traditionally divided into non-invasive and invasive types (4). Ductal carcinoma in situ (DCIS) is a heterogeneous disease, which is characterized by the clonal multiplication of epithelial breast cells, that have not yet penetrated the basement membrane (5). The most frequent histological type of breast carcinoma is invasive ductal breast carcinoma (IDC) - the infiltrative malignant neoplasm (4, 6).

Breast magnetic resonance imaging (MRI) is primarily used as the adjunct radiological technique, following mammography and breast ultrasound in the standard patient diagnostic algorithm. MRI is not designed to detect microcalcifications like mammography, however, breast MRI detects 10-15% of additional non-calcified lesions and lesions that are mammographically occult (7, 8). The percentage is even higher in dense breast, taking into account the suboptimal sensitivity of mammography, therefore breast MRI is regarded as the most sensitive imaging technique for breast screening, preoperative staging and therapy monitoring (7-10).

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) represents the advanced diagnostic imaging technique to detect suspicious breast lesions based on the analysis of structural and functional aspects, particularly on postcontrast series, based on the assessment of neoangiogenesis and the lesion microvascularization (8). The additional diagnostic value of DCE-MRI is based on the detection of neoangiogenesis in suspicious lesions, i.e., the increased number and permeability of tumor blood vessels. Following the administration of the contrast agent based on the compounds of gadolinium (Gd), the suspicious lesions tend to express increased permeability, which leads to higher contrast uptake (wash-in) and the subsequent washout (7, 9). Based on the above, the time-intensity (TIC) curve is defined as the increase of the single intensity (SI) and its change against time [s]. Based on the contrast wash-in and washout, it is possible to examine the morphodynamic characteristics of the lesions. DCE-MRI provides high sensitivity, but moderate specificity (7-10). Functional imaging is introduced with the aim of overcoming the moderate specificity: apparent diffusion coefficient (ADC), based on the diffusion-weighted imaging (DWI) and the positive enhancement integral (PEI) values, both representing functional MRI parameters that may further increase the specificity of the exam (9, 10). DWI aims to reflect tissue microstructure changes: the threedimensional mobility of water molecules, reflecting the organization of the tissue and the ADC [$mm^2/s \times 10^{-3}$] allows the quantification of the signal aiming to differentiate between benign and malignant lesions (10). PEI, as the semiquantitative parameter represents the endpoint in tissue perfusion assessment, providing the quantitative information based on the value of extracellular Gd contrast agent in different tissues (9).

The aim of this study is to examine the morphologic aspects: differences in percentage of initial wash-in, time-intensity curve (TIC), apparent diffusion coefficient (ADC), and positive enhancement integral (PEI) in postmenopausal female patients, in order to detect significant and clinically relevant differences in the predefined parameters between DCIS and IDC in postmenopausal female patients with pathohistologically proven diagnosis.

MATERIALS AND METHODS

In this retrospective study, there were 40 postmenopausal female patients, divided into two groups, based on the pathohistological diagnosis: IDC vs. DCIS. The first group (n_1) included 20 postmenopausal female patients, with pathohistologically confirmed pure ductal carcinoma in situ (DCIS). The second group (n_2) consisted of 20 female patients, with pathohistologically confirmed diagnosis of invasive ductal carcinoma (IDC). The MR exams and percutaneous biopsy procedures were conducted in our institution from 2018 to 2022, following the Institutional Review Board decision No 204412-01.

Prior to MRI exam, the initial radiological exam to detect and define the lesions was the full-field digital mammography (FFDM). All mammograms were defined as BI-RADS category 4 or 5. The patients were additionally examined using ultrasound and / or DCE-MRI with DWI in order to define the lesion detectability for the optimal image-guided biopsy procedure. All the lesions were biopsied either by ultrasound-guided core-needle biopsy (US-CNB), or MRI-guided vacuum-assisted biopsy (MR-VAB), or stereotaxic vacuum-assisted biopsy (SVAB). The nature of the lesions was confirmed pathohistologically.

Breast MRI exams in all patients were performed with the MRI-scanners 1.5 T (Avanto fit, Siemens Medical Solutions, Erlangen, Germany) and / or 3 T (Lumina, Siemens Medical Solutions, Erlangen, Germany) with the dedicated bilateral breast coil. Morphodynamic lesion features were defined with the full diagnostic protocol (FDP): T2W and pre- / postcontrast T1W sequences with the DWI with the b-gradients: b_{50}, b_{850} and the generated semiquantitative and ADC maps (10). FDP was used for the axial-plane images (slice thickness 2 mm): T2W STIR (TE/TR 60/7690, inversion time 180 ms, flip angle 150, field of view 340 × 340, image matrix 320 × 256); T2W TSE (TE/TR 70/5900, flip angle 180, field of view 340 × 340, image matrix 384 × 319); T1W-

TSE (TE/TR 12/910, flip angle 90, field of view 340 × 340, image matrix 320 × 234); T1W FLASH 3D (TE/TR 4.8/9.1, flip angle 25, field of view 340 × 340, image matrix 576 × 564) for one precontrast and five postcontrast series acquired every 1 min 23 s, after the bolus injection of 0.1 mmol/kg of body weight of gadobutrol (Gadovist, Bayer Pharma, Berlin, Germany) with the automatic injector (Mississippi, Ulrich Medical, Ulm, Germany) at the rate of 2 mL/s, with the flush of 20 mL saline (9, 10).

Breast MRI exmas were analyzed on the dedicated workstation (Leonardo, Siemens Medical Solutions, Erlangen, Germany / Carestream Vue PACS, Rochester, NY, USA) using Syngo (Syngo, Siemens Medical Solutions, Erlangen, Germany) and OsiriX (OsiriX, Pixmeo, Geneva, Switzerland) image processing software tools.

The following predefined parameters were computed and analyzed: a.) Lesion size [cm] of DCIS and IDC; b.) Dynamic features (wash-in / washout) were computed as the percentage of the signal intensity (SI) increase within 90 s (wash-in) and the type of time-intensity curve (TIC) was plotted against time [s] (10-12); c.) PEI values were computed as equal to the integral of the area under the curve of the increase in SI after contrast agent injection and expressed in numerical values (13) and d.) ADC values [$mm^2/s \times 10^{-3}$] were computed based on the two gradient values on DWI - at b_{50} and b_{850} (10-12).

The data were analyzed using the methods of descriptive and analytical statistics. To compare statistically significant differences between the examined subgroups, we used Chi-square test for the nominal data. Differences between numerical data were compared by Student's t-test or Mann-Whitney U test, depending on the distribution. The difference was considered statistically significant if $p \leq 0.05$, and highly statistically significant if $p \leq 0.001$. The SPSS software package (version 21.0, Chicago, Illinois, USA) was used for data processing.

RESULTS

The female postmenopausal patients in both subgroups (n_1 and n_2) were matched for age: 59,8 +/- 4,6 vs. 60,2 +/- 3,8 ($p=0.767$). The average tumor size (cm) was statistically significantly different between the two subgroups in favor of DCIS ($p < 0.001$), as presented in Table 1. For dynamic parameters, the wash-in was significantly higher during the first 90 s in the subgroup of patients with IDC ($p < 0.001$). For the functional parameters, both ADC and PEI values were significantly different between the patients with DCIS and IDC ($p < 0.001$), as shown in **Table 1**.

Regarding the distribution of the TIC: there was the predilection for the TIC type 2 in the subgroup of patients with DICS and for the TIC type 3 in the subgroup of patients with IDC, as shown in **Table 2**.

Table 2. Distribution of TIC types in n_1 and n_2.

TIC type	Subgroup		*p*
	n_1	n_2	
Type 1	3	4	0.004*
	15.0%	20.0%	
Type 2	14	4	
	70.0%	20.0%	
Type 3	3	12	
	15.0%	60.0%	

* Statistically significant difference ($p \leq 0.05$)

Based on the above, the postmenopausal female patients with higher initial wash-in values, TIC type 3 and lower values for ADC, with higher values for PEI, tend to belong to the group of patients with IDC **(Figure 1)**, while the patients with lower wash-in values, TIC type 2 and the higher values of ADC and lower values of PEI, tend to belong to the group of patients with DCIS **(Figure 2)**.

DISCUSSION

Breast MRI has long been a radiological method, which tended to overcome its moderate specificity with the introduction of the functional aspects in order to distinguish between the clinically relevant criteria: benign vs. malignant, responder vs. non-responder, in situ vs. invasive, etc. So far, large studies have tended to qualify and quantify the parameters of morphology, dynamic features and functional aspects separately and as groups of parameters with scores, having in mind radiological – pathological correlations, as the "golden standard" of assessment. The compound scores, sometimes related to as the "virtual biopsy" were supposed to lower the number

Table 1. Patient age (years), lesion size (cm), wash-in (% / 90s) positive enhancement integral value (PEI value) and apparent diffusion coefficient (ADC, [$10^{-3} mm^2/s$]).

Group	n_1	n_2	*p*
Age (years)	59.8 +/- 4.6	60.2 +/- 3.8	0.767
Lesion size (*cm*)	2.7 +/- 0.7	1.6 +/- 0.2	<0.001*
Wash-in (%/90*s*)	165.0 [96.0 – 345.0]	255.0 [145.0 – 452.0]	<0.001*
ADC ($10^{-3} mm^2/s$)	1.32 +/- 0.05	1.09 +/- 0.11	<0.001*
PEI values	435.0 [235.5 – 688.7]	1000.2 [654.2 – 1680.4]	<0.001*

* statistically significant difference ($p \leq 0.001$)

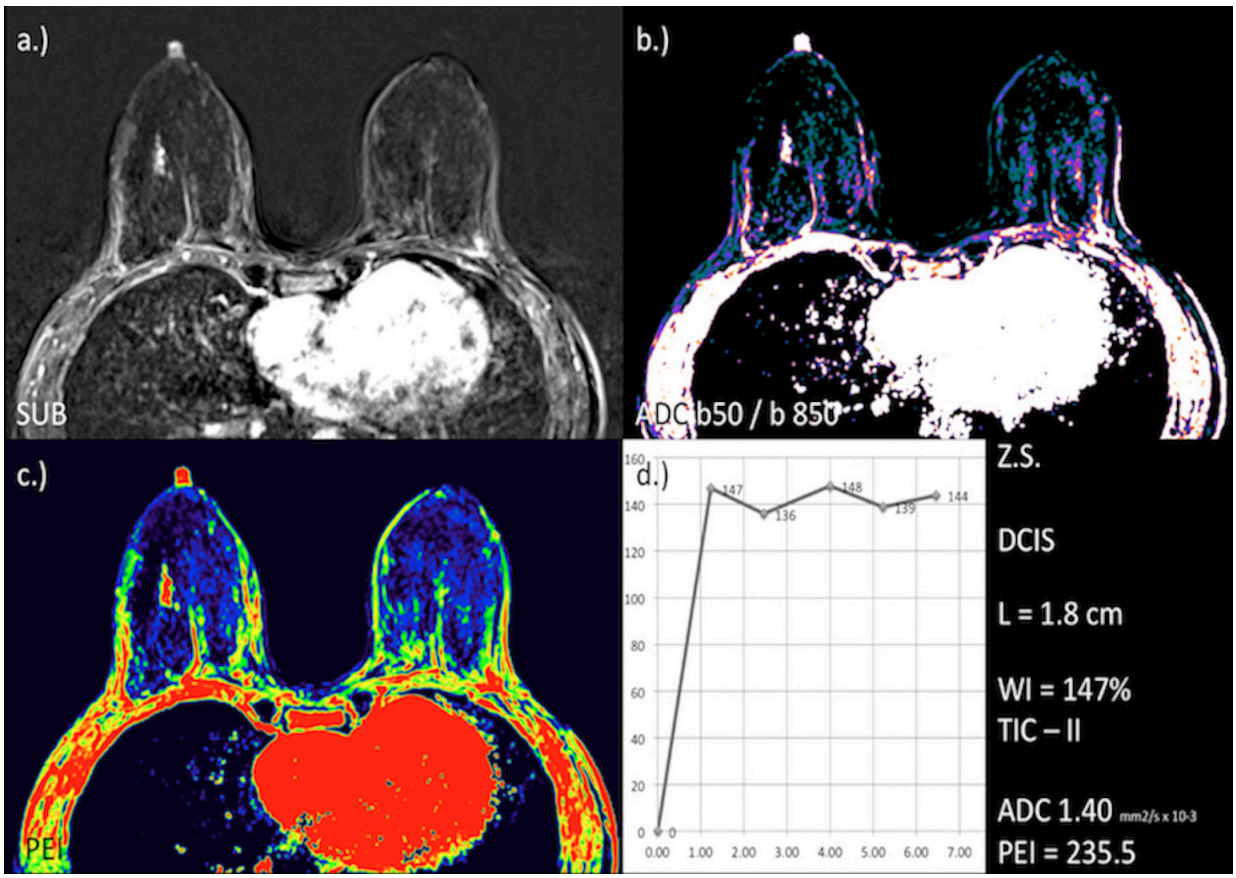


Figure 1. Morpho-dynamic and functional parameters of a 64-yr. old patient (Z.S.) with DCIS. (a) DCE-MRI, Subtraction (b) Diffusion Coefficient (ADC). (c) Positive Enhancement Integral (PEI). (d) Time-intensity curve.

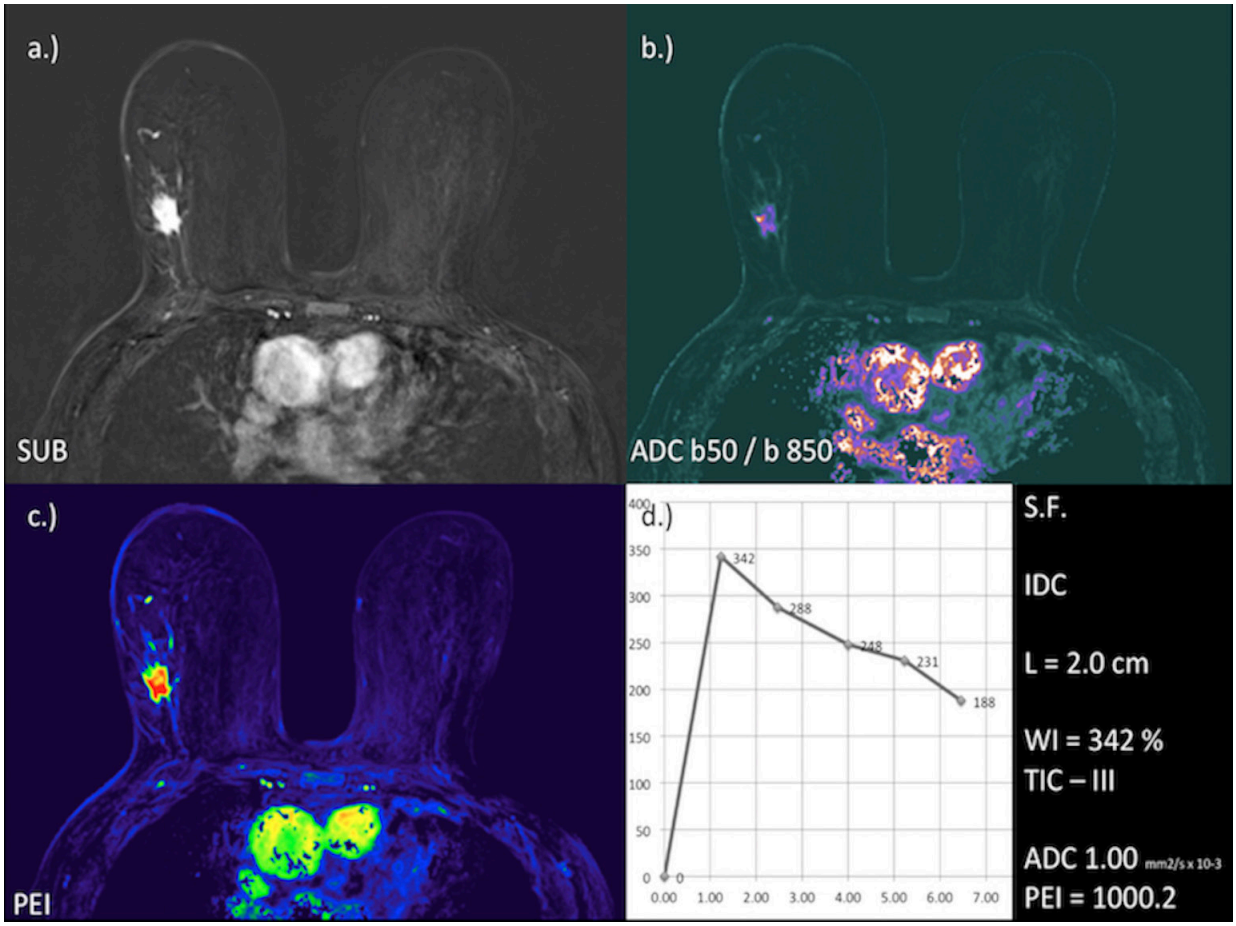


Figure 2. Morpho-dynamic and functional parameters of a 62-yr. old patient (S.F.) with IDC. (a) DCE-MRI, Subtraction (b) Diffusion Coefficient (ADC). (c) Positive Enhancement Integral (PEI). (d) Time-intensity curve.

of the overdiagnosed patients with unnecessary biopsies, confirming the benign nature of the disease. Additional scores, which introduced the algorithms (notably Fischer / Goettingen, and Kaiser score), aimed to define whether the lesion was suspicious based on the morphologic and dynamic featues additionally with the use of DWI-ADC (14, 15). In our research, we tried to define the morpho-dynamic features routinely assessed in the breast MRI exam, with the addition of two functional parameters aiming to cover different aspects of carcinogenesis: neoangiogenesis with contrast-enhanced MRI, kinetics of the contrast medium, with the assessment of dynamic parameters, lesion perfusion features with PEI and tissue organization and level of cellularity with DWI-ADC (9-12).

Female patients in the two subgroups were matched for age, all being postmenopausal with pathohistologically confirmed lesions. In terms of morphology, we decided to compare the lesion size with the largest diameter, as the general idea was not to include the bulky tumors and locally advanced tumors, taking into account the specific growth perutrbation in these tumors. Concerning the lesion morphology, the DCIS tend to show different levels of heterogeneity, however, the malignant lesions larger than 2 mm, produce growth factors stimulating angiogenesis and neoangiogenesis (16). This explains the increase in SI, as early as within the first two minutes following the contrast medium application (10, 16, 17). In our study, we obtained statistically significant higher wash-in values in the group of patients with pathohistologically confirmed IDC compared to the group with pathohistologically confirmed DCIS.

Jensen et al. reported results consistent with those obtained in this study regarding the TIC: TIC type 2 is most frequently detected in DCIS (18). There are studies that proved no regularity in TIC assessment (19). Our results confirm that TIC type 3 is more common in IDC, which was also confirmed in other studies (20-22). However, regarding the available references, there is no sufficiently defined data regarding the comparison of the predominant curve type in DCIS and IDC. We also need to mention that the heterogeneity may contribute to the lack of the common conclusion, taking into account the fact that the more heterogeneous lesions contain the pixels which do not necessarily show the enhcancement, therefore the region of interest may include the areas of the normal tissue, fiborcystic changes, in situ and or invasive areas, leading to the lack of TIC type specificity. The results of this study confirm the expected predominance of the type 2 in DCIS and type 3 in IDC, which is consistent with the malignant potential of invasive carcinoma and its infiltrative growth (4).

Expectedly, the tissue microstructure changes in more proliferative lesions, with higher level of cellularity, leading to the reduced water molecule thermal movements, which served as the basis for the application of DWI-ADC in oncologic imaging: the more malignant the lesions tend to be, the lower their ADC values become (21–23). It is worth mentioning that the ADC is considered to be the marker of cellularity (24). This specifically allows us to differentiate the lesions according to the grade: low vs. high grade DCIS, and DCIS vs. IDC (25–28). Our date confirmed that the ADC is significantly higher in DCIS compared to IDC (24–27). Based on this, the ADC increases the positive predictive value of breast MRI up to 96.6% and the specificity up to 93.3% (29).

Unlike mammography, DCE-MRI has greater sensitivity to determine the size of the DCIS lesion. The DCIS detectability relies on periductal and stromal vascular elements (30-33). The features of tissue perfusion, in addition to morphological criteria, may provide additional data about the underlying process and may contribute to better differentiation between in situ and invasive diseases. The sensitivity of DCE-MRI for the detection of DCIS ranges from 77 to 96% (30-33).

PEI values, as the integral part of the standardized DCE-MRI exam, give insight into the tissue perfusion and may contribute to better lesion characterization (9, 13, 31, 32). As a heterogeneous lesion, DCIS often appears as the non-mass enhancement (NME), with variable distribution and enhancement patterns. SI in DCIS may remain below the SI threshold typical for invasive carcinomas, which may also underestimate the extension of the lesion (14, 30-33). Based on the previous research in the field, we expected the degree of perfusion would be different in DCIS compared to IDC, which the results of this study confirmed (9). The PEI values in IDC were significantly higher compared to those in DCIS ($p<0.001$).

Our study has certain limitations: it is a single-center retrospective study. The relatively small number of patients certainly does not contribute to the generalized conclusions. However, the single-center study, contributes to the evaluation of the results based on uniform diagnostic protocols and the same technical conditions, which leads to standardized approach and better reproducibility. With the postmenopausal female patients in the study, the effects of hormonal changes were eliminated. Due to the limited number of subjects, it was not possible to define MRI parameters according to histological grades, however these data may serve as the basis for the prospective multicentric trial, aiming to differentiate between the histological grades and the criteria defined by immunostaining, which are related to the treatment selection.

CONCLUSION

The pre-defined morpho-dynamic and functional parameters in breast MRI, provide additional information in differentiation between invasive and in situ ductal breast carcinomas. The potential use of the additional parameters (DWI-ADC and PEI) in routine breast MRI

protocols may be of help in differentiation between DCIS and IDC, potentially increasing the specificity of MRI.

Acknowledgments

None.

Conflicts of interest

None to declare.

REFERENCES:

1. Breast cancer. World Health Organization. World Health Organization; 2018 [cited 2020Feb9]. Available from: https://www.who.int/cancer/prevention/diagnosis-screening/breast-cancer/en/
2. Hayes J, Richardson A, Frampton C. Population attributable risks for modifiable lifestyle factors and breast cancer in New Zealand women. *Intern Med J*. 2013;43(11):1198–204.
3. Reeder JG, Vogel VG. Breast cancer prevention. *Cancer Treat Res*. 2008;141:149-64.
4. Husain A. Sattar. Female Genital System and Breast. In: Kumar V, Abbas A.K, Aster J.C., editors. *Robbins basic pathology*. Ninth edition. Philadelphia, PA, USA. Saunders Elsevier; 2013. p. 710,711.
5. Bane A. Ductal Carcinoma In Situ : What the Pathologist Needs to Know and Why . *Int J Breast Cancer*. 2013;2013:1–7.
6. Sinn HP, Kreipe H. A brief overview of the WHO classification of breast tumors, 4th edition, focusing on issues and updates from the 3rd edition. *Breast Care*. 2013;8(2):149-54.
7. Radhakrishna S, Agarwal S, Purvish M. Parikh *et al*. Role of magnetic resonance imaging in breast cancer management. *South Asian J cancer*. 2018; 7(2):69–71.
8. Padhani AR. Dynamic contrast-enhanced MRI in clinical oncology: Current status and future directions. *J Magn Reson Imaging*. 2002;16(4):407–22.
9. Nadrljanski M, Maksimović R, Plešinac-Karapandžić V, Nikitović M, Marković-Vasiljković B, Milošević Z. Positive enhancement integral values in dynamic contrast enhanced magnetic resonance imaging of breast carcinoma: Ductal carcinoma in situ vs. invasive ductal carcinoma. *Eur J Radiol*. 2014;83(8):1363–7.
10. Nadrljanski M, Milošević Z, Plešinac-Karapandžić V, Goldner B. The role of breast magnetic resonance imaging in the diagnosis of ductal carcinoma in situ. *Srp Arh Celok Lek*. 2013;141(5–6):402–8.
11. Greenwood HI, Heller SL, Kim S, Sigmund EE, Shaylor SD, Moy L. Ductal Carcinoma in Situ of the Breasts: Review of MR Imaging Features . *Radiographics*. 2013;33(5):1569–88.
12. Christiane K. Kuhl. Dynamic Breast Magnetic Resonance Imaging. In: Elizabeth A. Morris, Laura Liberman, editors. *Breast MRI Diagnosis and Intervention*. New York, NY, USA. Springer Science+Business Media; 2005. p. 88-9.
13. Alicioglu B, Guler OT, Bulakbasi N, Akpinar S, Tosun O, Comunoglu C. Utility of semiquantitative parameters to differentiate benign and malignant focal hepatic lesions. *Clin Imaging*. 2013;37(4):692-6.
14. Liu D, Ba Z, Ni X, Wang L, Yu D, Ma X. Apparent Diffusion Coefficient to Subdivide Breast Imaging Reporting and Data System Magnetic Resonance Imaging (BI-RADS-MRI) Category 4 Lesions. Med Sci Monit. 2018;24:2180-2188.
15. Baltzer PA, Krug KB, Dietzel M. Evidence-Based and Structured Diagnosis in Breast MRI using the Kaiser Score. Fortschr Röntgenstr 2022; 194: 1216 -1228.
16. Raza S, Vallejo M, Chikarmane SA, Birdwell RL. Pure ductal carcinoma in situ: A range of MRI features. *Am J Roentgenol*. 2008;191(3):689–99.
17. Kuhl CK, Schild HH. Dynamic image interpretation of MRI of the breast. *J Magn Reson Imaging*. 2000;12(6):965–74.
18. Jansen SA, Newstead GM, Abe H, Shimauchi A, Schmidt RA, Karczmar GS. Pure ductal carcinoma in situ: Kinetic and morphologic MR characteristics compared with mammographic appearance and nuclear grade. *Radiology*. 2007;245(3):684–91.
19. Menell JH, Morris EA, Dershaw DD, Abramson AF, Brogi E, Liberman L. Determination of the presence and extent of pure ductal carcinoma in situ by mammography and magnetic resonance imaging. *Breast J*. 2005;11(6):382–90.
20. Kuhl CK, Mielcareck P, Klaschik S et al. Dynamic breast MR imaging: Are signal intensity time course data useful for differential diagnosis of enhancing lesions? *Radiology*. 1999;211(1):101–10.
21. Zhu Y, Zhang S, Liu P, Lu H, Xu Y, Yang WT. Solitary intraductal papillomas of the breast: MRI features and differentiation from small invasive ductal carcinomas. *Am J Roentgenol*. 2012;199(4):936–42.
22. Alduk AM, Brcic I, Podolski P, Prutki M. Correlation of MRI features and pathohistological prognostic factors in invasive ductal breast carcinoma. *Acta Clin Belgica Int J Clin Lab Med*. 2017;72(5):306–12.
23. Woodhams R, Ramadan S, Stanwell P, Sakamoto S, Hata H, Ozaki M, et al. Diffusion-weighted imaging of the breast: Principles and clinical applications. *Radiographics*. 2011;31(4):1059–84.
24. Padhani AR, Liu G, Mu-Koh D et al. Diffusion-weighted magnetic resonance imaging as a cancer biomarker: Consensus and recommendations. *Neoplasia*. 2009;11(2):102–25.
25. Bonekamp S, Corona-Villalobos CP, Kamel IR. Oncologic applications of diffusion-weighted MRI in the body. *J Magn Reson Imaging*. 2012;35(2):257–79.
26. Yoshikawa MI, Ohsumi S, Sugata S et al. Relation between cancer cellularity and apparent diffusion coefficient values using diffusion-weighted magnetic resonance imaging in breast cancer. *Radiat Med - Med Imaging Radiat Oncol*. 2008;26(4):222–6.
27. Yabuuchi H, Matsuo Y, Okafuji T et al. Enhanced mass on contrast-enhanced breast MR imaging: Lesion characterization using combination of dynamic contrast-enhanced and diffusion-weighted MR images. *J Magn Reson Imaging*. 2008;28(5):1157–65.
28. Partridge SC, Mullins CD, Kurland BF et al. Apparent diffusion coefficient values for discriminating benign and malignant breast MRI lesions: Effects of lesion type and size. *Am J Roentgenol*. 2010;194(6):1664–73.
29. Costantini M, Belli P, Rinaldi P et al. Diffusion-weighted imaging in breast cancer: Relationship between apparent diffusion coefficient and tumour aggressiveness. *Clin Radiol*. 2010;65(12):1005–12.
30. Plein S, Schwitter J, Suerder D, Greenwood JP, Boesiger P, Kozerke S. k-Space and time sensitivity encoding-accelerated myocardial perfusion MR imaging at 3.0 T: comparison with 1.5 T. *Radiology*. 2008 Nov;249(2):493-500.
31. Peter SC, Wenkel E, Weiland E et al. Combination of an ultrafast TWIST-VIBE Dixon sequence protocol and diffusion-weighted imaging into an accurate easily applicable classification tool for masses in breast MRI. *Eur Radiol*. 2020; 54(2):3413–9.
32. Yamada T, Mori N, Watanabe M et al. Radiologic-pathologic correlation of ductal carcinoma in situ. *Radiographics*. 2010;30(5):1183–98.
33. Khiat A, Gianfelice D, Amara M, Boulanger Y. Influence of post-treatment delay on the evaluation of the response to focused ultrasound surgery of breast cancer by dynamic contrast enhanced MRI. *Br J Radiol*. 2006;79(940):308–14.
34. Kuhl CK. Science to practice: Why do purely intraductal cancers enhance on breast MR images? *Radiology*. 2009;253(2):281–3.

MORFO-DINAMSKE I FUNKCIONALNE KARAKTERISTIKE MRI DOJKI U PROCENI DUKTLANOG KARCINOMA IN SITU I INVAZIVNOG DUKTALNOG KARCINOMA KOD POSTMENOPAUZALNIH ISPITANICA

Marko Mihajlović[1], Anđela Đajić[1], Iva Krušac[1], Dejan Dimitrijević[1], Luka Raspopović[1], Mirjan Nadrljanski[1, 2]

Sažetak

Uvod: Najčešći histološki tipovi karcinoma dojke podrazumevaju invazivni duktalni karcinom (IDC), kao i njegov najčešći prekursor – duktalni karcinom in situ (DCIS). Evaluacija morfo-dinamskih i funkcionalnih parametara na MRI dojki: veličina lezije, inicijalno postkontrastno povećanje intenziteta signala (wash-in), definisanje tipa krive promene intenziteta signala u jedinici vremena (TIC), koeficijent difuzije (ADC), kao i procena pozitivne vrednosti integrala postkontrastnog povećanja intenziteta signala kod postmenopauzalnih ispitanica, omogućavaju diferencijaciju između IDC i in situ lezije.

Metode: U retrospektivno ispitivanje sprovedeno u jednom centru, uključeno je 40 postmenopauzalnih ispitanica sa potvrđenom histopatološkom dijagnozom (DCIS n_1=20; IDC n_2=20), kod kojih su analizirane lezije na MRI dojki sa primenom standardnog - kompletnog dijagnostičkog protokola na apartima 1.5T i 3T. Softverski paket SPSS 21.0 je korišćen u statističkoj analizi – proceni razlike predefinisanih parametara u dve grupe ispitanica.

Rezultati: Veličina tumora je bila statistički značajno veća ($p<0.001$) kod ispitanica sa DCIS. U podgrupi ispitanica sa IDC, postoji statistički značajna razlika – veće vrednosti "wash-in" i PEI ($p<0.001$). Vrednost ADC je bila statistički značajno veća u grupi ispitanica sa DCIS ($p<0.001$). Postoji i značajna razlika u distribuciji TIC ($p<0.05$): TIC tip 2 je najviše zastupljena kod ispitanica sa DCIS, dok je TIC tip 3 dominantno zastupljena kod ispitanica sa IDC.

Zaključak: Na osnovu predefinisanih morfo-dinamskih i funkcionalnih parametara, MRI dojki može da omogući diferencijaciju između dva tipa duktalnog karcinoma: IDC i DCIS. Histopatološka potvrda ostaje „zlatni standard" u diferencijaciji, uzimajući u obzir prirodu bolesti..

Ključne reči: MRI dojki, duktalni karcinom in situ, invazivni duktalni karcinom, morfo-dinamski parametri, funkcionalni parametri

Primljen: 20.06.2023. I **Revizija:** 30.04.2024. I **Prihvaćen:** 30.04.2024.

Medicinska istaživanja 2024; 57(3):1-7

Medicinska istaživanja 2024; 57(3):9-17 | DOI: 10.5937/medi57-48108

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

The Association of Pain with Walking Speed and Functional Abilities in Patients Suffering from progressive forms of multiple sclerosis

Sindi Mitrović[1,2], Aleksandra Vidaković[1,2], Olivera Đorđević[1,2], Zoran Bukumirić[1,3], Suzana Dedijer-Dujović[1,2], Tijana Dimkić Tomić[1,2], Ljubica Konstantinović[1,2]

[1] University of Belgrade, Faculty of Medicine, Belgrade, Serbia
[2] Clinic for rehabilitation „Dr Miroslav Zotović“, Belgrade, Serbia
[3] Institute for Medical Statistics and Informatics, Belgrade, Serbia

Recived: 26 December 2023
Revised: 09 April 2024
Accepted: 23 April 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Sindi Mitrović
Clinic for rehabilitation „Dr Miroslav Zotović“
13 Sokobanjska Street, 11000 Belgrade, Serbia
E-mail: sindizmitrovic@gamil.com

Summary

Introduction/Aim: In progressive forms of MS, the frequency of pain increases as the disease progresses affecting patients' functional abilities and making the disease much more complex. We conducted a cross-sectional study to examine the association of pain with walking speed and functional abilities in patients who suffer from progressive forms of multiple sclerosis.

Material and methods: The cross-sectional study was conducted at the Clinic for rehabilitation "Dr Miroslav Zotović" in the period from January 2020 to May 2023. The research included 55 patients with PPMS and SPMS consecutively admitted to this Clinic for rehabilitation. Demographic and socio-epidemiological data and disease-related data were collected from all the patients. Pain intensity was assessed using *Numeric Rating Scale* (NRS). Since all patients experienced spasticity, pain was also assessed using the Pain/Discomfort (PD) subscale of the Multiple Sclerosis Spasticity Scale 88 (MSSS-88). The subjective perception of gait impairment was assessed using a subscale of the same questionnaire, MSSS 88, related to walking, namely the Walk (WL). Walking speed was measured by *The Timed 25 Foot Walk* (T25FW). The functional assessment and all questionnaires were completed in the morning hours over a 24-hour period from the day patients were admitted to rehabilitation.

Results: There is a significant strong correlation between WL and P/D (rho=0. 770; p<0.001) and between WL and NRS (rho=0. 825 p<0.001). There is a statistically significant moderate negative correlation between T25FW and NRS pain (p<0.001). There is no statistically significant correlation between T25FW and pain intensity measured by PD (p=0.033). There is a statistically significant correlation between EDSS and pain intensity (NRS p=0.002; PD p=0.006) either.

Conclusion: The results of this research indicated a significant negative impact of pain on walking speed and functional disability.

Key words: pain, walking speed, functional disability, progressive forms of multiple sclerosis

Cite this article as: Mitrović S, Vidaković A, Đorđević O, Bukumirić Z, Dedijer-Dujović S, Dimkić Tomić T, Konstantinović LJ. The Association of Pain with Walking Speed and Functional Abilities in Patients Suffering from progressive forms of multiple sclerosis; Medicinska istaživanja 2024; 57(3):9-17 DOI: 10.5937/medi57-48108

INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated and neurodegenerative disease of the central nervous system (CNS) whose etiology remains unknown (1). Since it affects the most productive population and leads to long-term physical disability over time, MS is not only a health problem, but a social and economic problem as well (2). Recognizing the dominant cause of functional impairment in MS patients is of great importance for tailoring an individualized, effective treatment plan and improving the quality of life.

Throughout the course of MS, pain is a common, varying symptom which has significant effects on an individual's functional capacity and quality of life (3,4). It is strong predictor of activity limitations and participation restrictions in MS patients (5,6). One third of people with MS describes pain as one of their worst symptoms (7). Pain can appear as an acute syndrome, but chronic pain occurs in 50–75% of MS patients in different stages of disease progression (8). The most frequent types of pain are spasticity pain and headache (prevalence rates of 43%–60%), central neuropathic pain (CNP, 5%–28%), and back pain (10%–20%) (9). According to literature data, pain is treated in only 38% of patients, and treatment satisfaction has been verified in 61% of patients (10). Poor analgesic outcomes indicates that pain control in these patients is still insufficient (10) and may result from pain heterogeneity in MS (11,12). Pain relief is not important only because of the frequency of pain and its impact on functional disability during the disease, but also because of the consequences it has on various daily aspects of patient's life. Recognized risk factors for pain in MS are as follows: older age, longer disease duration, higher EDSS score, accompanying depression or mental disorders, unstable course of the disease, lower level of education, progressive disease phenotypes (5,13). In progressive forms of MS, the frequency of pain and the degree of spasticity increase with disease progression, which affects the gait and has negative consequences on patients' functional status and the quality of life (14). Rehabilitation requirements are derived from severe functional impairment, where the treatment of spasticity, pain and muscle weakness is of greatest importance (2).

Functionality is a complex process that includes an interaction between a patient's mental and physical abilities and is not linearly correlated with the impairment. Walking ability is the basic characteristic of functional independence, so one of the greatest challenges in rehabilitation of patients with MS is to preserve it. The results of some studies showed that 70% of patients with walking difficulties perceived this aspect of the disease as the main functional problem in performing daily activities (15). A large number of patients experience walking difficulties in early stages of the disease (2). In the first month upon the diagnosis, 45% of patients experience impaired mobility, while after a decade of living with the disease 93% of patients have walking difficulties (16). Walking difficulties can be related to reduced walking capacity alone or combined with MS symptoms, such as reduced muscle strength, spasticity, poor balance, fatigue, pain, and depression (17,18).

Although there is considerable medical and scientific interest, the association between pain intensity and walking speed and functional disability has not been fully established in MS patients yet. We conducted a cross-sectional study with the aim of examining the association of pain with walking speed and functional disability in MS patients.

MATERIAL AND METHODS

The research included 55 patients diagnosed with primary progressive multiple sclerosis (PPMS) and secondary progressive multiple sclerosis (SPMS) consecutively admitted for rehabilitation at the Clinic for rehabilitation "Dr Miroslav Zotović". The inclusion criteria were as follows: (a) Patients 18-65 years of age with a confirmed form of SPMS or PPMS, (b) Presence of back pain and/or painful spasms in lower extremities for more than six months (in order to exclude the presence of pain due to current exacerbations of MS), (c) Degree of functional disability (*Expanded Disability Status Scale* - EDSS) < 6.5 and (d) PainDETECT questionare score <19 (19). The exclusion criteria were as follows: clinical worsening in the past 30 days, other medical conditions that interfered with walking, headaches, serious associated diseases (malignancy, cardiovascular disease).

The research was conducted in the period from January 2020 to May 2023.

The sample size was based on the assessment of the correlation between variables in patients with multiple sclerosis. According to the literature, a correlation between the variables EDSS and Pain/Discomfort (PD) was found in patients with multiple sclerosis (correlation coefficient 0.38)

(20). The minimum number of participants needed to estimate the correlation of these variables of interest with a statistical power of 80% and a significance level of 0.05 is 52.

Each patient was introduced to all the details of the protocol and their written consent was required. Demographic and socio-epidemiological data (age, gender, marital status and level of education) as well as the disease-related data (disease duration, disease form, EDSS, presence of spasticity for six months, representation of pain, analgesic and spasmolytic therapy, satisfaction with pain relief) were collected. The *Numerical Rating Scale* (NRS) was used for the assessment of pain intensity. Since all patients experienced spasticity, pain was also assessed using the Pain/Discomfort (PD) subscale of the

Multiple Sclerosis Spasticity Scale 88 (MSSS-88). The subjective perception of gait impairment was assessed using a subscale of the same questionnaire, MSSS 88, related to walking, namely the Walk (WL). Both subscales were used particularly since authors suggest they could be used as stand-alone subscales (20,21). Quantitative assessment of mobility and leg function performance was measured according to walking speed using the clinical instrument *The Timed 25 Foot Walk* (T25FW). T25FW was used by a physiotherapist while other measurements were done by doctors. The functional assessment and all questionnaires were completed in the morning hours over a 24-hour period from the day patients were admitted to rehabilitation.

All participants signed an informed consent for taking part in the research, which was approved by the Clinic's Ethics Committee (No.32-2226/2).

Pain intensity was assessed using the following scales:

Numeric Rating Scale (NRS) represents a subjective assessment of pain intensity in patients. The 11-point numeric scale ranges from 0 to 10, where 0 indicates the absence of pain and 10 indicates the most intense pain (unbearable pain). Mild pain is graded as 1, 2 or 3, moderate pain is graded as 4, 5 or 6, while severe pain is graded as 7, 8 or 9 and the most intense pain is graded as 10. Patients were given verbal instructions to choose a number on the scale that corresponded to pain intensity they experienced in the past 24h or, more precisely, to choose three ratings on the scale that corresponded to the current, best, and worst pain intensity experienced in the past 24h. The mean value of these three values represented the final grade (3,22).

Pain/Discomfort (PD) – a subscale of the *Multiple Sclerosis Spasticity Scale88* (MSSS-88) questionnaire. The subscale contains 9 questions. Answer categories are graded according to Likert in the following way: 1. does not bother me at all, 2. it bothers me a little, 3. it bothers me moderately, 4. it bothers me a lot. We calculated the score of each subscale separately and the total score by simply adding up the responses. The subscale values can be scored as an independent measurement instrument, and possible values are 9 to 36. This scale correlated significantly with MSWS-12 and also corelated with EDSS (20,23).

Gait was assessed by the following test and scale:

T25FW (*theTimed 25 Foot Walk*)–a clinical instrument for quantitative assessment of mobility and leg function performance based on a timed 25-foot walk (7.62m). Subjects are instructed to walk a marked 7.62m-long path quickly and safely. The time is calculated from the moment instructions are given to the end of the marked 7.62 m-long path. The second T25FW performance is done immediately after the first, having the patient walk the same distance. Patients are allowed to use aids during the test. T25FW score is the average of two performance tests (24,25).

Walk (WL - Perception of gait impairment)–a subscale of the *Multiple Sclerosis Spasticity Scale88* (MSSS-88) questionnaire. The subscale contained 10 questions. The response categories were graded according to Likert in the following way: 1. it does not bother me at all, 2. it bothers me a little, 3. it bothers me moderately, 4. it bothers me a lot. We calculated the score of each scale separately, as well as the overall score by simply adding up the responses. Subscale values can be scored as an independent measurement instrument, possible values being 10-40. This scale correlated significantly with EDSS, MAS (Modified Ashworth Scale) and MSWS-12 (20,23).

The degree of functional impairment was assessed using the following scales:

Expanded Disability Status Scale (EDSS)is a scale used for assessing physical impairment in MS patients. According to this scale and the neurological examination, the impairment is presented using a numeric value. The degree of impairment in 8 functional systems (visual, brainstem, pyramidal tract, sensory, cerebellar, sphincter function, cerebral or mental function) and the patient's ability to walk were assessed. This score can range from 0 (normal neurological finding despite the symptoms) to 10 (death due to MS). EDSS 1.0 to 4.5 corresponds to patients who can walk independently. EDSS 5.0 to 9.5 includes the presence of a severe mobility disorder. The scale has good validity and reliability, whereas some studies found its sensitivity inadequate. Another disadvantage is a significant impact of walk on the overall score (26).

STATISTICAL ANALYSIS

Depending on the type of variables and normality of distribution, the description of the data is shown as n (%), arithmetic mean ±standard deviation, or median (range, min-max). The Pearson correlation coefficient and Spearman's rank correlation coefficient are used to analyze correlations. Statistical hypotheses were tested at a statistical significance level (alpha level) of 0.05. All data were processed in IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) software package.

RESULTS

The study included 55 patients with progressive forms of MS. The basic demographic characteristics of the patients are shown in **Table 1**.

The majority of patients were female (80%), had secondary education (72.7%), were married (65.5%), and retired (58.2%). The average age of the respondents was 46.3±9,4 years. **(Table 1)**

Table 1. The basic demographic characteristics of the patients

Variable	Respondents (n=55)
Gender	
• male	11 (20.0%)
• female	44 (80.0%)
Age	45.96±9.4 (29-67)
Level of education	
• high school	40 (72.7%)
• university	15 (27.3%)
Marital status	
• married	36 (65.5%),
• divorced	7 (12.7%)
• single	10 (18.2%)
• widowed	2 (3.6%)
Employment	
• employed	16 (29.1%)
• retired	32 (58.2%)
• unemployed	7 (12.7%)

[a] arithmetic mean ±standard deviation

Table 2. The basic clinical characteristics of patients

Variable	Respondents (n=55)
Disease form	
- primary progressive	32 (58.2%)
- secondary progressive	23 (41.8%)
Disease duration[a] (years)	11,56±6,271 (2-24)
Spasticity	
Yes	55 (100%)
No	0
Representation of pain	
Back pain	25 (45.45%)
Painful spasms in lower limb	17 (30.9%)
Back pain and painful spasms in lower limb	13 (23.6%)
Analgesics	25 (45%)
NSAIL	15 (60%)
Anticonvulsants	9 (36%)
Other (homeopathic remedies)	1 (4%)
Spasmolytics	20 (36.4%)
Satisfaction with pain relief	26 (47.27%)
NRS pain[a]	5 (3-8)
P/D[b]	17.6±5.3 (9-28)
T25FW[b]	0.76±0.25 (0.32-1.33)
WL[b]	24.2±7.5 (11-40)
EDSS score[a]	5(3-6,5)

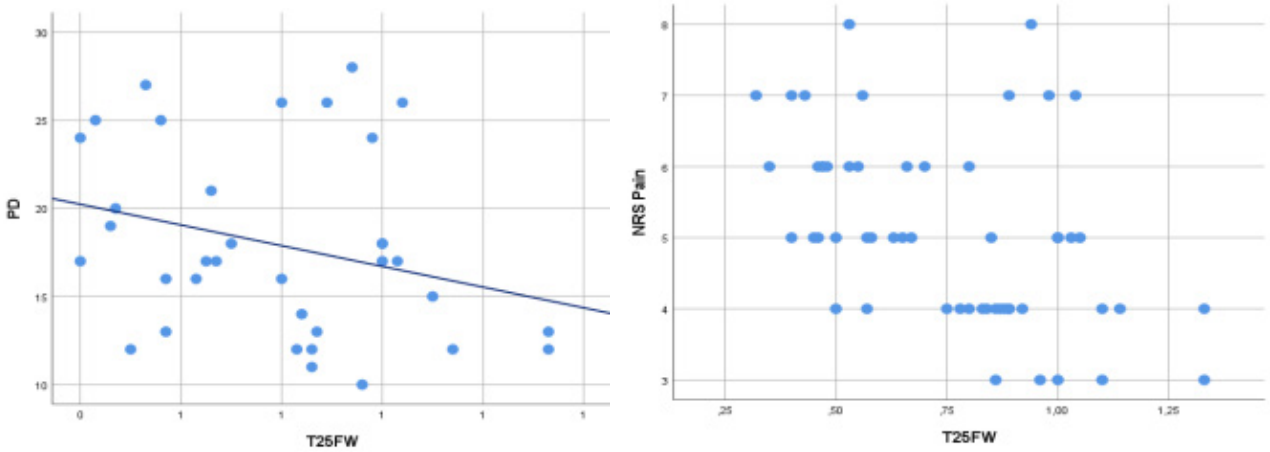
[a]median±interquartile range (range); [b]arithmetic median±standard deviation (range) EDSS – Expanded Disability Status Scale; T25FW – The Timed 25 Foot Walk; NRS – Numeric Rating Scale for pain assessment; P/D - pain and discomfort; WL – Walk

According to clinical characteristics, the majority of patients had the primary progressive (PP) form of MS (58.2%) with median disease duration of 11 years (range, 2-22). Median NRS pain was 5 (range, 3-8), while the average PD value in all respondents was 17.6±5.3 (the lowest value was 9, and the highest value was 28). The total number of patients who used analgesics was 25 (45%), of which 25 (60%) used NSAIL, 9 (36%) used anticonvulsants, and 1 (4%) used homeopathic remedies; 26 (47.27%) patients were satisfied with pain relief they obtained. All patients had spasticity lasting more than six months but only 36.4% of them used spasmolytics. Most of the patients 25 (45.45%) had back pain while painful spasms in lower limb had 17 (30.9%) and both symptoms had 13 (23.6%) of the patients. The average walking speed, measured by T25FW, of all participants was 0.76±0.25 (range 0.32 – 1.33). Subjective assessment of walking difficulty measured by WL was 24.2±7.5 (range 11-40). Median EDSS score was 5 (range, 3-6.5). **(Table 2)**

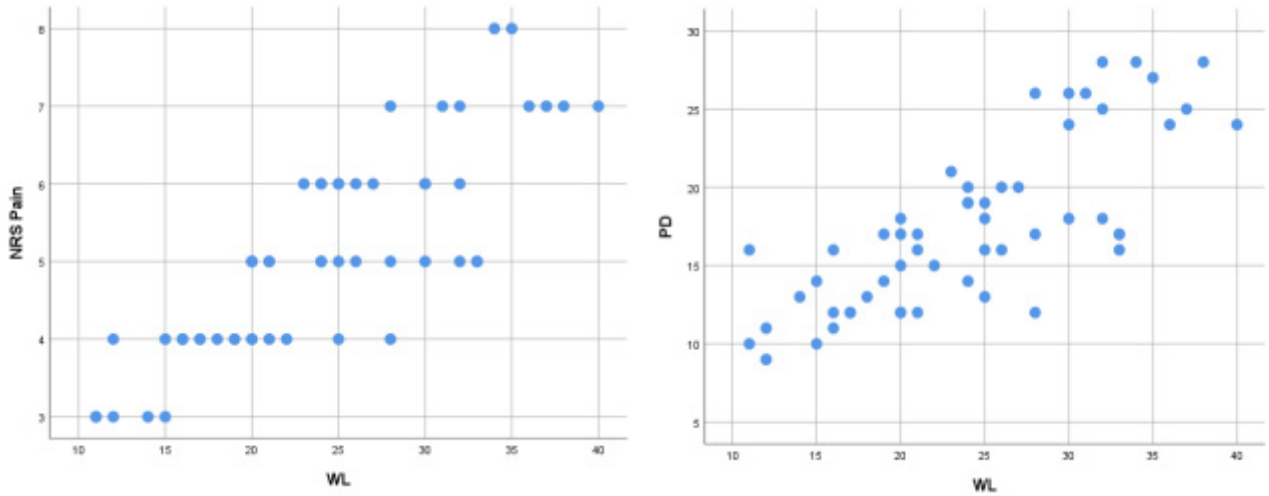
There is no statistically significant correlation between T25FW and P/D (r=-0.288; p=0.033). There is a statistically significant moderate negative correlation between T25FW and NRS pain score (rho=-0.475; p<0.001). Lower values of T25FW are associated with higher values of NRS pain score.

There is a statistically significant strong correlation between WL and NRS pain score (rho=0.825; p<0.001), as well as between WL and P/D (rho=0.770; p<0.001). Higher values of WL are associated with higher values of NRS and P/D.

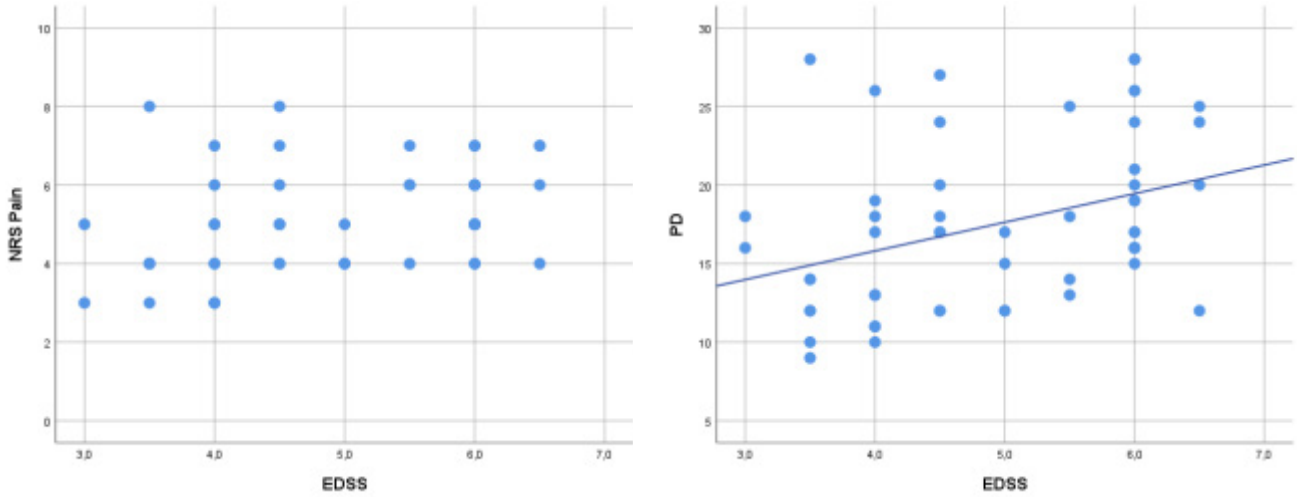
There is a statistically significant mild correlation between EDSS and NRS (rho=0.418; p=0.002) and between EDSS and PD (rho=0.392; p=0.003).



Graph 1. The relation between walking speed (T25FW) and pain intensity (P/D, NRS)



Graph 2. The relation between walk (WL) and pain intensity (NRS, P/D)



Graph 3. The relation between expanded disability status scale (EDSS) and pain intensity (NRS, P/D)

Summarizing the presented results, we can say that there is a significant strong correlation between WL and P/D (rho=0. 770; p<0.001) and between WL and NRS (rho=0. 825 p<0.001). In other words, the greater the intensity of pain, the more difficulty walking is percieved by patients. It has been shown that there is a statistically significant moderate negative correlation between T25FW and NRS pain (p<0.001). However, there is no statistically significant correlation between T25FW and pain intensity measured by PD (p=0.033). There is a statistically significant correlation between EDSS and pain intensity (NRS p=0.002; PD p=0.006) either. **Table 3.** The association of pain and walking speed and the degree of functional disability

DISCUSSION

Our research aimed at examining the association of pain with walking speed and functional disability in patients suffering from progressive forms of MS. According to literature (PubMed/Medline, Scopus and search engine of Google Scholar) the influence of pain on walking speed and functional disability regardless of the form of the disease has not been examined simultaneously yet. Our results indicated the presence of a significant strong correlation between subjective perception of walk (WL) and pain intensity (NRS)/feeling of discomfort (P/D). It was shown that there was a statistically significant moderate negative correlation between walking speed (T25FW) and pain intensity measured by NRS with no significant correlation with pain intensity measured by PD. There

Measuring instruments	T25FW	WL	EDSS
NRS	rho=-0.36; p=0.035	rho=0.831; p<0.001	rho=0.29; p=0.086
P/D	r=-0.288; p=0.033	rho=0.770; p<0.001	r=0.367; p=0.006

Rho – Spearman's rank correlation coefficient; r – correlation coefficient; p – p value; NRS – pain numeric rating scale; P/D – pain and discomfort; W – walk; T25FW – the timed 25 foot walk; EDSS – Expanded Disability Status Scale

was statistically significant correlation between pain intensity (NRS) and the feeling of pain and discomfort and the degree of physical disability (EDSS). This type of correlation indicates that pain therapy should be an integral part of the treatment these patients receive.

In our study we used T25FW as a clinical instrument for quantitative assessment of leg mobility and function and it proved to be valid for assessing walking speed in patients with MS (25). The respondents' average speed in relation to the degree of disability was slightly lower compared to the results of the study conducted by Langeskov-Christensen and colleagues (27). The aim of their study was to quantify walking impairment and perceived impact of MS on walking according to EDSS scores and to examine the relations between these parameters in 474 patients with MS. In the study, in moderate MS group (EDSS ≥ 4.5 and ≤6.5) most patients had progressive forms of MS (RR108, SP116, PP 50) with T25FW m/s: 0.93 ± 0.36 m/s. In this group walking speed correlates more closely to EDSS (-0.47) than in the mild group with lower EDSS (-0.37) with majority of patients with relapse remitting form of MS (27). The walking speed and functional disability of our patient were similar to patients in moderate group form Langeskov-Christensen and colleagues study. To assess gait, from patients' with spasticity point of view, we used a specific subscale of the WL questionnaire MSSS88 whose values indicated that our participants perceived a greater degree of gait impairment. Pain intensity was moderate in all participants in our study. There was a statistically significant strong correlation between NRS and subjective and objective measuring instruments for gait assessment. When it comes to subjective assessment of pain intensity using the P/D scale, there was a significant correlation with the subscale related to perceived gait impairment, whereas there was no correlation with walking speed. Freeman J et al., in their longitudinal study, show that P/D has moderate correlation with EDSS (20).

Based on a literature review (PubMed/Medline, Scopus and search engine of Google Scholar) there are no studies that examined a correlation between pain and walking speed in patients with MS, but gait was mostly viewed through a set of symptoms (muscle weakness, spasticity, tremor, emotional/mood disturbance, fatigue, or pain) that affect one's physical activity. Some studies showed that pain reduction together with spasticity reduction led to an improvement in walking speed after various treatments and interventions, and they thus indirectly showed that pain reduction affected the improvement of walking speed (28,29). The results of a study conducted by Jesse and colleagues are another indirect indicator of an association between walking speed and pain perception (30). They examined the association between fatigue perception and gait impairment with reference to pain through the subscale of Short form health survey (SF-36) in patients with MS who have a lower degree of disability (EDSS 2.6±0.7, 2-6). The results of SF-36 scales Physical functioning and Bodily pain, which measure the overall perception of general health, had negative correlations with gait measures in MS patients, i.e., self-perception of functional ability was reduced, and self-perception of bodily pain was increased in MS patients compared to healthy population (30). In addition, Motl and colleagues examined whether worsening of symptoms affected the level of physical activity in patients with all three forms of MS and the results showed that worsening of symptoms, including an increase in bodily pain, led to a significant decrease in the level of physical activity and functional ability (18). In a subsequent study, Motl and colleagues showed that fatigue and depression, but not pain, directly affected the level of overall physical activity on a sample of 269 patients with relapsing-remitting multiple sclerosis (RRMS). Based on the result of our research and according to the previous above-mentioned studies, the presence of pain significantly affects the performance and perception of impaired lower-extremity function in patients with progressive forms of MS.

The median EDSS score of our participants is between moderate and severe disability. It should be emphasized that EDSS score values and disease duration in our respondents are in accordance with the results of other studies conducted in our population, and that they are similar to the results obtained in groups of respondents with progressive forms of MS examined in other studies (31, 32). Our results are in line with the results obtained in several previous studies that indicated pain correlates with pain and was more common in case of increased disability and longer disease duration (6,10,33,34). Some clinical studies did not find any correlation but most of them included patients with lower EDSS scores and mostly with RRMS (35, 36, 37, 38).

The results of research conducted by Shahrbanian and colleagues indicated that the degree of disability in MS (measured by EDSS) was an important predictor of the presence and intensity of pain, patients with more severe disability were more likely to experience pain (39). Furthermore, compared to patients with MS, the results indicated that patients who experienced pain were more likely to have a more severe disability than those with no pain. These results suggest that the onset of pain and variation in pain intensity cannot be predicted solely on the basis of the disease or personal characteristics, but that other factors play an important role as well.

When it comes to a longitudinal follow-up of pain syndrome, there is inconsistency in the results of the studies that examined the association of pain and functional disability. Stenager and colleagues reported a higher prevalence of several pain syndromes with disease progression (initial mean of EDSS 3.4), especially in subjects with worsening EDSS (40). On the other hand, Brochet and colleagues obtained results that indicated a statistically insignificant reduction in the prevalence of pain in patients with a lower degree of disability in the early stages of the disease (median EDSS 2) (37).

One of the possible explanations of the association between pain intensity and functional disability was provided by Grau-López and colleagues (10). Evaluating other clinical characteristics associated with pain in MS, they observed that patients with progressive forms of the disease and a higher degree of disability (measured by EDSS scale) reported pain symptoms much more often than patients with minor neurological damage. When they analyzed the relationship between pain and neuroradiological findings they followed, they observed that patients who experienced greater pain intensity had a greater myelin disruption in the central nervous system and a greater likelihood of paresis, spasticity, and abnormal posture, so they were more likely to suffer from neuropathic and nociceptive pain as well. A special contribution of this study is the finding that patients with more spinal cord lesions (regardless of their location) are more likely to experience pain. This is supported by the results of the studie that dealt with the association between spinal cord injuries of various etiologies (with a special emphasis on traumatic lesions) and neuropathic pain (41). It was shown that spinal cord lesions may cause changes in survival, function and excitability of pathways responsible for pain transmission (the spinothalamic tract) secondary due to the reduction in inhibitory neurotransmitters such as GABA and glutamate and due to the release of inflammatory mediators such as free radicals, nitric oxide, and pro-inflammatory cytokines. These changes create an environment suitable for the possible development of pain at different levels.

A better understanding of the prevalence, nature, and course of MS-related pain, as well as the identification of the groups of patients who experience most severe pain may help to evaluate the real extent of this problem. Furthermore, a better understanding of MS-related pain epidemiology may contribute to a better understanding of the mechanisms of symptoms and potentially to the development of targeted treatment strategies.

Despite all the effort researchers and doctors have been investing in solving this problem for decades, the etiology of MS remains unknown, so treatment itself cannot be directed at the cause. There is a multidisciplinary approach to the treatment and therapeutic modalities are pharmacological and non-pharmacological and they are applied individually or are combined, depending on the needs of the patient. The basic postulate of MS treatment is that the therapy should be started as soon as possible, immediately after establishing the diagnosis, as any delay contributes to further deterioration and a faster development of neurologic and functional deficits. The presence of pain in MS patients contributes to the complexity of choosing the treatment concerning its efficacy, safety and costs. It is necessary to have a multimodal approach which includes pharmacotherapy, rehabilitation, psychotherapy, interventional procedures (transcranial direct-current stimulation, spinal cord stimulation, deep brain stimulation), and lifestyle modification in order to achieve the best treatment outcome and improve the quality of life of patients and their families.

Several limitations should be considered when interpreting the results of this study. We did not consider degree of spasticity, disease form and patients are not classified according to the degree of functional disability that can has effect on the measured outcomes. We only included patients with back pain and painful spasms in lower extremities without considering other types of pain. Our study included only participants with progressive forms of MS. Pain was only evaluated with questionnaires without supporting it with magnetic resonance imaging findings. We did not compare outcome measures between patients with PPMS and SP MS because of small number of patients.

CONCLUSIONS

The results of our study indicated a significant negative impact of pain on walking speed and on functional disability. The association between pain and other factors in MS has been widely studied but remains controversial due to inconsistencies regarding numerous clinical and personal factors. The small number of available studies and the diversity of their design presented an obstacle and made it difficult for us to compare our results with the results of previous studies.

Acknowledgments: None.

Conflicts of Interest: The authors declare no conflict of interest.

Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki. The Clinic for rehabilitation „Dr Miroslav Zotović“ Ethics Committee approved this study (No.32-2226/2).

REFERENCES

1. Walton C, King R, Rechtman L, Kaye W, Leray E, Marrie RA, et al. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. Multiple Sclerosis Journal. 2020 Dec 1;26:1816–21. doi: 10.1177/1352458520970841
2. PMID: 33174475
3. Sutliff MH. Contribution of impaired mobility to patient burden in multiple sclerosis. Curr Med Res Opin. 2010 Jan;26(1):109-19. doi: 10.1185/03007990903433528. PMID: 19916707.
4. Alschuler KN, Jensen MP, Ehde DM. Defining mild, moderate, and severe pain in persons with multiple sclerosis. Pain Med 2012; 13:1358–65,
5. Failde I, Dueñas M, Ribera MV, Gálvez R, Mico JA, Salazar A, de Sola H, Pérez C. Prevalence of central and peripheral neuropathic pain in patients attending pain clinics in Spain: factors related to intensity of pain and quality of life. J Pain Res. 2018 Sep 12; 11:1835-1847. doi: 10.2147/JPR.S159729. Erratum in: J Pain Res. 2018 Oct 17; 11:2397. PMID: 30254486; PMCID: PMC6140696

6. Drulovic J, Basic-Kes V, Grgic S, Vojinovic S, Dincic E, Toncev G, Kezic MG, Kisic-Tepavcevic D, Dujmovic I, Mesaros S, Miletic-Drakulic S, Pekmezovic T. The Prevalence of Pain in Adults with Multiple Sclerosis: A Multicenter Cross-Sectional Survey. Pain Med. 2015 Aug;16(8):1597-602. doi: 10.1111/pme.12731. Epub 2015 Jun 18. PMID: 26087108.
7. Solaro C, Brichetto G, Amato MP, Cocco E, Colombo B, D'Aleo G, Gasperini C, Ghezzi A, Martinelli V, Milanese C, Patti F, Trojano M, Verdun E, Mancardi GL; PaIMS Study Group. The prevalence of pain in multiple sclerosis: a multicenter cross-sectional study. Neurology. 2004 Sep 14;63(5):919-21. doi: 10.1212/01.wnl.0000137047.85868.d6. PMID: 15365151.
8. Stenager E, Knudsen L, Jensen K. Acute and chronic pain syndromes in multiple sclerosis. Acta Neurol Scand 1991; 84:197–200
9. Racke MK, Frohman EM, Frohman T. Pain in Multiple Sclerosis: Understanding Pathophysiology, Diagnosis, and Management Through Clinical Vignettes. Front Neurol. 2022 Jan 13; 12:799698. doi: 10.3389/fneur.2021.799698. PMID: 35095742; PMCID: PMC8794582
10. Rivel M, Achiron A, Dolev M, Stern Y, Zeilig G, Defrin R. Unique features of central neuropathic pain in multiple sclerosis: Results of a cluster analysis. Eur J Pain. 2022 May;26(5):1107-1122. doi: 10.1002/ejp.1934. Epub 2022 Mar 22. PMID: 35263811; PMCID: PMC9313873.
11. Grau-López L, Sierra S, Martínez-Cáceres E, Ramo-Tello C. Analysis of the pain in multiple sclerosis patients. Neurologia. 2011 May;26(4):208-13. English, Spanish. doi: 10.1016/j.nrl.2010.07.014. Epub 2010 Oct 2. PMID: 21163193.
12. Ferraro D, Plantone D, Morselli F, Dallari G, Simone AM, Vitetta F, Sola P, Primiano G, Nociti V, Pardini M, Mirabella M, Vollono C. Systematic assessment and characterization of chronic pain in multiple sclerosis patients. Neurol Sci 2018; 39:445–53.
13. Solaro C, Cella M, Signori A, Martinelli V, Radaelli M, Centonze D, Sica F, Grasso MG, Clemenzi A, Bonavita S, Esposito S, Patti F, D'Amico E, Cruccu G, Truini A. Neuropathic Pain Special Interest Group of the Italian Neurological S. Identifying neuropathic pain in patients with multiple sclerosis: a cross-sectional multicenter study using highly specific criteria. J Neurol 2018; 265:828–35
14. Kalia LV, O'Connor PW. Severity of chronic pain and its relationship to quality of life in multiple sclerosis. Mult Scler. 2005 Jun;11(3):322-7. doi:10.1191/1352458505ms1168oa. PMID: 15957515.
15. Kratz AL, Whibley D, Alschuler KN, Ehde DM, Williams DA, Clauw DJ, Braley TJ. Characterizing chronic pain phenotypes in multiple sclerosis: a nationwide survey study. Pain. 2021 May 1;162(5):1426-1433. doi: 10.1097/j.pain.0000000000002136. PMID: 33196577; PMCID: PMC8054538.
16. Larocca NG. Impact of walking impairment in multiple sclerosis: perspectives of patients and care partners. Patient. 2011;4(3):189-201. doi: 10.2165/11591150-000000000-00000. PMID: 21766914.
17. Hartung HP. Impact of mobility impairment in Multiple sclerosis 1-healthcare professionals' perspectives. European Neurological Review. 2011 Jul; 6:110–4.https://doi.org/10.17925/ENR.2011.06.02.110
18. Motl RW, Snook EM, McAuley E, Gliottoni RC. Symptoms, self-efficacy, and physical activity among individuals with multiple sclerosis. Res Nurs Health. 2006 Dec;29(6):597-606. doi: 10.1002/nur.20161. PMID: 17131278.
19. Motl RW, Arnett PA, Smith MM, Barwick FH, Ahlstrom B, Stover EJ. Worsening of symptoms is associated with lower physical activity levels in individuals with multiple sclerosis. MultScler. 2008 Jan;14(1):140-2. doi: 10.1177/1352458507079126. PMID: 18089672.
20. Freynhagen R, Baron R, Gockel U, T ¨olle TR. painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. Curr Med Res Opin 2006; 22:1911–20.
21. Freeman J, Gorst T, Ofori J, Marsden J. Evaluation of the Multiple Sclerosis Spasticity Scale 88: A Short Report. Rehabil Process Outcome. 2019 Jan 16;8:1179572718823510. doi:10.1177/1179572718823510
22. Hobart JC, Riazi A, Thompson AJ, Styles IM, Ingram W, Vickery PJ, Warner M, Fox PJ, Zajicek JP. Getting the measure of spasticity in multiple sclerosis: the Multiple Sclerosis Spasticity Scale (MSSS-88). Brain. 2006 Jan;129(Pt 1):224-34. doi: 10.1093/brain/awh675. Epub 2005 Nov 9. PMID: 16280352.
23. Hawker GA, Mian S, Kendzerska T, French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). Arthritis Care Res (Hoboken). 2011 Nov;63 Suppl 11:S240-52. doi: 10.1002/acr.20543. PMID: 22588748.
24. Rodic SZ, Knezevic TI, Kisic-Tepavcevic DB, Dackovic JR, Dujmovic I, Pekmezovic TD, Drulovic JS, Konstantinovic LM. Validation of the Serbian Version of Multiple Sclerosis Spasticity Scale 88 (MSSS-88). PLoS One. 2016 Jan 15;11(1):e0147042. doi: 10.1371/journal.pone.0147042. PMID: 26771661; PMCID: PMC4714847.
25. Kaufman M, Moyer D, Norton J. The significant change for the Timed 25-foot Walk in the multiple sclerosis functional composite. Mult Scler. 2000 Aug;6(4):286-90. doi: 10.1177/135245850000600411. PMID: 10962550.
26. Motl, R.W.; Cohen, J.A.; Benedict, R.; Phillips, G.; LaRocca, N.; Hudson, L.D.; Rudick, R. Validity of the timed 25-foot walk as an ambulatory performance outcome measure for multiple sclerosis. Mult. Scler. J. 2017, 23, 704–710
27. Amiri B, Sahebozamani M, Sedighi B. The effects of 10-week core stability training on balance in women with multiple sclerosis according to Expanded Disability Status Scale: a single-blinded randomized controlled trial. Eur J Phys Rehabil Med. 2019 Apr;55(2):199-208. doi: 10.23736/S1973-9087.18.04778-0. Epub 2018 Jan 2. PMID: 29307152.
28. Langeskov-Christensen D, Feys P, Baert I, Riemenschneider M, Stenager E, Dalgas U. Performed and perceived walking ability in relation to the Expanded Disability Status Scale in persons with multiple sclerosis. J Neurol Sci. 2017 Nov 15; 382:131-136. doi: 10.1016/j.jns.2017.09.049. Epub 2017 Oct 3. PMID: 29111007.
29. Marinelli L, Mori L, Solaro C, Uccelli A, Pelosin E, Currà A, Molfetta L, Abbruzzese G, Trompetto C. Effect of radial shock wave therapy on pain and muscle hypertonia: a double-blind study in patients with multiple sclerosis. MultScler. 2015 Apr;21(5):622-9. doi: 10.1177/1352458514549566. Epub 2014 Sep 25. PMID: 25257616.
30. Marinaro C, Costantino C, D'Esposito O, Barletta M, Indino A, De Scorpio G, Ammendolia A. Synergic use of botulinum toxin injection and radial extracorporeal shockwave therapy in Multiple Sclerosis spasticity. Acta Biomed. 2021 Jan 28;92(1):e2021076. doi: 10.23750/abm.v92i1.11101. PMID: 33682833; PMCID: PMC7975967.
31. Huisinga JM, Filipi ML, Schmid KK, Stergiou N. Is there a relationship between fatigue questionnaires and gait mechanics in persons with multiple sclerosis? Arch Phys Med Rehabil. 2011 Oct;92(10):1594-601. doi: 10.1016/j.apmr.2011.05.017. Epub 2011 Aug 27. PMID: 21872838.
32. Lević ZM, Dujmović I, Pekmezović T, Jarebinski M, Marinković J, Stojsavljević N, Drulović J. Prognostic factors for survival in multiple sclerosis. MultScler. 1999 Jun;5(3):171-8. doi: 10.1177/135245859900500306. PMID: 10408717.
33. Confavreux C, Vukusic S. Natural history of multiple sclerosis: a unifying concept. Brain. 2006 Mar;129(Pt 3):606-16. doi: 10.1093/brain/awl007. Epub 2006 Jan 16. PMID: 16415308.
34. Martinelli Boneschi F, Colombo B, Annovazzi P, Martinelli V, Bernasconi L, Solaro C, Comi G. Lifetime and actual prevalence of pain and headache in multiple sclerosis. MultScler. 2008 May;14(4):514-21. doi: 10.1177/1352458507085551. PMID: 18562506.
35. Douglas C, Wollin JA, Windsor C. Illness and demographic correlates of chronic pain among a community-based sample of people with multiple sclerosis. Arch Phys Med Rehabil. 2008 Oct;89(10):1923-32. doi: 10.1016/j.apmr.2008.03.022. PMID: 18929020.
36. Amer KA, Aldosari AA, Somaily MY, Shawkhan RA, Almuhsini RA, Al Mater MA, Al Saleh AI, Alshabeeb MS, Alshahrani FS. The Assessment of the Prevalence and Disability Severity of Musculoskeletal Pain in Patients With Multiple Sclerosis in Saudi Arabia. Cureus. 2022 Dec 11;14(12):e32413. doi: 10.7759/cureus.32413. PMID: 36644051; PMCID: PMC9833628.
37. Beiske AG, Pedersen ED, Czujko B, Myhr KM. Pain and sensory complaints in multiple sclerosis. Eur J Neurol. 2004 Jul;11(7):479-82. doi: 10.1111/j.1468-1331.2004.00815.x. PMID: 15257687.

38. Brochet B, Deloire MS, Ouallet JC, Salort E, Bonnet M, Jové J, Petry KG. Pain and quality of life in the early stages after multiple sclerosis diagnosis: a 2-year longitudinal study. Clin J Pain. 2009 Mar-Apr;25(3):211-7. doi: 10.1097/AJP.0b013e3181891347. PMID: 19333171.

39. Grasso MG, Clemenzi A, Tonini A, Pace L, Casillo P, Cuccaro A, Pompa A, Troisi E. Pain in multiple sclerosis: a clinical and instrumental approach. MultScler. 2008 May;14(4):506-13. doi: 10.1177/1352458507085553. PMID: 18562505.

40. Shahrbanian S, Duquette P, E Mayo N. Impairment, disability and fatigue in multiple sclerosis. Caspian J Intern Med. 2018 Summer;9(3):244-251. doi: 10.22088/cjim.9.3.244. PMID: 30197769; PMCID: PMC6121335.

41. Stenager E, Knudsen L, Jensen K. Acute and chronic pain syndromes in multiple sclerosis. A 5-year follow-up study. Ital J Neurol Sci. 1995 Dec;16(9):629-32. doi: 10.1007/BF02230913. PMID: 8838789.

42. Yezierski RP. Spinal cord injury pain: spinal and supraspinal mechanisms. J Rehabil Res Dev. 2009;46(1):95-107. PMID: 19533523.

POVEZANOST BOLA SA BRZINOM HODA I FUNKCIONALNOM SPOSOBNOŠĆU KOD PACIJENATA OBOLELIH OD PROGRESIVNIH FORMI MULTIPLE SKLEROZE

Sindi Mitrović[1,2], Aleksandra Vidaković[1,2], Olivera Đorđević[1,2], Zoran Bukumirić[1,3], Suzana Dedijer-Dujović[1,2], Tijana Dimkić Tomić[1,2], Ljubica Konstantinović[1,2]

Sažetak

Uvod/Cilj: Kod progresivnih formi MS učestalost bola raste sa tokom bolesti, utiče na funkcionalnu sposobnost pacijenata i čini je mnogo kompleksnijom. Sproveli smo studiju preseka u cilju ispitivanja povezanosti bola sa brzinom hoda i funkcionalnom sposobnošću pacijenata obolelih od progresivnih formi multiple skleroze.

Metod: U periodu od januara 2020. do maja 2023.godine u Klinici za rehabilitaciju „Dr Miroslav Zotović" sprovedena je studija preseka. Istraživanjem je obuhvaćeno 55 ispitanika sa dijagnostikovanom PPMS i SPMS, konsekutivno primljenih na rehabilitaciju. Prikupljani su demografski i socio-epidemiološki podaci i podaci vezani za bolest. Intenzitet bola je procenjivan *Numeričkom skalom* (NRS). Obzirom da su svi pacijenti imali spasticitet bol je procenjivan i subskalom upitnika *Multiple Sclerosis Spasticity Scale 88 (MSSS-88)* koja se odnosi na Bol/Nelagodnost (*Pain/Discomfort*, P/D). Subjektivna percepcija oštećenja hoda procenjivana je subskalom istog upitnika koja se odnosi na hod (WL – Walk). Brzinu hoda smo merili kliničkim instrumentom – *The Timed 25 Foot Walk* (T25FW). Funkcionalna procena i svi upitnici su popunjavani u jutarnjim časovima u toku 24h od dana prijema pacijenata na rehabilitaciju.

Rezultati: Postoji značajna jaka pozitivna povezanost između WL i P/D (rho=0,770; p<0,001) i WL i NRS (rho=0,825 p<0,001). Pokazano je da postoji statistički značajna osrednja negativna povezanost T25FW i NRS bol (p<0.001). Ne postoji statistički značajna povezanost između T25FW i intenziteta bola merenog P/D (p=0,033). Takođe, pokazana je statistički značajna povezanost EDSS i intenziteta bola (NRS p=0,0002; PD p=0,006).

Zaključak: Rezultati istraživanja su ukazali na značajan negativan uticaj bola na brzinu hoda i na stepen funkcionalne onesposobljenosti.

Ključne reči: MRI dojki, duktalni karcinom in situ, invazivni duktalni karcinom, morfo-dinamski parametri, funkcionalni parametri

Primljen: 26.12.2023. I **Revizija:** 09.04.2024. I **Prihvaćen:** 23.04.2024.

Medicinska istaživanja 2024; 57(3):9-17

Medicinska istaživanja 2024; 57(3):19-25 | DOI: 10.5937/medi57-48168

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Neurological complications of hepatitis C in Serbian population

Stojan Perić[1,2], Luka Nikolić[2], Jelena Zlatar[2], Nikola Mitrović[2,3], Milica Dajević[4], Ivo Božović[1], Branislav Ralić[5], Ivana Basta[1,2], Aleksandra Kačar[1,2]

[1] University Clinical Center of Serbia, Neurology Clinic, Belgrade, Serbia
[2] University of Belgrade, Faculty of Medicine, Belgrade, Serbia
[3] University Clinical Center of Serbia, Clinic for Infectious and Tropical Diseases, Belgrade, Serbia
[4] Clinical Center of Montenegro, Neurology Clinic, Podgorica, Montenegro
[5] Clinical Hospital Center Zvezdara, Department of Neurology, Belgrade, Serbia

Recived: 10 December 2023
Revised: 05 April 2024
Accepted: 05 April 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Stojan Perić
University Clinical Centre of Serbia, Neurology Clinic
6 Dr Subotića Street, 11 000 Belgrade, Serbia
E-mail: stojanperic@gmail.com

Summary

Introduction: Approximately 58 million people live with chronic hepatitis C virus (HCV) infection across the globe. Over half of the patients develop at least one extrahepatic complication throughout the disease. Neuropsychiatric disorders have been described in up to 50% of HCV patients. Peripheral neuropathies seem to be the most common complication.

Aim: To explore a wide range of neurological complications of chronic HCV infection in Serbian patients.

Materials and Methods: From the medical electronic system of the Neurology Clinic, a sample of 79 HCV patients was obtained (57% were male, average age was 59.0 ± 13.7 years, and average hepatitis duration was 12.4 ± 7.8 years).

Results: Of the 79 registered HCV patients, 14 (17.7%) were newly diagnosed at the Neurology Clinic. There were 29 different primary neurological diagnoses on record. The most frequent complication was polyneuropathy (PNP) found in 28 (35.4%) patients. The most common type was distal symmetric PNP. The average age of patients with PNP was significantly higher compared to those without it. Prevalence of diabetes mellitus and heart disease was more common in patients with PNP. Furthermore, glomerulonephritis was registered only in HCV patients with PNP.

Conclusion: Elderly HCV patients with comorbidities such as diabetes mellitus and/or heart disease seem to be at an increased risk of polyneuropathies and should be screened accordingly.

Key words: hepatitis C virus (HCV), chronic infection, neurological complications, peripheral neuropathy, polyneuropathy

Cite this article as: Perić S, Nikolić L, Zlatar J, Mitrović N, Dajević M, Božović I, Ralić B, Basta I, Kačar A. Neurological complications of hepatitis C in serbian population; Medicinska istaživanja 2024; 57(3):19-25 DOI: 10.5937/medi57-48168

INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded positive sense RNA virus that belongs to the Flaviviridae family. As an RNA virus, it has a high mutation rate, and an incubation period ranging from 2 weeks to 6 months with roughly 80% of cases being asymptomatic. Only 15-45% of those with acute infection have the infection cleared within 6 months, with the remainder remaining chronically infected (1). HCV is not only hepatotropic, but lymphotropic and neurotropic as well, with an estimated 40-74% of patients developing at least one extrahepatic complication (EHC) during the disease (2). Many EHCs are postulated to be immunologic in origin, with one cause thought to be due to infected B lymphocytes producing monoclonal or polyclonal autoantibodies with cryoglobulin (CG) or rheumatoid factor (RF) properties (3). This may also be due to the activation of autoreactive T cells with cross-reactivity against neuronal and other tissues (4). The most prevalent and well documented EHCs are nephropathy, diabetes mellitus type 2, cardiovascular diseases, non-Hodgkin lymphomas and neuropsychiatric disorders (2).

Among the neurological EHCs of HCV, distal symmetric sensorimotor axonal peripheral neuropathy is the most common and best-established entity, present in around 10% of patients (5). This is particularly true among CG positive individuals, with peripheral neuropathies present in up to 86% of CG positive patients (6), and in approximately 65% of those who develop vasculitis (7). Other neuropathies associated with HCV infection include polyradiculopathies, as well as small fiber neuropathies with paresthesia and autonomic dysfunction (8).

Less commonly, the central nervous system (CNS) can be involved. The pathophysiology is incompletely understood but may involve IL-1β altering the blood-brain barrier, active viral replication within the CNS, as well as direct and indirect HCV neurotoxicity. CNS complications include encephalopathies, myelopathies, hemorrhagic stroke in younger patients, and psychiatric symptoms (9).

Use of immunosuppressive agents, such as steroids and cytotoxic drugs are not recommended in those with HCV because of the threat of increases in viral load (10). Pegylated interferon (PEG IFN) and ribavirin (RBV) combination, an immunomodulatory therapy, has been used for decades but with a low rate of success in achieving a stable virological response (SVR) of about 50%. From 2014, direct-acting antiviral (DAA) medications began to be used in therapy, which significantly improved the treatment success, over 90% nowadays. The problem is the price of these drugs, which is why they are unavailable in a significant number of countries around the world (11). Patients treated with an interferon-based regimen have different neuropsychiatric manifestations such as the development of irritability in 30-40%, severe insomnia and depression requiring antidepressant therapy in 25%, so they need to be screened for neuropsychiatric diseases prior to starting this therapy (4). These are thought to occur due to interferon induced cytokine production and inhibition of serotonin synthesis. Sensory and motor neuropathies and autoimmunity have been described in relation to interferon therapy, but these side effects are rare (12). With the use of new DAA medications for treatment of chronic HCV infection as IFN free regimes, these manifestations have become less significant.

The aim of this paper was to further investigate the broad spectrum of neurological complications of chronic HCV infection from the perspective of a neurologist, while shedding more light toward peripheral neuropathies as the most common manifestations.

MATERIALS AND METHODS

Data were collected from a sample of patients with the diagnosis of chronic hepatitis C and a history of hospitalization at the Neurology Clinic, University Clinical Center of Serbia, between January 1, 2010 and December 31, 2019. Retrospective and completely anonymous data were used, thus signed informed consent was not requested.

Inclusion into the sample was based on a diagnosis matching one of the following codes according to the international classification of diseases, 10th revision, (ICD-10): B18 (Chronic viral hepatitis), B18.2 (Chronic viral hepatitis C), B18.8 (Other chronic viral hepatitis), B18.9 (Chronic viral hepatitis, unspecified), B19 (Unspecified viral hepatitis), K73 (Chronic hepatitis, not elsewhere classified), K73.0 (Chronic persistent hepatitis, not elsewhere classified), K73.8 (Other chronic hepatitis, not elsewhere classified), and K73.9 (Other chronic hepatitis, not elsewhere classified). We checked if the diagnosis of HCV infection was confirmed by serological test and/or positive quantitative HCV polymerase chain reaction (PCR) test in patients' electronic medical records. Furthermore, patients' electronic medical records were studied in detail to confirm the presence of at least one neurological diagnosis, justifying their inclusion into the sample. This resulted in a sample of 165 individuals. In 38 patients diagnoses of hepatotropic viruses other than hepatitis C were found, and in 48 patients no specific infectious causes of hepatitis were found, and these patients were hence excluded from the sample.

Electronic medical records were investigated for variables of interest. These included sociodemographic parameters such as age, gender, and presumed way of transmission. The clinical parameters related to chronic hepatitis C included duration of illness, whether it was newly diagnosed, current activity of disease expressed through the degree of hepatocellular necrosis, previous antiviral treatment, and the presence of hepatic complications. We assessed neurological diagnoses, duration of

neurological disease, and temporal association between HCV infection and neurological disease. Peripheral neuropathies were of particular interest. The type and degree of PNP was further described based on the patients' clinical picture and electrophysiological testing trough nerve conduction studies (NCS). PNP was defined as sensory, motor, or sensorimotor; symmetrical or asymmetrical; axonal, demyelinating, or axonal-demyelinating; and its severity was graded in relation to the patients' ability to walk. Data were also collected regarding other chronic illnesses, such as glomerulonephritis, thyroid dysfunction, diabetes, heart disease, lung disease, connective tissue diseases, hematological diseases, cancer, and others.

The data was analyzed using the arithmetic mean, standard deviation, and proportions, as descriptive methods. Fisher test, Chi-squared test and Students t test were used to compare subgroups of patients, with the level of statistical significance set at $p<0.05$. Statistical analyses were carried out with Statistical Package for the Social Sciences (SPSS) version 23 (IBM Corp, Armonk, NY, USA).

RESULTS

After excluding duplicates and those with non-HCV hepatitis, the sample yielded 79 patients that fit the inclusion criteria. Of these, 45 (57%) were male, and the average age was 59.0 ± 13.7 years, ranging between 31 and 91 years **(Table 1)**. The most frequent known route of transmission was intravenous drug abuse in 17 (21.5%) patients, with a single (1.3%) patient with known routes of transmission, namely via transfusion, occupationally, or from a partner. Of all patients, there were 14 (17.7%) newly diagnosed, i.e. diagnosis of chronic HCV infection was made as a part of their work-up during their stay at the Neurology Clinic. In patients with previous diagnosis of HVC, average duration of hepatitis was 12.4 ± 7.8 years. The disease was found to be active in the observed period in 39 (49.4%) patients, and inactive in 21 (26.6%) patients. The majority of patients (59.5%) had no treatment for hepatitis C on record. Among those treated, the most commonly used drug both alone and in combination was PEG IFN, in 6 (7.6%) alone and in 9 (11.4%) in combination with a DAA. RBV was used in 5 (6.3%) patients, all in combination with PEG IFN at least. Finally, DAA alone or combined were used in 14 (17.7%) patients - sofosbuvir (5, or 6.3%), velpatasvir (4, or 5.1%), grazeprovir (4, or 5.1%), ledipasvir (3, or 3.8%), elbasvir (3, or 3.8%), with boceprevir, telaprevir, pibrentasvir, and glekaprevir only being used in single patients (1.3%). DAA were also often used in combination with PEG IFN (5, or 6.3%).

Neurological complications most often occurred after the diagnosis of HCV infection, as was found in 55 (69.6%) patients. The remainder of those with a known specific chronology evenly divided into 9 (11.4%) with concomitant hepatitis and neurological complications, and 9 (11.4%) with a neurological diagnosis before the diagnosis of hepatitis. On average, each patient had 1.8 ± 1.0 neurological diagnoses in their history, ranging between 1 and 5, and 2.8 ±2.0 other chronic illnesses, ranging between 0 and 9. There were 29 different primary neurological diagnoses within the sample **(Table 2)**.

The most frequent neurological complication present in the sample was PNP, occurring in 28 (35.4%) patients, for 24 (30.4%) of whom it was the primary neurological diagnosis. Headache was the second most frequent complication, documented in 20 patients (25.3%) of whom 8 patients (10%) had it as their primary neurological diagnosis. Mononeuropathy occurred in 3 (3.8%) patients, as a compressive ulnar lesion, optic neuritis, and trigeminal neuritis, respectively. Brachial plexitis was found as a primary neurological diagnosis in 1 (1.3%) patient, while another had a secondary diagnosis of lumbosacral plexitis. The next most frequent neurological diagnosis was epilepsy, present in 17 (21.5%) patients, for 11 (13.9%) of whom it was the primary diagnosis. In those for whom epilepsy was a secondary diagnosis, the history of seizures was either a direct consequence of the primary neurological diagnosis, e.g. hemorrhagic stroke in 2, or superseded by a more progressive clinical entity, e.g. amyotrophic lateral sclerosis and progressive multifocal encephalopathy in another 2, or with inadequate medical history on the seizures as it was in the final 2. While cognitive deficit was the primary neurological diagnosis in only 1 patient, 12 patients

Table 1. Sociodemographic, clinical and laboratory features of HCV patients with neurological diseases, page 7.

Feature	Mean ± SD or number (%) of patients
Male gender	45 (57.0%)
Age	59.0 ± 13.7 years
Route of transmission	
Intravenous drug use	17 (21.5%)
Transfusion	1 (1.3%)
Occupational	1 (1.3%)
Sexual route	1 (1.3%)
Duration of HCV infection	12.4 ± 7.8 years
Newly diagnosed	14 (17.7%)
Disease activity	
Active	39 (49.4%)
Inactive	21 (26.6%)
Treatment	
No treatment	47 (59.5%)
Interferon alone	6 (7.6%)
DAA*	9 (11.4%)
Combination	10 (12.7%)
Unspecified treatment	7 (8.9%)
Positive HCV serology and/or PCR	57 (70.9%)

* direct-acting antiviral therapy

Table 2. Frequency of primary neurological diagnoses in patients with chronic HCV, page 8.

Primary Neurological Diagnosis	**Number (%) of patients**
Polyneuropathy	24 (30.4%)
Brachial plexitis	1 (1.3%)
Mononeuropathy	3 (3.8%)
Chronic back pain	1 (5.1%)
Encephalopathy	4 (1.3%)
Idiopathic cognitive deficit	1 (1.3%)
Vascular dementia	1 (1.3%)
Epilepsy	11 (13.9%)
Hemorrhagic stroke	1 (1.3%)
Ischemic stroke	2 (2.5%)
Ischemic optic neuropathy	1 (1.3%)
Recurrent hemiplegia	1 (1.3%)
Hemihypesthesia	1 (1.3%)
Spastic paraplegia	2 (2.5%)
Spastic quadriplegia	1 (1.3%)
Headache	8 (10.1%)
Hydrocephalus	1 (1.3%)
Intracranial tumor	1 (1.3%)
Myopathy	2 (2.5%)
Myesthenia gravis	1 (1.3%)
Parkinsonism	2 (2.5%)
ALS	1 (1.3%)
Cerebellar ataxia	1 (1.3%)
Vertigo	1 (1.3%)
Multiple sclerosis	3 (3.8%)
Clinically isolated syndrome	1 (1.3%)
Progressive multifocal encephalopathy	1 (1.3%)
Acute disseminated encephalomyelitis	1 (1.3%)

had it as a secondary finding, for a total prevalence of 13 (16.5%). A total of 8 (10.1%) patients had encephalopathy, half (5.1%) of whom had it as a primary neurological diagnosis. Altogether, there were 8 (10.1%) patients with a history of stroke, of whom 5 (6.3%) were hemorrhagic and 3 (3.8%) were ischemic. Only 2 (2.5%) ischemic strokes and 1 hemorrhagic stroke were primary neurological diagnoses. Multiple sclerosis occurred in 3 (3.8%) patients exclusively as a primary neurological diagnosis. Finally, parkinsonism was documented in 2 (2.5%) patients. Other conditions classified as primary neurological diagnosis that occurred in single patients (1.3%) included the following: progressive multifocal encephalopathy, acute disseminated encephalomyelitis, clinically isolated syndrome, vascular dementia, ischemic optic neuropathy, spastic paraplegia, spastic quadriplegia, recurrent hemiplegia, hemihypoesthesia, amyotrophic lateral sclerosis, cerebellar ataxia, vertigo, hydrocephalus, intracranial tumor, myopathy, mitochondrial myopathy, myasthenia gravis, and chronic back pain. Non-neurological diagnoses in hepatitis C patients are presented in **Table 3**.

Table 3. Frequency of non-neurological diagnoses in patient with chronic HCV infection, page 9.

Primary Neurological Diagnosis	**Number of patients**
Cirrhosis	21
Hepatocellular Carcinoma	6
Gammopathy	5
Anemia	4
Pancytopenia	1
Hemophilia A	1
Immune Thrombocytopenia	1
Non-Hodgkin Lymphoma	1
Vasculitis	8
Glomerulonephritis	6
Concomitant Infection	4
Hyperthyroidism	1
Hypothyroidism	3
Sjogren Syndrome	2
Sjogren Syndrome with Rheumatoid Arthritis	1
Arthropathy	3
Purpura	3
Angioedema	1

In patients with PNP, normal gait was observed in 10 (35.7%) of 28 patients, difficulty walking was found in 11 (39.3%), one-sided support was necessary for one (3.6%), bilateral support was needed for four (14.3%) patients, and one (3.6%) subject was unable to walk. Sensorimotor impairment was the most prevalent, seen in 19 patients (67.9%), while isolated sensory and isolated motor were present in 3 (10.7%) each. Pathophysiological typing was not specified in 14 (50%) patients, the remaining half being demyelinating (21.5%), and axonal-demyelinating or pure axonal (14.3% patients each). Furthermore, symmetric changes were far more prevalent (71.4%) compared to asymmetric (10.7%). Serological testing in these patients showed positive CG in 4 (14.3%) of the 7 (25.0%) patients tested, 1 (3.6%) positive for ANA of the 5 (17.9%) patients tested, and neither of the 2 (7.1%) patients tested for ANCA were positive. As would be expected from the findings above, the most common subtype observed was distal symmetric PNP, found in nine patients (32.1%). The other two types included small fiber PNP in one and Guillain-Bare Syndrome in another patient (3.6% each).

There was a statistically significant difference between the average age of patients with PNP when compared to those without PNP (64.4 ± 9.0 vs. 56.1 ± 15.0 years, $p<0.01$) (Table 4). Additionally, 35.7% of patients with PNP were found to have diabetes as opposed to only 7.8% of those without PNP ($p<0.05$). Similarly, there was a statistically significant difference in the frequency of the heart disease in patients with PNP vs. those without PNP (42.9% vs. 19.6%, $p<0.05$). Additionally, there were no glomerulonephritis cases among the patients without PNP, whereas 21.4% of those with PNP had glomerulone-

phritis ($p<0.05$). Lastly, it is interesting to note that while 17.9% of those with PNP were found to have vasculitis compared to only 5.7% of those without PNP ($p=0.09$).

DISCUSSION

The finding of PNP as the most frequent neurological disorder in the setting of chronic HCV infection (35%) with neurological comorbidity is very much in line with literature where it was reported in 40% to 75% of all HCV patients (4). The effects of CG on the peripheral nerves are thought to be the most common cause of PNP in this setting. Types 2 and 3 CG, also known as mixed CGs, are most often found in the setting of chronic HCV infection (13). Our own findings, where just over half of tested PNP patients were positive for CG, are much greater than the expected 25% to 30%, and almost certainly accounted for by the very low sample size of those tested in our cohort. Though various peripheral neuropathies have been described in association with CG, those most found are PNP of the distal, symmetric, sensory or sensorimotor type (5). This type was also most frequently present in our cohort.

The reality at play is more complex, as PNP is also a frequent finding in those without circulating mixed CGs (8). In these patients, PNP is similarly thought to be due to the lymphotropic nature of HCV, but due to its effect on infected T cells (14). One of the mechanisms that connect HCV infections and PNP, which does not include CG, could be in immune response dysfunction during chronic hepatitis C. Thus, HCV causes disorders of the innate and adaptive arms of the immune response, which includes dendritic cells (DC), macrophages (Kupffer cells), natural killer (NK) cells, CD4+ and CD8+ T cells, such as B cells and peripheral blood mononuclear cells (PBMC) which are also reservoirs for HCV (15, 16). Impaired synthesis of various cytokines such as interleukin 1, 2, 6, 12, 15, 18, as well as interferon (type I and III) can be of importance in the pathogenesis of PNP (17).

The association that was found between glomerulonephritis and PNP in our cohort of patients may also be supported by the link between CG and PNP. The presence of mixed CGs in the setting of either systemic vasculitis or glomerulonephritis has been termed "mixed cryoglobulinemia syndrome" (MCS) (18), and can be thought of as a symptomatic outcome of high circulating CG levels. Again, while not the only etiological agent that results in MCS, the relationship between chronic HCV infection and MCS has been well established (19), as well as the relationship between MCS of all etiologies and PNP (20). Thus, we can conclude that, while a significant association between PNP and vasculitis was not made at this sample size, the association that was found between PNP and glomerulonephritis was significant and potentially hinting at CGs as the underlying cause of both. Alternatively, the strong association between glomerulonephritis and PNP may be due to the reduced renal clearance of toxic metabolites (21). Unfortunately, this study lacked adequate data on the chronological relationship between these two conditions. Namely, if glomerulonephritis were to emerge earlier, the resultant uremia and other toxic metabolites might in fact, be causing a uremic PNP (22), whereas, if they occur concomitantly or there was no temporal pattern, a shared pathophysiological mechanism such as MCS would be more likely. It is of note that the majority of our patients had length dependent neuropathy, while in uremia it is typical for short fibers to be affected. Thus, it is less likely that observed neuropathy in hepatitis C is of toxic origin. This would be an interesting avenue for future study.

Correlation that was found between PNP and diabetes is likely the result of synergy between the pathological mechanisms through which HCV and diabetes bring about PNP. At the level of the pancreatic β cells, HCV has the ability to promote diabetes as an EHC (2). The effects of diabetes could be explained in several ways. Some patients with diabetes may have pre-existing nerve injury due to ischemia, inflammation, and metabolic changes, so further injury from chronic hepatitis C makes things worse (23,24). Reduced rates of nerve regeneration were found early in diabetes even before symptoms and signs of neuropathy appeared, while the presence of diabetic neuropathy was associated with a further decrease in the capacity to regenerate (25). In accordance with this, regenerative deficit with reduced axonal sprouting and Schwann cell migration was shown to be common in diabetes and supposed to underlie the development of neuropathy (23). Diabetes may also increase inflammation in chronic hepatitis C since both diseases are associated with systemic inflammation including an increased level of different cytokines (24,26).

We also noticed correlation between PNP and older age of patients, independent of the duration of hepatitis C. This could be a result of synergism between the biological age of the patient and other comorbidities such as diabetes (11). An additional downside of age may be due to the side effect profile of the only therapeutic options available in the past - PEG IFN with RBV. While usually better known for their neuropsychiatric and physiological side effects, several case reports associate their use with worsening or induction of peripheral neuropathies, among a number of other clinical entities (27–30).

Other than PNP, the broad scope of neurological complications encountered in the study is also in line with the literature. Cognitive deficit is known to occur in up to 50% of those with chronic HCV infection (31). It can also mask the initial stages of encephalopathy, which may be both hepatic and directly due to active viral replication in the CNS. Stroke, both ischemic and hemorrhagic, is also reported to occur more frequently in this population, at younger ages as well (32). While the number of stroke

Table 4. Comparison of chronically HCV infected patients with and without polyneuropathy, page 10.

Feature	non-PNP Mean ± SD or number (%) of patients	PNP Mean ± SD or number (%) of patients	p
Number	51	28	
Age	56.1 ± 15.0	64.4 ± 9.0	p<0.01
Glomerulonephritis	0 (0%)	6 (21.4%)	p<0.05
Diabetes	4 (7.8%)	10 (35.7%)	p<0.05
Heart disease	10 (19.6%)	12 (42.9%)	p<0.05
Vasculitis	3 (5.4%)	5 (17.9%)	p=0.09

Sociodemographic, laboratory and clinical features without statistical difference were not presented in the Table.

patients within our sample was admittedly too small for any significant associations to be made, it is interesting to note that the average age of patients with a history of hemorrhagic stroke was almost nearly 8 years lower than both the sample average, and the average of those with ischemic strokes. This correlation is thought to potentially result from MCS or an increased rate of atherosclerosis associated with HCV (33).

CONCLUSION

In summary, peripheral neuropathies are common manifestations of chronic hepatitis C present in approximately one third of patients and should not be overlooked since they can affect patients' abilities. Physicians, including neurologists, should also think of other extrahepatic complications of this infection, thus requesting multidisciplinary approach in this chronic disorder.

Acknowledgments

None.

Conflicts of interest

None to declare.

Ethical approval

In this paper only retrospective data were used from medical records, thus Ethical approval was not obtained. Corresponding author and his collaborators undertake that the processed data in this research are presented in a way that does not allow the identification of an individual subject. All data are related only and exclusively to the topic of the research and without the possibility of connecting the data with the identity of the persons.

REFERENCES

1. Alter MJ. Epidemiology of hepatitis C. Hepatology. 1997 1;26(S3):62S-65S. doi: 10.1002/HEP.510260711
2. Mazzaro C, Quartuccio L, Adinolfi LE, Roccatello D, Pozzato G, Nevola R, et al. A Review on Extrahepatic Manifestations of Chronic Hepatitis C Virus Infection and the Impact of Direct-Acting Antiviral Therapy. Viruses. 2021 1;13(11). doi: 10.3390/V13112249
3. Maslennikov R, Ivashkin V, Efremova I, Shirokova E. Immune disorders and rheumatologic manifestations of viral hepatitis. World J Gastroenterol. 2021 14;27(18):2073–89. doi: 10.3748/WJG.V27.I18.2073
4. Faccioli J, Nardelli S, Gioia S, Riggio O, Ridola L. Neurological and psychiatric effects of hepatitis C virus infection. World J Gastroenterol. 2021 8;27(29):4846. doi: 10.3748/WJG.V27.I29.4846x
5. Cacoub P, Renou C, Rosenthal E, Cohen P, Loury I, Loustaud-Ratti V, et al. Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC. Groupe d'Etude et de Recherche en Medecine Interne et Maladies Infectieuses sur le Virus de l'Hepatite C. Medicine. 2000;79(1):47–56.'doi: 10.1097/00005792-200001000-00005
6. Adinolfi LE, Nevola R, Lus G, Restivo L, Guerrera B, Romano C, et al. Chronic hepatitis C virus infection and neurological and psychiatric disorders: An overview. World Journal of Gastroenterology : WJG. 2015 2;21(8):2269. doi: 10.3748/WJG.V21.I8.2269
7. Saadoun D, Terrier B, Semoun O, Sene D, Maisonobe T, Musset L, et al. Hepatitis C virus-associated polyarteritis nodosa. Arthritis Care Res (Hoboken). 2011;63(3):427–35. doi: 10.1002/ACR.20381
8. Yoon MS, Obermann M, Dockweiler C, Assert R, Canbay A, Haag S, et al. Sensory neuropathy in patients with cryoglobulin negative hepatitis-C infection. J Neurol. 2011;258(1):80–8. doi: 10.1007/S00415-010-5686-1
9. Kleefeld F, Arendt G, Neuen-Jacob E, Maschke M, Husstedt I, Obermann M, et al. Neurological complications of hepatitis C infections. Nervenarzt. 2021 1;92(2):144–9. doi: 10.1007/S00115-020-00999-6/FIGURES/1
10. Cacoub P, Saadoun D, Limal N, Léger JM, Maisonobe T. Hepatitis C virus infection and mixed cryoglobulinaemia vasculitis: A review of neurological complications. AIDS. 2005;19(3). doi: 10.1097/01.AIDS.0000192081.33938.2F
11. Xia H, Lu C, Wang Y, Zaongo SD, Hu Y, Wu Y, Yan Z, Ma P. Efficacy and Safety of Direct-Acting Antiviral Therapy in Patients With Chronic Hepatitis C Virus Infection: A Real-World Single-Center Experience in Tianjin, China. Front Pharmacol 2020; 11:710. doi: 10.3389/fphar.2020.00710
12. Cacoub P, Terrier B, Saadoun D. Hepatitis C virus-induced vasculitis: therapeutic options. Ann Rheum Dis. 2014 1;73(1):24–30. doi: 10.1136/ANNRHEUMDIS-2013-203883
13. Desbois AC, Cacoub P, Saadoun D. Cryoglobulinemia: An update in 2019. Joint Bone Spine. 2019 1;86(6):707–13. doi: 10.1016/J.JBSPIN.2019.01.016
14. Nemni R, Sanvito L, Quattrini A, Santuccio G, Camerlingo M, Canal N. Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. J Neurol Neurosurg Psychiatry. 2003 1;74(9):1267. doi: 10.1136/JNNP.74.9.1267
15. Rios DA, Casciato PC, Caldirola MS, Gaillard MI, Giadans C, Ameigeiras B, De Matteo EN, Preciado MV, Valva P. Chronic Hepatitis C Pathogenesis: Immune Response in the Liver Microenvironment

and Peripheral Compartment. Front Cell Infect Microbiol. 2021 3; 11:712105. doi: 10.3389/fcimb.2021.712105

16. Chigbu DI, Loonawat R, Sehgal M, Patel D, Jain P. Hepatitis C Virus Infection: Host–Virus Interaction and Mechanisms of Viral Persistence. Cells. 2019;8(4):376. doi: 10.3390/cells8040376
17. Shin E.C., Sung P.S., Park S.H. Immune responses and immunopathology in acute and chronic viral hepatitis. Nat. Rev. Immunol. 2016; 16:509–523. doi: 10.1038/nri.2016.69
18. Ramos-Casals M, Stone JH, Cid MC, Bosch X. The cryoglobulinaemias. Lancet. 2012 28;379(9813):348-60. doi: 10.1016/S0140-6736(11)60242-0
19. Rutledge SM, Chung RT, Sise ME. Treatment of hepatitis C virus infection in patients with mixed cryoglobulinemic syndrome and cryoglobulinemic glomerulonephritis. Hemodial Int. 2018 1;22 Suppl 1:S81–96.
20. Galli M, Monti G, Marson P, Scaini P, Pietrogrande M, Candela M, et al. Recommendations for managing the manifestations of severe and life-threatening mixed cryoglobulinemia syndrome. Autoimmun Rev. 2019 1;18(8):778–85.
21. Rutledge SM, Chung RT, Sise ME. Treatment of hepatitis C virus infection in patients with mixed cryoglobulinemic syndrome and cryoglobulinemic glomerulonephritis. Hemodial Int. 2018 1;22 Suppl 1:S81–96. doi: 10.1111/HDI.12649
22. Bolton CF. Peripheral neuropathies associated with chronic renal failure. Can J Neurol Sci. 1980;7(2):89–96. doi: 10.1017/S0317167100023453
23. Bae JS, Kim YJ, Kim JK. Diabetes mellitus exacerbates the clinical and electrophysiological features of Guillain-Barré syndrome. Eur J Neurol. 2016 Mar 1;23(3):439–46. doi: 10.1111/ENE.12885
24. Berciano J, García A, Figols J, Muñoz R, Berciano MT, Lafarga M. Perineurium contributes to axonal damage in acute inflammatory demyelinating polyneuropathy. Neurology. 2000;55(4):552–9. doi: 10.1212/WNL.55.4.552
25. Polydefkis M, Hauer P, Sheth S, Sirdofsky M, Griffin JW, McArthur JC. The time course of epidermal nerve fibre regeneration: studies in normal controls and in people with diabetes, with and without neuropathy. Brain. 2004;127(Pt 7):1606–15. doi: 10.1212/WNL.55.4.552
26. Ebenezer GJ, O'Donnell R, Hauer P, Cimino NP, McArthur JC, Polydefkis M. Impaired neurovascular repair in subjects with diabetes following experimental intracutaneous axotomy. Brain. 2011;134(Pt 6):1853–63. doi: 10.1093/BRAIN/AWR086
27. Hartge MM, Unger T, Kintscher U. The endothelium and vascular inflammation in diabetes. Diab Vasc Dis Res. 2007;4(2):84–8. doi: 10.3132/DVDR.2007.025
28. Barut S, Karaer H, Oksuz E, Eken AG, Basak AN. Bell's palsy and choreiform movements during peginterferon α and ribavirin therapy. World Journal of Gastroenterology : WJG. 2009 8;15(29):3694. doi: 10.3748/WJG.15.3694
29. Khiani V, Kelly T, Shibli A, Jensen D, Mohanty SR. Acute inflammatory demyelinating polyneuropathy associated with pegylated interferon alpha 2a therapy for chronic hepatitis C virus infection. World J Gastroenterol. 2008 14;14(2):318–21. doi: 10.3748/WJG.14.318
30. Jones MR, Urits I, Wolf J, Corrigan D, Colburn L, Peterson E, et al. Drug-Induced Peripheral Neuropathy: A Narrative Review. Curr Clin Pharmacol. 2020 22;15(1):38. doi: 10.2174/1574884714666190121154813
31. Monaco S, Ferrari S, Gajofatto A, Zanusso G, Mariotto S. HCV-Related Nervous System Disorders. Clin Dev Immunol. 2012;2012. doi: 10.1155/2012/236148
32. Lee MH, Yang HI, Wang CH, Jen CL, Yeh SH, Liu CJ, et al. Hepatitis C virus infection and increased risk of cerebrovascular disease. Stroke. 2010;41(12):2894–900. doi: 10.1161/STROKEAHA.110.598136
33. Petta S, Adinolfi LE, Fracanzani AL, Rini F, Caldarella R, Calvaruso V, et al. Hepatitis C virus eradication by direct-acting antiviral agents improves carotid atherosclerosis in patients with severe liver fibrosis. J Hepatol. 2018 1;69(1):18–24. 10.1016/J.JHEP.2018.02.015

NEUROLOŠKE KOMPLIKACIJE HEPATITISA C U SRPSKOJ POPULACIJI

Stojan Perić[1,2], Luka Nikolić[2], Jelena Zlatar[2], Nikola Mitrović[2,3], Milica Dajević[4], Ivo Božović[1], Branislav Ralić[5], Ivana Basta[1,2], Aleksandra Kačar[1,2]

Sažetak

Uvod: Oko 58 miliona ljudi širom sveta boluje od hroničnog hepatitisa C. Preko polovine pacijenata razvije barem jednu ekstrahepatičnu komplikaciju u toku bolesti. Neurološki i psihijatrijski poremećaji centralnog i perifernog nervnog sistema opisani su u sklopu HCV infekcije kod čak oko polovine pacijenata. Od neuroloških komplikacija, najčešće su periferne neuropatije.

Cilj: Ispitivanje širokog opsega neuroloških komplikacija hronične hepatitis C infekcije u srpskoj populaciji.

Materijali i metode: Uzorak od 79 pacijenata prikupljen je iz elektronskog sistema Klinike za neurologiju Univerzitetskog kliničkog centra Srbije. Četrdeset pet (57%) pacijenata bilo je muškog pola, a prosečna životna dob iznosila je 59.0 ± 13.7 godina. Hepatitis C infekcija je u proseku trajala 12.4 ± 7.8 godina.

Rezultati: Od ukupno 79 pacijenata, kod 14 (17.7%) je novodijagnostikovan hepatitis C za vreme boravka na Klinici. Opisano je 29 različitih primarnih neuroloških dijagnoza. Polineuropatija je bila najčešća neurološka komplikacija opisana kod 28 (35.4%) pacijenata. Distalna simetrična polineuropatija je bila najčešća forma. Pacijenti sa polineuropatijom su bili stariji. Učestalost dijabetesa i učestalost srčanih oboljenja bili su znatno češći kod pacijenata sa polineuropatijom u odnosu na ostatak uzorka. Glomerulonefritisi su uočeni isključivo kod pacijenata sa polineuropatijom.

Zaključak: U neurološkoj praksi često se susreću pacijenti sa HCV. Stariji pacijenti, koji pored hroničnog HCV boluju i od drugih oboljenja poput dijabetesa i/ili srčanih oboljenja u povećanom su riziku da razviju polineuropatiju.

Ključne reči: hepatitis C, hronična infekcija, neurološke komplikacije, periferne neuropatije, polineuropatije.

Primljen: 10.12.2023. I **Revizija:** 05.04.2024. I **Prihvaćen:** 05.04.2024.

Medicinska istaživanja 2024; 57(3):19-25

Medicinska istaživanja 2024; 57(3):27-33 | DOI: 10.5937/medi57-49227

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Climbing as a measurement of locomotion ability in the *Drosophila* model of fragile X syndrome

Vedrana Makević[1], Maja Stojković[2], Marko Biorac[1], Sara Milojević[2], Maria Capovilla[3], Dragana Protić[2,4]

[1]University of Belgrade, Faculty of Medicine, Department of Pathophysiology, Belgrade, Serbia

[2]University of Belgrade, Faculty of Medicine, Department of Pharmacology, Clinical Pharmacology and Toxicology, Belgrade, Serbia

[3]UMR7275 CNRS-INSERM-UniCA, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne Sophia Antipolis, France

[4]Fragile X Clinic, Special Hospital for Cerebral Palsy and Developmental Neurology, 11000 Belgrade, Serbia

Recived: 12 February 2024

Revised: 04 April 2024

Accepted: 04 April 2024

Check for updates

Funding information:
This research was funded by the Science Fund of the Republic of Serbia, Program IDEA, GRANT No. 7673781. The authors of the publication are responsible for the content of this publication and this content does not express the attitudes of the Science Fund of the Republic of Serbia. In addition, this study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia Project No. 451-03-66/2024-03/200110.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Dragana Protic,

Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade,

1, Dr Subotića Starijeg Street, 11000 Belgrade, Serbia

E-mail: dragana.protic@med.bg.ac.rs

Summary

Introduction: Fragile X syndrome (FXS) is the most common monogenetic cause of intellectual disability (ID) and autism spectrum disorder (ASD) in humans. The *Drosophila melanogaster* model of FXS (*dFMR1* mutants) is an excellent model for research in the field of FXS. The aim of this study was a comprehensive investigation of climbing abilities, as a measurement of locomotion, in the $dFMR1^{B55}$ line as a *Drosophila* model of FXS.

Methods: In this study, control w^{1118} and $dFMR1^{B55}$ lines of fruit flies were used. The climbing performance of flies was examined using a climbing performance assay for groups of flies as well as for individual flies. Parameters that represent climbing ability, speed and endurance were determined. Females and males were analyzed separately.

Results: This study revealed the following: (i) worse climbing performance of $dFMR1^{B55}$ males in comparison to w^{1118} males; (ii) worse climbing success of $dFMR1^{B55}$ females in comparison to w^{1118} females; (iii) better climbing performance of top performer males in comparison to top performer females in the group climbing test in both $dFMR1^{B55}$ and w^{1118} groups; (iv) better, but not statistically significant, climbing performance (based on the time needed for 50% of flies to complete the task), and a higher success rate in $dFMR1^{B55}$ females in comparison to $dFMR1^{B55}$ males. **Conclusion**: According to the results of the current study, climbing impairment was proved only in $dFMR1^{B55}$ males, while $dFMR1^{B55}$ females had climbing abilities similar to control w^{1118} females.

Keywords: fragile X syndrome, *Drosophila melanogaster*, climbing assay

Cite this article as: Makević V, Stojković M, Biorac M, Milojević S, Capovilla M, Protić D. Climbing as a measurement of locomotion ability in the drosophila model of fragile X syndrome; Medicinska istaživanja 2024; 57(3):27-33 DOI: 10.5937/medi57-49227

INTRODUCTION

Drosophila melanogaster as a model organism has contributed enormously to the field of biomedical research, especially to genetics (1). The genome homology between fruit flies and humans is approximately 60% for all genes and 75% for the disease-causing genes (2, 3). Classes of neurotransmitters, molecular pathways, synaptic plasticity, and neuronal signaling are highly conserved in fruit flies (4-6). In addition, some human body structures have counterparts in *Drosophila,* such as brain parts involved in learning and memory, like the human hippocampus and the *Drosophila* mushroom bodies (7). The ease of maintenance, small size, short generation time and life span, large progeny, fewer ethical concerns, and the availability of genetic tools make *Drosophila melanogaster* an excellent model organism for neurobehavioral investigations (1, 8, 9). Numerous *Drosophila* models have been developed primarily for the exploration of genes related to human diseases (8, 10-12). An example of such disorders is fragile X Syndrome (FXS) (13, 14).

With a prevalence of 1:5000 in males and 1:8000 in females, FXS is the most common hereditary cause of intellectual disability (ID) and autism spectrum disorder (ASD) in humans (15). Expansion of CGG trinucleotides repeats (more than 200 CGG repeats) within the *Fragile X Messenger Ribonucleoprotein 1* (*FMR1*) gene, which is located at Xq27.3, its hypermethylation and transcriptional silencing lead to deficiency or absence of the encoded *FMR1* protein (FMRP). This protein is an RNA-binding protein which is involved in the regulation of numerous mRNAs in postsynaptic neurons (16). Moreover, FMRP is essential for neural development and it is implicated in post-transcriptional regulation and in the microRNA and Piwi-interacting RNA pathways. Additionally, it is a part of P-bodies and stress granules (reviewed in (17)). Deficiency of FMRP is the main cause of the clinical features in individuals with FXS. Core symptoms associated with the absence of functional FMRP in affected individuals (18, 19) reviewed in (20) are: ASD (presented in almost 60% of males (21)), shyness, abnormal eye contact and social anxiety, attention deficit hyperactivity disorder (ADHD), sensory hyperarousal, aggressive behavior, sleep problems, repetitive behaviors, and hand flapping. In addition, physical characteristics including elongated faces, prominent ears, joint hypermobility, soft skin, flat feet, high palate, and macroorchidism are also observed in these individuals (16, 22-24). Motor impairments, like delayed motor development and atypical motor behaviors, common in FXS, usually represent the first signs of impaired development in affected children (25-28).

Various animal models of this syndrome have been developed, including the *Fmr1* knock-out (KO) mice (29), zebrafish (30) and the *Drosophila melanogaster* model of FXS (14, 31, 32). *Fmr1* (FBgn0028734); (33), the *Drosophila* homolog of the human *FMR1* gene herein called *dFMR1,* is highly conserved with 35% identity and 60% similarity in two KH domains (34) (reviewed in: (17)). Thus, the fruit fly model of FXS (*dFMR1* mutants) is an excellent model for research in the field of FXS. Namely, this model of flies exhibits altered sleep and circadian rhythm (13, 14, 35-37), defects in learning, memory (38-40) and locomotion (35, 41-43), and changes in social interactions (13, 44) and repetitive behavior (45). Defects in locomotor activity include alternated larva crawling (42, 43), impairment in climbing (45, 46) and in flight (41). As mentioned above, genotype/phenotype overlap makes the *dFMR1* mutants an excellent model for studying FXS and a great tool for pharmacological research in this field. However, there are several different *dFMR1* mutant lines, but the exact differences among their behavioral characteristics have not been clarified yet and only very few studies have been conducted on molecular or phenotypic sex-differences in FXS model organisms.

The aim of this study was a comprehensive investigation of climbing abilities, as a measurement of locomotion, in the $dFMR1^{B55}$ line as a *Drosophila* model of FXS, analyzing females and males separately. Current research will enable more intensive use of this specific model in future preclinical research in the field of FXS.

MATERIALS AND METHODS

Flies

In this study, the $dFMR1^{B55}$ mutant (FX group) was used for researching climbing ability. This line was generated by Inoue and his group (2002) by imprecise excision of the EP(3)3422 P-element that caused a deletion of *dFMR1* genomic DNA containing exons 2, 3, and 4 and creating a protein null allele (14). As control, wild type w^{1118} flies were employed (WT group).

All experimental groups of flies were grown on standard cornmeal/agar/molasses medium at 25°C with 60% humidity under a 12-h light cycle which starts at 7 am and a dark cycle starting at 7 pm. Both sexes of seven-day-old virgin flies, were used separately for all experiments. All assays were performed in the dark under a dim red light to avoid the phototaxis effect (47), between noon and 3 pm to prevent potential circadian rhythm effect on climbing performance.

Climbing Performance Assay for Groups of Flies

For climbing trials, seven-day-old virgin male and female flies from the $dFMR1^{B55}$ and w^{1118} stocks previously separated by sex and genotype were transferred into empty tubes that contained 10 flies each (48). The tubes were constructed from two vials joined at their openings and connected with clear tape in order to make them longer (49). Flies were accustomed to dark conditions for an hour before the experiment.

Table 1. Results of analyses of climbing in the groups of wild-type flies and *dFMR1* mutants

Group climbing						
	Males			Females		
	WT	FX	p value	WT	FX	p value
N1	11	11		11	10	
Failure rate (%)	18.2	27.3	0.500[¥]	9.1	0.0	0.524[¥]
N2	9	8		10	10	
t1 (s) mean ± SD	6.557 ± 1.580	7.250 ± 0.886	0.290	8.908 ± 2.038	9.450 ± 1.072	0.467
t2 (s) mean ± SD	16.213 ± 4.351	35.281 ± 20.480	**0.015**	17.308 ± 10.820	24.292 ± 13.199	0.212
SR mean ± SD (%)	79.167 ± 10.607	64.375 ± 4.580	**0.002**	85.583 ± 8.231	74.015 ± 14.649	**0.043**

Student's t-test is used unless indicated otherwise. [¥]Chi square test. The failure rate was calculated as percentage of cases that were excluded from further analysis because half of a tested population failed to reach the goal of 17.5 cm during three minutes of observation in three or more experiments.

Abbreviations: WT, wild-type; FX, fragile X; N1, total number of experimental groups; N2, number of experimental groups used in analyses after exclusion of experimental groups in which half of the flies in the group did not pass the 17.5 cm mark in three minutes in three out of four trials; t1(s) , the time, in seconds, needed for the first fly in the group to pass the mark drawn at 17.5 cm from the bottom of the tube; t2(s), the time, in seconds, needed for half of the flies in the group (5 flies) to pass the 17.5 cm mark; SR, Success Rate represents the percentage of flies that passed the 17.5 cm mark within three minutes. Bold: statistically significant p values.

Each tube was gently tapped on a soft surface making flies fall to the bottom and initiate the negative geotaxis reflex (disturbed flies start to climb opposite to the gravitation vector (47)). The following parameters were recorded: (1) the time for the first fly (top performer) in the group to pass a mark drown at 17.5 cm from the bottom of the tube; (2) the time for half of the flies in the group (5 flies) to pass the 17.5 cm mark; (3) the percentage of experiments in which half of the flies in the group did not climb to the 17.5 cm mark within three minutes; (4) the percentage of flies that passed the 17.5 cm mark within three minutes (success rate). The whole procedure was repeated four times for each group, with three-minute intervals between measurements, and their average was taken for statistical analysis. At least 10 samples per genotype were analyzed. Experiments in which half of the flies in the group did not pass the 17.5 cm mark in three minutes in three out of four trials were excluded from data analyses. This number is represented as the parameter 'failure rate'(46).

Climbing Performance Assay for Individual Flies

An assay for individual climbing performance of flies was used to measure climbing speed and endurance (50). Individual flies were transferred in the modified serological pipette with marks at distances of nine and 27 cm from the bottom. Flies were knocked down to the bottom of the tube to initiate the negative geotaxis reflex. The time that a fly took to reach the 9 cm mark was recorded and used for the estimation of climbing speed. For the estimation of endurance, we measured the distance that the fly climbed within 15 s (27 cm was considered maximal distance and we stopped the time when the fly reached it). Average of three measurements per tube were used for statistical analysis. In the analysis, we entered just the cases in which the fly passed the 9 cm mark (50).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA). All data are shown as mean +/- SD or median (range). Student's t-test or Mann Whitney U test were used for analyzing differences between the compared groups depending on the normality of the distribution, except for the failure rate for which the Chi square test was used. This failure rate was calculated as percentage of cases that were excluded from further analysis because half of the tested population failed to reach the goal of 17.5 cm during the period of three minutes of observation in three or more experiments. $p<0.05$ was considered statistically significant.

RESULTS

Results of Climbing Performance for Groups of Flies

The results of climbing performance for groups of flies are shown in **Table 1** and **Table 2**. In both sexes, the first fly of the wild-type (WT) group climbed the assigned height of 17.5 cm faster than the first fly of the FX group, but this difference was not statistically significant (males p=0.290, females p=0.467, **Table 1**). However, the first half of WT males (five flies) reached 17.5 cm significantly faster than first half of FX males (p=0.015, **Table 1**). Results for females were in the same direction but without statistical significance (p=0.212, **Table 1**). The flies were observed for 3 minutes and the percentage of flies that succeeded to climb 17.5 cm during that period (success rate) was significantly lowerin the FX group compared to the WT group in both sexes (males p=0.002, females p=0.043, **Table 1**).

Table 2. Sex comparison of climbing in the groups of wild-type flies and *dFMR1* mutants

Group climbing sex comparison						
	WT			FX		
	Males	Females	p value	Males	Females	p value
N1	11	11		11	10	
Failure rate (%)	18.2	9.1	0.534[¥]	27.3	0.0	0.074[¥]
N2	9	10		8	10	
t1 (s) mean ± SDI	6.556 ± 1.580	8.908 ± 2.038	**0.013**	7.250 ± 0.886	9.4500 ± 1.0728	**<0.001**
t2 (s) median	16.750	14.0000	0.487[§]	31.292	19.917	0.076[§]
t2 (s) range	(10.25 - 24.00)	(9.00 - 46.75)	NA	(16.50 - 83.00)	(13.00 - 49.25)	NA
SR mean ± SD (%)	79.167 ± 10.607	79.167 ± 8.231	0.157	64.375 ± 4.581	74.015 ± 14.649	0.075

Student's t-test is used unless indicated otherwise. [§]Mann Whitney U test was used; [¥]Chi square test. The failure rate was calculated as percentage of cases that were excluded from further analysis because half of a tested population failed to reach the goal of 17.5 cm during three minutes of observation in three or more experiments.

Abbreviations: WT, wild-type; FX, *dFMR1*^{B55}; N1, total number of experimental groups; N2, number of experimental groups used in analyses after exclusion of experimental groups in which half of the flies in the group did not pass the 17.5 cm mark in three minutes in three out of four trials; t1(s), the time, in seconds, needed for the first fly in the group to pass the mark drown at 17.5 cm from the bottom of the tube; t2(s), the time, in seconds, needed for half of the flies in the group (5 flies) to pass the 17.5 cm mark; SR, Success Rate represents the percentage of flies that passed the 17.5 cm mark within three minutes. Bold: statistically significant p values.

The comparison between sexes revealed that the first male fly was significantly faster than the first female fly in both groups (WT group: p=0.013, FX group: p<0.001, **Table 2**). Conversely, the first half of the female group came to the goal faster than the first half of the male group in WT flies and especially in FX flies. Although in this case the difference between WT and FX females and males was not significant, a positive trend is observed in the FX group (p=0.487 and p=0.076, respectively, **Table 2**). Furthermore, FX females had higher success rate than FX males (p=0.075, **Table 2**). This failure rate was consistent among all investigated groups, with the exception of FX males that failed much more than FX females, but without statistical significance (p=0.074, **Table 2**).

Results of Climbing Performance for Individual Flies

As described above, in the next climbing assay, every fly is tested separately and climbing speed and endurance of an individual fly are obtained. The results are represented in **Tables 3** and **4**. The results undoubtedly show that FX males are slower and less endurant than WT males (p=0.018, p=0.001; respectfully, **Table 3**). However, WT and FX females had similar speed and endurance (p>0.05, both). Further, WT and FX males were faster and more endurant than females (p<0.001, all. **Table 4**).

DISCUSSION

In the current investigation, *dFMR1*^{B55} mutant climbing abilities were examined in groups of flies, as presented in previous studies (45, 46, 51). Furthermore, this study described, for the first time, climbing abilities in *dFMR1*^{B55} mutants using individual flies. This research, for the first time, compared *dFMR1*^{B55} mutant climbing performance in females and males separately, and provided information about *dFMR1*^{B55} mutant endurance. Finally, this study revealed the following: (i) worse climbing performance of *dFMR1*^{B55} males in comparison to w^{1118} males; (ii) worse climbing success of *dFMR1*^{B55} females in comparison to w^{1118} females; (iii) better climbing performance of top performer males in comparison to top performer females in the group climbing test in both *dFMR1*^{B55} and w^{1118} groups; (iv) better, but not statistically significant, climbing performance (based on the time needed for 50% of flies to complete the task) and higher success rate in *dFMR1*^{B55} females in comparison to *dFMR1*^{B55} males.

Table 3. The results of analyses of climbing of individual wild-type flies and *dFMR1* mutants

Individual climbing						
	Males			Females		
	WT	FX	p value	WT	FX	p value
N	29	33		18	22	
speed	1.865 ± 0.372	1.614 ± 0.431	**0.018**	1.238 ± 0.396	1.256 ± 0.299	0.867
endurance	22.138 ± 3.592	18.581 ± 4.341	**0.001**	14.880 ± 3.091	14.712 ± 2.271	0.845

Student's t-test is used. Speed (cm/s) was calculated based on time in seconds that a fly took to reach the 9 cm mark; endurance (cm), the distance a fly climbed within 15 s.

Abbreviations: N, number of individual flies; WT, wild-type; FX, *dFMR1*^{B55} mutants. Bold: statistically significant p values.

Table 4. Sex comparison of climbing of individual wild-type flies and *dFMR1* mutants

Individual climbing sex comparison						
	WT			FX		
	Males	Females	p value	Males	Females	p value
N	29	18		33	22	
speed	1.864 ± 0.372	1.238 ± 0.396	**<0.001**	1.614 ± 0.431	1.256 ± 0.299	**0.001**
endurance	22.138 ± 3.592	14.879 ± 3.090	**<0.001**	18.580 ± 4.341	14.712 ± 2.271	**<0.001**

Student's t-test is used. Speed (cm/s) was calculated based on time in seconds that a fly took to reach the 9 cm mark; endurance (cm), the distance a fly climbed within 15 s.

Abbreviations: WT, wild-type; FX, *dFMR1*^{B55} mutants; N, number of individual flies. Bold: statistically significant p values.

Our study is in accordance with the results of other studies that investigated the motor capabilities of *dFMR1* mutants and also found a motor impairment. They investigated motor capability like flight (41), larva crawling (42, 43), and average motor activity (35). On the other hand, the study conducted by Dockendorff *et al.* (2002) found that *dFMR1* fly total motor activity observed during nine days in the dark was similar with motor activity in control WT flies. However, this discrepancy could be explained by different conditions/methods which were used in this study such as dark during nine days of experiments (13). Interestingly, *Fmr1*-KO male mouse performance on standard motor tests (including climbing) was similar to their WT counterparts with an exception of the raised-beam test in which *Fmr1* mice performed worse (52). However, motor learning is proven to be impaired in *Fmr1* mice (53).

Additional studies examined this ability in *dFMR1* mutants only in males (45, 46). Martinez and colleagues (2007) found that the first *dFMR1* male in the group climbed with a similar speed as the first male in the control group but the first half of *dFMR1* males was slower than their WT counterpart and the success rate of *dFMR1* was decreased compared to Canton S controls, but not to the Oregon red control line (46). The study of Tauber and colleagues (2011) obtained similar results, but they found differences in favor of controls even between the fastest male climbers in the two groups (45). However, this study used genetically rescued mutant flies as controls.

The current study investigated climbing ability of both sexes in *dFMR1*^{B55} mutants. Two assays used in the current study consistently showed that *dFMR1*^{B55} males are poorer climbers in comparison to WT. However, apart from the lower success rate in group climbing, *dFMR1*^{B55} females did not show differences in climbing ability compared with control flies. Therefore, the current research revealed that the mutation affects climbing abilities in a sex-specific manner in the *dFMR1*^{B55} mutants. Similarly, a recent study described that lead (plumb, Pb) exposure worsened climbing abilities of *Drosophila* Oregon-R in a sex-dependent manner. Namely, Pb provoked climbing impairment in both sexes, but climbing ability in male flies was more affected than in females. It is important to mention that Pb also induced other human-autistic-like behavior in fruit flies which is similar to the features of *dFMR1* mutants which were used in our study (9). Niveditha et al. (2017) found that females had lower reactive oxygen species levels and higher antioxidant levels than males (54). Thus, we could suggest that better oxidative stress handling in fly females might be responsible for their better climbing performance in general (54).

In conclusion, according to the results of the current study, climbing impairment was proved only in *dFMR1*^{B55} males, while *dFMR1*^{B55} females had similar climbing abilities to control WT. Thus, we could recommend that *dFMR1*^{B55} male mutants might present an excellent model for further research of locomotion impairment in the field of FXS. Also, *dFMR1*^{B55} male mutants could be an important 'tool' for pharmacological research and investigations of pharmacological agent effects to this kind of behavior. On the other hand, investigation of different aspects of climbing performance in *dFMR1*^{B55} female mutants could be unreliable. In addition, we can suggest always using both assays ('group' and 'individual') based on different parameters that can be obtained. Briefly, the current study demonstrates that *dFMR1*^{B55} male mutants are a very useful tool for research on locomotion and motility in the field of fragile X.

Conflicts of interest

None to declare

Ethical approval

This study includes research only on alternative model, i. e. Drosophila fragile X model. According to the National Centre for the Replacement Refinement & Reduction of Animal in Research, partial replacement includes the use of some animals that, based on current scientific thinking, are not considered capable of experiencing suffering. This includes invertebrates such as Drosophila, nematode worms and social amoebae, and immature forms of vertebrates (https://www.nc3rs.org.uk/the-3rs). According to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (https://eur-lex.europa.eu/eli/dir/2010/63/oj) and Serbia Law on animals' welfare (Sl. list RS 41/09 at: https://

www.paragraf.rs/propisi/zakon_o_dobrobiti_zivotinja.html), ethical review permissions are not needed for scientific research which include alternative models such as Drosophila.

Author Contributions

V.M. - acquisition, analysis, and interpretation of data, preparing the draft of the manuscript, M. S. - acquisition, analysis, and interpretation of data, M. B. - preparing the draft of the manuscrip, S. M. - acquisition and analysis of data, M. C. - interpretation of revised version of manuscript, D. P. - conception and design of the work and interpretation of revised version of manuscript.

REFERENCES

1. Jennings BH. Drosophila – a versatile model in biology & medicine. Materials Today. 2011;14(5):190-5.
2. Yamamoto S, Jaiswal M, Charng WL, Gambin T, Karaca E, Mirzaa G, et al. A drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases. Cell. 2014;159(1):200-14.
3. Ugur B, Chen K, Bellen HJ. Drosophila tools and assays for the study of human diseases. Dis Model Mech. 2016;9(3):235-44.
4. Littleton JT, Ganetzky B. Ion channels and synaptic organization: analysis of the Drosophila genome. Neuron. 2000;26(1):35-43.
5. O'Kane CJ. Drosophila as a model organism for the study of neuropsychiatric disorders. Curr Top Behav Neurosci. 2011;7:37-60.
6. Nitta Y, Sugie A. Studies of neurodegenerative diseases using Drosophila and the development of novel approaches for their analysis. Fly (Austin). 2022;16(1):275-98.
7. Campbell RA, Turner GC. The mushroom body. Curr Biol. 2010;20(1):R11-2.
8. Cotterill S, Yamaguchi M. Role of Drosophila in Human Disease Research 3.0. Int J Mol Sci. 2023;25(1).
9. Shilpa O, Anupama KP, Antony A, Gurushankara HP. Lead (Pb)-induced oxidative stress mediates sex-specific autistic-like behaviour in Drosophila melanogaster. Molecular Neurobiology. 2021;58(12):6378-93.
10. Xiong Y, Yu J. Modeling Parkinson's Disease in Drosophila: What Have We Learned for Dominant Traits? Front Neurol. 2018;9:228.
11. Hegde KN, Srivastava A. Drosophila melanogaster as a Tool for Amyotrophic Lateral Sclerosis Research. J Dev Biol. 2022;10(3).
12. Ueoka I, Pham HTN, Matsumoto K, Yamaguchi M. Autism Spectrum Disorder-Related Syndromes: Modeling with Drosophila and Rodents. Int J Mol Sci. 2019;20(17).
13. Dockendorff TC, Su HS, McBride SM, Yang Z, Choi CH, Siwicki KK, et al. Drosophila lacking dfmr1 activity show defects in circadian output and fail to maintain courtship interest. Neuron. 2002;34(6):973-84.
14. Inoue S, Shimoda M, Nishinokubi I, Siomi MC, Okamura M, Nakamura A, et al. A role for the Drosophila fragile X-related gene in circadian output. Curr Biol. 2002;12(15):1331-5.
15. Tassone F, Iong KP, Tong TH, Lo J, Gane LW, Berry-Kravis E, et al. FMR1 CGG allele size and prevalence ascertained through newborn screening in the United States. Genome Med. 2012;4(12):100.
16. Hagerman RJ, Berry-Kravis E, Hazlett HC, Bailey DB, Jr., Moine H, Kooy RF, et al. Fragile X syndrome. Nat Rev Dis Primers. 2017; 3:17065.
17. Trajković J, Makevic V, Pesic M, Pavković-Lučić S, Milojevic S, Cvjetkovic S, et al. Drosophila melanogaster as a Model to Study Fragile X-Associated Disorders. Genes (Basel). 2022;14(1).
18. Kaufmann WE, Kidd SA, Andrews HF, Budimirovic DB, Esler A, Haas-Givler B, et al. Autism Spectrum Disorder in Fragile X Syndrome: Cooccurring Conditions and Current Treatment. Pediatrics. 2017; 139(Suppl 3):S194-s206.
19. Bailey DB, Jr., Raspa M, Olmsted M, Holiday DB. Co-occurring conditions associated with FMR1 gene variations: findings from a national parent survey. Am J Med Genet A. 2008; 146a(16):2060-9.
20. Protic DD, Aishworiya R, Salcedo-Arellano MJ, Tang SJ, Milisavljevic J, Mitrovic F, et al. Fragile X Syndrome: From Molecular Aspect to Clinical Treatment. Int J Mol Sci. 2022;23(4).
21. Harris SW, Hessl D, Goodlin-Jones B, Ferranti J, Bacalman S, Barbato I, et al. Autism profiles of males with fragile X syndrome. Am J Ment Retard. 2008;113(6):427-38.
22. Hagerman, R. J. in Fragile X Syndrome: Diagnosis,Treatment and Research (eds Hagerman, R. J. & Hagerman, P. J.) 3–109 (Johns Hopkins Univ. Press,2002).
23. Kidd SA, Lachiewicz A, Barbouth D, Blitz RK, Delahunty C, McBrien D, et al. Fragile X syndrome: a review of associated medical problems. Pediatrics. 2014;134(5):995-1005.
24. Heulens I, Suttie M, Postnov A, De Clerck N, Perrotta CS, Mattina T, et al. Craniofacial characteristics of fragile X syndrome in mouse and man. Eur J Hum Genet. 2013;21(8):816-23.
25. Baranek GT, Danko CD, Skinner ML, Bailey DB, Jr., Hatton DD, Roberts JE, et al. Video analysis of sensory-motor features in infants with fragile X syndrome at 9-12 months of age. J Autism Dev Disord. 2005;35(5):645-56.
26. Zhang D, Kaufmann WE, Sigafoos J, Bartl-Pokorny KD, Krieber M, Marschik PB, et al. Parents' initial concerns about the development of their children later diagnosed with fragile X syndrome. J Intellect Dev Disabil. 2017;42(2):114-22.
27. Hinton R, Budimirovic DB, Marschik PB, Talisa VB, Einspieler C, Gipson T, et al. Parental reports on early language and motor milestones in fragile X syndrome with and without autism spectrum disorders. Dev Neurorehabil. 2013;16(1):58-66.
28. Will EA, Bishop SL, Roberts JE. Developmental divergence: motor trajectories in children with fragile X syndrome with and without co-occurring autism. Journal of Neurodevelopmental Disorders. 2019;11(1):23.
29. Bakker CE, Verheij C, Willemsen R, Helm Rvd, Oerlemans F, Vermey M, et al. Fmr1 knockout mice: A model to study fragile X mental retardation. Cell. 1994;78(1):23-33.
30. den Broeder MJ, van der Linde H, Brouwer JR, Oostra BA, Willemsen R, Ketting RF. Generation and characterization of FMR1 knockout zebrafish. PLoS One. 2009;4(11):e7910.
31. Drozd M, Bardoni B, Capovilla M. Modeling Fragile X Syndrome in Drosophila. Front Mol Neurosci. 2018;11:124.
32. Dahlhaus R. Of Men and Mice: Modeling the Fragile X Syndrome. Front Mol Neurosci. 2018; 11:41.
33. Gramates LS, Agapite J, Attrill H, Calvi BR, Crosby MA, Dos Santos G, et al. FlyBase: a guided tour of highlighted features. Genetics. 2022;220(4).
34. Wan L, Dockendorff TC, Jongens TA, Dreyfuss G. Characterization of dFMR1, a Drosophila melanogaster homolog of the fragile X mental retardation protein. Mol Cell Biol. 2000;20(22):8536-47.
35. Morales J, Hiesinger PR, Schroeder AJ, Kume K, Verstreken P, Jackson FR, et al. Drosophila fragile X protein, DFXR, regulates neuronal morphology and function in the brain. Neuron. 2002;34(6):961-72.
36. Sekine T, Yamaguchi T, Hamano K, Siomi H, Saez L, Ishida N, et al. Circadian phenotypes of Drosophila fragile x mutants in alternative genetic backgrounds. Zoolog Sci. 2008;25(6):561-71.

37. Bushey D, Tononi G, Cirelli C. The Drosophila fragile X mental retardation gene regulates sleep need. J Neurosci. 2009;29(7):1948-61.
38. McBride SM, Choi CH, Wang Y, Liebelt D, Braunstein E, Ferreiro D, et al. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. Neuron. 2005;45(5):753-64.
39. Choi CH, McBride SM, Schoenfeld BP, Liebelt DA, Ferreiro D, Ferrick NJ, et al. Age-dependent cognitive impairment in a Drosophila fragile X model and its pharmacological rescue. Biogerontology. 2010;11(3):347-62.
40. Banerjee P, Schoenfeld BP, Bell AJ, Choi CH, Bradley MP, Hinchey P, et al. Short- and long-term memory are modulated by multiple isoforms of the fragile X mental retardation protein. J Neurosci. 2010;30(19):6782-92.
41. Zhang YQ, Bailey AM, Matthies HJ, Renden RB, Smith MA, Speese SD, et al. Drosophila fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. Cell. 2001;107(5):591-603.
42. Xu K, Bogert BA, Li W, Su K, Lee A, Gao FB. The fragile X-related gene affects the crawling behavior of Drosophila larvae by regulating the mRNA level of the DEG/ENaC protein pickpocket1. Curr Biol. 2004;14(12):1025-34.
43. Günther MN, Nettesheim G, Shubeita GT. Quantifying and predicting Drosophila larvae crawling phenotypes. Sci Rep. 2016;6:27972.
44. Bolduc FV, Valente D, Nguyen AT, Mitra PP, Tully T. An assay for social interaction in Drosophila fragile X mutants. Fly (Austin). 2010;4(3):216-25.
45. Tauber JM, Vanlandingham PA, Zhang B. Elevated levels of the vesicular monoamine transporter and a novel repetitive behavior in the Drosophila model of fragile X syndrome. PLoS One. 2011;6(11):e27100.
46. Martinez VG, Javadi CS, Ngo E, Ngo L, Lagow RD, Zhang B. Age-related changes in climbing behavior and neural circuit physiology in Drosophila. Dev Neurobiol. 2007;67(6):778-91.
47. Ferreiro MJ, Pérez C, Marchesano M, Ruiz S, Caputi A, Aguilera P, et al. Drosophila melanogaster White Mutant w(1118) Undergo Retinal Degeneration. Front Neurosci. 2017;11:732.
48. Cao W, Song L, Cheng J, Yi N, Cai L, Huang FD, et al. An Automated Rapid Iterative Negative Geotaxis Assay for Analyzing Adult Climbing Behavior in a Drosophila Model of Neurodegeneration. J Vis Exp. 2017(127).
49. Barone MC, Bohmann D. Assessing neurodegenerative phenotypes in Drosophila dopaminergic neurons by climbing assays and whole brain immunostaining. J Vis Exp. 2013(74):e50339.
50. Gabrawy MM, Campbell S, Carbone MA, Morozova TV, Arya GH, Turlapati LB, et al. Lisinopril Preserves Physical Resilience and Extends Life Span in a Genotype-Specific Manner in Drosophila melanogaster. J Gerontol A Biol Sci Med Sci. 2019;74(12):1844-52.
51. Novak SM, Joardar A, Gregorio CC, Zarnescu DC. Regulation of Heart Rate in Drosophila via Fragile X Mental Retardation Protein. PLoS One. 2015;10(11):e0142836.
52. Roy S, Zhao Y, Allensworth M, Farook MF, LeDoux MS, Reiter LT, et al. Comprehensive motor testing in Fmr1-KO mice exposes temporal defects in oromotor coordination. Behav Neurosci. 2011;125(6):962-9.
53. Padmashri R, Reiner BC, Suresh A, Spartz E, Dunaevsky A. Altered structural and functional synaptic plasticity with motor skill learning in a mouse model of fragile X syndrome. J Neurosci. 2013;33(50):19715-23.
54. Niveditha S, Deepashree S, Ramesh SR, Shivanandappa T. Sex differences in oxidative stress resistance in relation to longevity in Drosophila melanogaster. J Comp Physiol B. 2017;187(7):899-909.

TEST PENJANJA KAO MERA LOKOMOTRONE SPOSOBNOSTI NA MODELU VINSKE MUŠICE ZA FRAGILNI X SINDROM

Vedrana Makević[1], Maja Stojković[2], Marko Biorac[3], Sara Milojević[2], Maria Capovilla[4], Dragana Protić[2]

Sažetak

Uvod: Fragilni X sindrom (FXS) je najčešći monogenetski uzrok intelektualne zaostalosti i poremećaja iz spektra autizma kod ljudi. Odličan model za istraživanje u ovoj oblasti je *Drosophila melanogaster* model FXS (*dFMR1* mutanti). Cilj ove studije bio je sveobuhvatno istraživanje penjanja, kao mere lokomocije, linije *dFMR1*^{B55}, koja je model FXS kod vinske mušice.

Metode: U ovoj studiji korišćene su linije vinskih mušica *w*^{1118} i *dFMR1*^{B55}. Sposbnost penjanja muva ispitana je testovima penjanja za grupe muva i za pojedinačne muve. Određivani su parametri koji se odnose na sposobnost penjanja, brzinu i izdržljivost muva. Ženke i mužjaci su zasebno analizirani.

Rezultati: Ova studija je pokazala: (i) lošiju sposobnost penjanja *dFMR1*^{B55} mužjaka u poređenju sa *w*^{1118} mužjacima, (ii) slabiju stopu uspeha u penjanju *dFMR1*^{B55} ženki u poređenju sa *w*^{1118} ženkama, (iii) bolju penjačku sposobnost mužjaka u odnosu na ženke u obe ispitivane linije pokazanu u individualnom i grupnom testu penjanja u poređenju najbržih muva, (iiii) bolju sposobnost penjanja (baziranu na vremenu potrebnom da 50% muva izvrši zadatak) i veću stopa uspeha *dFMR1*^{B55} ženki u poređenju sa *dFMR1*^{B55} muškarcima, premda ove razlike nisu bile statistički značajne.

Zaključak: U ovoj studiji, poremećaj penjanja je dokazan samo kod *dFMR1*^{B55} mužjaka, dok su *dFMR1*^{B55} ženke imale slične sposobnosti penjanja sa kontrolnim *w*^{1118}.

Ključne reči: fragilni X sindrom, *Drosophila melanogaster*, test penjanja

Primljen: 12.02.2024. I **Revizija:** 04.04.2024. I **Prihvaćen:** 04.04.2024.

Medicinska istaživanja 2024; 57(3):27-33

Medicinska istaživanja 2024; 57(3):35-40 | DOI: 10.5937/medi57-49963

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Histopathological findings in enteric nervous plexuses in children with intestinal motility disorders – a single center experience

Radmila Janković[1,3], Sanja Sinđić-Antunović[2,3], Miloš Đuknić[1,3], Dragana Vujović[2,3], Nina Ristić[3,4], Jovan Jevtić[1,3], Đorđe Topličić[2], Milena Backović[3,5], Nenad Zdujić[2], Dunja Putniković[3], Marija Lukač[2,3]

[1] University of Belgrade, Faculty of Medicine, Institute of Pathology "Prof. dr Đorđe Joannović", Belgrade, Serbia

[2] Department of Pediatric Surgery, University Children´s Hospital, Belgrade, Serbia

[3]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

[4]Department of Gastroenterology, University Children´s Hospital, Belgrade, Serbia

[5]Institute for Oncology and Radiology of Serbia, Belgrade, Serbia

Recived: 21 March 2024

Revised: 11 May 2024

Accepted: 11 May 2024

Check for updates

Funding information:

This work has been supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia through Grant Agreement with University of Belgrade, Faculty of Medicine No: 451-03-66/2024-03/200110

Competing interests:

The authors have declared that no competing interests exist

Correspondence to:

Jankovic Radmila

University of Belgrade, Faculty of Medicine, Institute of Pathology "Prof. Dr. Đorđe Joannović",

1 Dr Subotića Street, 11000 Belgrade, Serbia

E-mail: radmila.jankovic@med.bg.ac.rs radmila.jankovic011@gmail.com

Summary

Introduction/Aim: The aim of the study was to determine the frequency of various histopathological findings in biopsies of children with intestinal hypomotility and the incidence and characteristics of Hirschsprung disease (HD).

Methods: Biopsies of colon and rectum taken due to intestinal hypomotility and chronic constipation at the Department of Pediatric Surgery of the University Children´s Hospital in Belgrade over the 10-year period (from 2009 to 2018) were reviewed using pathology reports from the archive of the Institute of pathology, Faculty of Medicine, University of Belgrade.

Results: A total of 287 patients with intestinal motility disorder were identified, with 554 biopsy samples. Of the total number of patients, 56% (161/287) were without any morphological changes in enteric nervous system (ENS). The most common histopathological findings were HD (69/287; 24%) and immaturity of ganglion cells (29/287; 10%). Isolated hypoganglionosis of ENS was found in 5 (2%) cases. Heterotopia of ganglion cells was the only finding in 8 (3%) cases. Rare causes of intestinal dysmotility were: eosinophilic proctitis/colitis (EPC) (4/287), neuronal intestinal dysplasia B (2/287), unclassified disganglionoses (3/287). Rectosigmoid variant of HD was the most frequent HD variant (80.3%). Acetylcholinesterase method and immunohistochemical staining were used in 19.5% cases.

Conclusions: HD and immaturity of ganglion cells were the most common pathological findings in ENS of constipated children. It is important to differentiate EPC from other lesions of enteric plexuses due to different natural history and therapy.

Key words: Hirschsprung disease, suctional biopsy, full thickness biopsy, intestinal motility disorders

Cite this article as: Janković R, Sindjić-Antunović S, Đuknić M, Vujović D, Ristić N, Jevtić J, Topličić Đ, Backović M, Zdujić N, Putniković D, Lukač M. Histopathological findings in enteric nervous plexuses in children with intestinal motility disorders – a single center experience; Medicinska istaživanja 2024; 57(3):35-40 DOI: 10.5937/medi57-49963

INTRODUCTION

Intestinal motility disorders present a relatively common problem in children, particularly in the first year of life (1). The typical clinical presentation of intestinal dysmotility, including dyspepsia, vomiting, abdominal pain and distension, bloating, and constipation is nonspecific (2). The most prevalent pathological finding causing motor intestinal disorders in infancy and childhood is Hirschsprung disease (HD) (3). HD is a congenital aganglionosis affecting the rectum and a variable length of the bowel segment proximal to the rectum. The delayed passage of meconium in the first days of life is a crucial clinical sign that raises suspicion of HD. The absence of ganglion cells in the segment of the enteric nervous system (ENS) leads to pseudoobstruction. An early diagnosis of HD is crucial for preventing severe enterocolitis, a complication with a potentially fatal outcome (4).

On the other hand, there are other rare disorders of the ENS that can clinically manifest as chronic constipation or symptoms resembling HD. The aim of this study was to analyze the frequency of various histopathological findings in biopsies of neonates, infants, and children with intestinal hypomotility and chronic constipation, as well as to determine the incidence and characteristics of Hirschsprung disease (HD) in a Serbian regional center.

METHODS

We conducted an analysis at the Institute of Pathology in Belgrade, examining histopathological slides and pathology reports of colon and rectum biopsies taken due to intestinal hypomotility and chronic constipation at the Department of Pediatric Surgery, University Children's Hospital, Belgrade. This study covers a 10-year period, from 2009 to 2018. In cases where there was suspicion of Hirschsprung disease (HD), fresh suction biopsies were stained using the acetylcholinesterase (AchE) histochemical method. The majority of biopsies were routinely fixed in formalin, embedded in paraffin, cut into 5 μm thick sections, and stained with hematoxylin & eosin (H&E). Additionally, in some cases, immunohistochemical staining was performed using various antibodies. The antibodies and their dilutions commonly used are listed in **Table 1**.

Table 1. Details of antibodies used in the analysis of suction intestinal biopsies

Antibody	Clone	Source	Dilution
Calretinin	DAK-calret 1	DAKO	1:50
MAP-2	HM-2	Abcam	1:1000
Glut-1	SPM948	Lab Vision	1:200
S-100	Z0311	DAKO	1:400
GFAP	GF2	DAKO	1:50
Synaptophysin	SY38	DAKO	RTU
SOX-10	BC34	Abcam	1:100
Bcl-2	Bcl-2/100/D5	Novocastra	1:100
CD117	104 D2	DAKO	1:300

RESULTS

Over the 10-year period, we identified 287 patients with intestinal motility disorders, comprising a total of 554 biopsy samples. The majority of the patients were males (178/287, 62%). The median age at the time of the first biopsy was 5 months, ranging from the first day of life to 209 months. More than half of all patients underwent only one biopsy (165/287; 57.5%) **(see Figure 1)**. In 6 cases (2%), the samples were deemed inadequate for analysis.

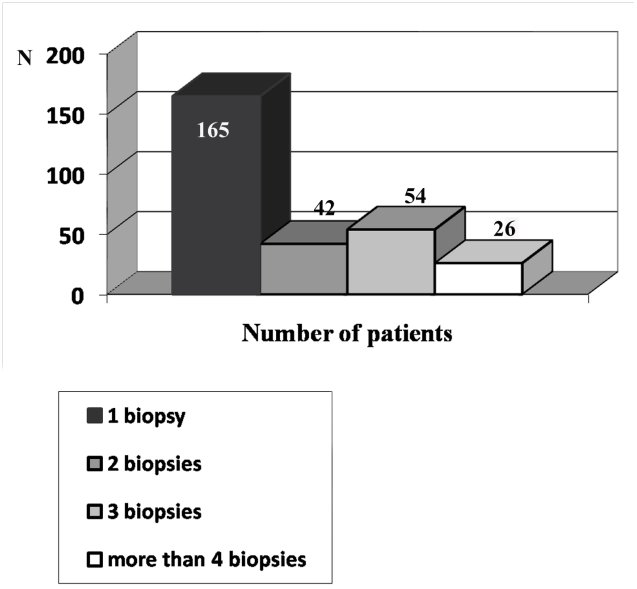


Figure 1. The distribution of the total number of biopsies in children with intestinal dysmotility problems

About half of the patients exhibited symptoms suggestive of Hirschsprung disease (HD) (151/287, 53%). One-fifth of the patients (56/287; 19.5%) had intestinal dysmotility caused by anal atresia. The remaining patients presented with intestinal hypomotility and various clinical manifestations, such as chronic constipation, subocclusion, and intestinal perforation.

All biopsies underwent routine H&E staining. The acetylcholinesterase method was applied in 22 patients (7.6%): in 16 patients as the sole additional staining and in 6 patients in conjunction with immunohistochemical staining. Immunohistochemical staining was applied in 53 biopsies, with 30 patients receiving it as the only additional staining. The most commonly used antibodies were calretinin, MAP-2, and Glut-1 **(Table 2)**. Notably, there were no false positive or false negative results.

The majority of patients (161/287, 56%) with intestinal motility disorders did not exhibit any morphological changes in the ENS. HD was the most frequent pathological finding (69/287; 24%). In 46% (69/151) of clinically suspicious HD cases, the diagnosis was confirmed by histopathology. The rectosigmoid variant of HD (RS-HD) was the most prevalent HD subtype (49/61; 80.3%). Less common variants included long segment HD (8/61;

Table 2. The frequency of usage of primary antibodies for immunohistochemical staining in the biopsies of children with intestinal dysmotility problems

Antibody	Number of biopsies (N)	Percentage of all immunostained biopsies (%)
Calretinin	42	79%
MAP-2	40	75%
Glut-1	33	62%
S-100	8	15%
GFAP	2	4%
Synaptophysin	6	11%
SOX-10	2	4%
Bcl-2	7	13%
CD117	10	19%

13.1%), total colonic aganglionosis (3/61; 4.9%), and ultra-short HD (US-HD), diagnosed in only one case.

The median age of patients at the time of histopathological diagnosis of HD was 19.5 days (ranging from the first day of life to 140 months). The median age at the time of surgical resection of the agangliotic colon was 4.5 months (ranging from 24 days to 11 years and 9 months). In 6 cases, reoperation was necessary, with a median of 36.1 months (ranging from 8 months to 50.6 months). Primary surgical resection in two patients was performed at another institution. In all cases requiring a redo operation, aganglionosis of the colon affected a long segment – TCA was present in one case, while hypoganglionosis was associated with HD in another case.

Long-segment HD was diagnosed in 13.1% of patients. US-HD was diagnosed in one patient, while TCA was diagnosed in 3 (4.9%) patients. The transanal endorectal pull-through resection was the most common surgical technique applied in all cases with classical RS-HD and US-HD. In other cases, various procedures such as the Soave, Duhamel, or Swenson technique were applied. Immaturity of ganglion cells was the second most common finding (29/287; 10%) in the ENS in children with intestinal motility disorders in our center, particularly within the first year of life **(see Figure 2)**.

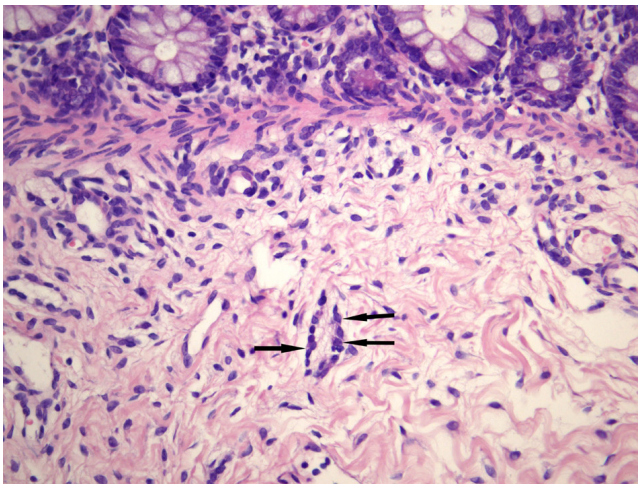


Figure 2. Immaturity of ganglion cells in the submucosal nervous plexus (H&E, x400). The ganglion cells (arrows) exhibit small basophilic nuclei and a limited amount of cytoplasm – it is difficult to distinguish them from glial cells on H&E slides.

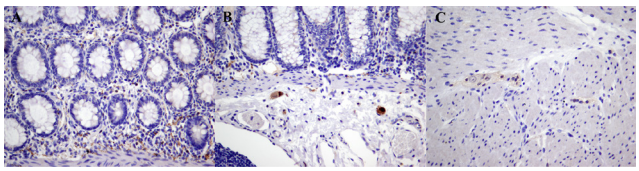


Figure 3. The biopsy from a patient with hypoganglionosis associated with Hirschsprung disease (HD) revealed the following features: in the lamina propria of hypoganglionic segment, intrinsic nerve fibers were present (A), small ganglia were found in the submucosa (B), and rare small ganglia were noted without calretinin expression in ganglion cells in the myenteric nervous plexus (C) (A, B, C: Calretinin, x400).

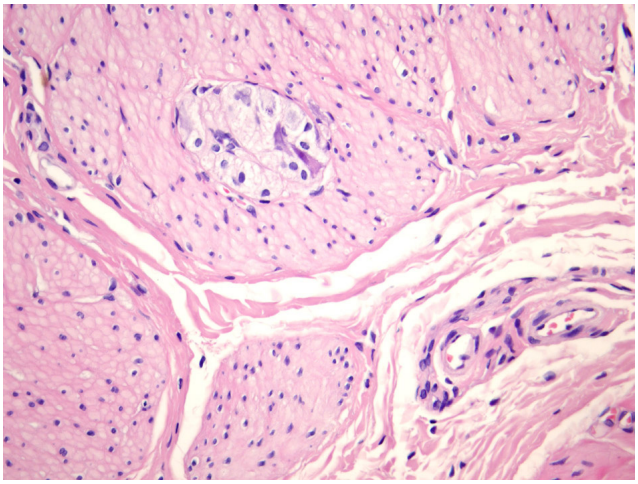


Figure 4. Heterotopic ganglia in the circular muscular layer: it is surrounded by muscular fibers (H&E, x400).

Hypoganglionosis of the enteric nervous system was identified as the sole pathological finding in 5 (2%) cases, while in one patient, it was associated with HD **(Figure 3)**.

Heterotopic ganglia were the exclusive finding in 8 (3%) cases. Ectopic ganglia were identified in the muscular coat, surrounded by muscular fibers **(Figure 4)**.

Rare causes of intestinal dysmotility included eosinophilic proctitis or colitis (4/287), intestinal neuronal dysplasia B (IND B) (2/287) and non-classified disgan-

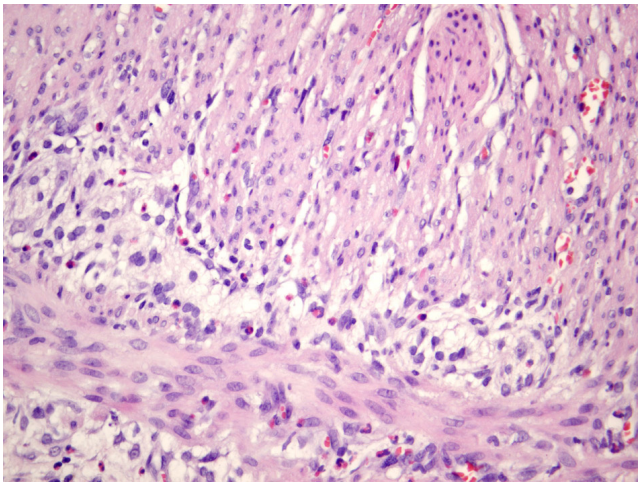


Figure 5. Rare findings in enteric nervous plexuses as a cause of intestinal dysmotility include eosinophilic ganglionitis within eosinophilic colitis. Eosinophils surround myenteric ganglia and are also present within ganglia, as well as between muscular fibers (HE, x400).

glionoses (3/287) **(Figure 5)**. In both cases of IND B, it was an isolated finding. The histological findings in enteric nervous plexuses in dolichocolon were variable, and we categorized them as non-classified disganglionosis. This included the presence of thick nerve bundles with ganglion cells in the submucosa of the proximal rectum and sigmoid colon, several megaganglia in the submucosa (less than 20%), relative hyperplasia of glial cells within myenteric ganglia, and the presence of heterotopic ganglia within muscular layers.

DISCUSSION

Constipation in children, especially in the first year of life, is a common problem. The majority of constipated children experience functional issues, but it is crucial to rule out organic causes of chronic constipation (5,6). The primary cause of intestinal dysmotility in the first year of life is HD, consistent with larger global studies (7). The absence of meconium stool within first 48 hours of life may indicate possible rectal aganglionosis and serve as an indication for rectal biopsy. In our study, the median age at the time of histopathological confirmation of HD was 19.5 days. In rare cases, the diagnosis was established later, with the oldest HD patient being 11.5 years old. Late HD diagnosis is often associated with complications and a long recovery (8,9).

The "gold standard" for HD diagnosis is histopathological analysis of suction rectal biopsy (10). In previous years, frozen sections stained with H&E and AchE method were considered the "gold standard" (3). However, due to limitations of AchE usage, calretinin immunohistochemical staining has become a new standard in many laboratories (4). Calretinin immunohistochemistry has several advantages, being applicable on formalin-fixed tissue and superficial biopsies (11,12). However, it is not an ideal marker, especially as HD diagnosis is based on the absence of its expression. Additionally, in euganglionic intestines in patients with Down syndrome, calretinin expression may be absent (13). Different recommendations exist for utilizing immunostaining in HD diagnostics, suggesting the application of several antibodies (10,14,15). In our center, over a 10-year period, both AchE and immunohistochemical staining were used with similar success in HD diagnosis. With the advancements in artificial intelligence, deep learning approaches have shown potential to standardize and facilitate the diagnostic process (16).

The majority of children with HD in our study were treated using the transanal endorectal pull-through technique, which has demonstrated superior effectiveness compared to alternative methods. Earlier onset, lower incidence, and mild complications make this procedure more favorable, reducing the need for additional surgeries (17). Previous surgical methods had more long-term complications, with fecal and urinary incontinence being significantly more common compared to the general population in long-term follow-up studies. Earlier surgical methods were also associated with reduced fertility in female patients (18).

Most children with intestinal hypomotility in our group were male, consistent with larger global series (18). The distribution of HD variants was similar to other large studies (15), and it did not differ from a previous study in the same institution during a different period (19).

Immaturity of ganglion cells can be a cause of intestinal dysmotility, often presenting in the first year of life, usually in premature babies, and typically does not require intervention (15). It is an obligatory pathological finding after the fourth year of life (20).

Isolated hypoganglionosis is a rare pathological finding, often with a clinical presentation similar to HD. It can also be associated with HD (2). Typical histopathological findings include rare small myenteric ganglia and damaged AChE activity (20). The estimation of the number of ganglion cells is delicate, without an exact cut-off. Recommendations suggest that each laboratory establishes its own cut-off values related to age (21).

Ectopic ganglia within the muscular layers are considered a pathological finding in the majority of the gastrointestinal tract (21), while their presence in the mucosal lamina propria in children is considered normal (2,21).

Clinical manifestations of eosinophilic enterocolitis could include chronic constipation or pseudoobstruction, similar to HD (22,23). However, the significance of eosinophils in the aganglionic segment and in the transition zone is not clear (24).

IND B is a controversial diagnosis, often associated with HD (24,25). Its clinical presentation can be very similar to HD, but histopathological features are distinctive. While HD is more of a qualitative diagnosis, IND is more of a quantitative diagnosis, based on the estimation of the percentage of megaganglia in the submucosa (25). Non-classified dysganglionoses are complex, with different features of ganglionic abnormalities. Although "unclassified," there is a complex classification of non-classified disganglionoses (26) to provide better treatment. In rare cases with chronic constipation, disorders of Cajal cells should be considered as a possible cause of intestinal pseudoobstruction (27). Also, transitory dysfunction of Cajal cells can be the cause of postoperative dysmotility in patients with surgically treated Hirschsprung's disease (28). In some cases, routine histopathological methods may not be helpful, and the underlying pathological mechanisms are sometimes unclear. A very few associated genetic mutations have been identified (29). In some rare cases, extraintestinal causes such as syringomyelia could be identified as the cause of chronic constipation (30).

CONCLUSION

More than half of patients with intestinal motility disorders did not show any morphological changes in the ENS. The most prevalent causes of chronic constipation in the pediatric age group in our center are HD and immaturity of ganglion cells. Furthermore, rare causes of intestinal hypomotility, such as hypoganglionosis, heterotopic ganglia, IND B, or eosinophilic colitis, should be considered as potential contributors to intestinal hypomotility.

Conflicts of interest

None

Author contributions

Conception and design: RJ, SSA, ML, MĐ, DV, JJ; data collection: JJ, MĐ, DV, MB, DP, ĐT; writing the article: RJ, SSA, MĐ, DV, NR; critical revision of the article: RJ, SSA, DV, MĐ, NZ, NR, ML; final approval of the article: RJ, SSA, MĐ, DV, NR, JJ, ĐT, MB, NZ, DP, ML

Ethical approval

This study positively stated by the local Ethics committee (University of Belgrade, Medical School-29/VII-2, 1st July 2015 and University Children's Hospital Tiršova, Belgrade (26/185, 4th June 2015).

REFERENCES

1. Pashankar DS. Childhood constipation: evaluation and management. Clin Colon Rectal Surg 2005; 18(2):120.
2. Schäppi MG, Staiano A, Milla AJ, Smith VV, Dias JA, Heuschkel R, Husby S, Mearin ML, Papadopoulou A, Ruemmele FM, Vandenplas Y, Koletzko S. A practical guide for the diagnosis of primary enteric nervous system disorders. J Pediatr Gastroenterol Nutr 2013; 57(5): 677-686.
3. Gfroerer S, Rolle U. Pediatric intestinal motility disorders. World J Gastroenterol 2015; 21(33):9683-9687.
4. Kapur R. Practical pathology and genetics of Hirschsprung's disease. Seminars Ped Surg 2009; 18(4):212-223.
5. Ho JMD, How CH. Chronic constipation in infants and children. Singapore Med J 2020;61(2):63-68.
6. Wallace C, Sinopoulou V, Gordon M, Akobeng AK, Llanos-Chea A, Hungria G, Febo-Rodriguez L, Fifi A, Fernandez Valdes L, Langshaw A, Saps M. Probiotics for treatment of chronic constipation in children. Cochrane Database Syst Rev. 2022; 3(3):CD014257.
7. Khan AR, Vujanic GM, Huddart S. The constipated child: how likely is Hirschsprung's disease? Pediatr Surg Int 2003; 19(6):439-442.
8. Tan YW, Chacon CS, Geoghegan N, Saxena A, Clarke S, Haddad M, Choudhry M. Late Diagnosis of Hirschsprung's Disease: Definition and Implication on Core Outcomes. Eur J Pediatr Surg 2022; 32(6):512-520.
9. Huerta CT, Ramsey WA, Davis JK, Saberi RA, Gilna GP, Parreco JP, Sola JE, Perez EA, Thorson CM. Nationwide outcomes of newborns with rectosigmoid versus long-segment Hirschsprung disease. J Pediatr Surg. 2023; 58(5):849-855.
10. Ambartsumyan L, Smith C, Kapur RP. Diagnosis of Hirschsprung Disease. Pediatr Dev Pathol. 2020; 23(1):8-22.
11. Gonzalo DH, Plesec T. Hirschsprung disease and use of calretinin in inadequate rectal suction biopsies. Arch Pathol Lab Med 2013; 137(8):1099-1102.
12. Singh SK, Gupta UK, Aggarwal R, Rahman RA, Gupta NK, Verma V. Diagnostic Role of Calretinin in Suspicious Cases of Hirschsprung's Disease. Cureus. 2021; 13(2):e13373.
13. Warren M, Kaul A, Bove KE. Calretinin-immunoreactive hypoinnervation in Down syndrome (DS): report of an infant with very short-segment Hirschsprung disease and comparison to biopsy findings in 20 normal infants and 11 infants with DS and chronic constipation. Pediatr Dev Pathol 2016; 19(2):87-93.
14. Bachmann L, Besendörfer M, Carbon R, Lux P, Agaimy A, Hartmann A, Rau TT. Immunohistochemical Panel diagnostics of Hirschsprung's disease with Map2, calretinin, Glut1 and S100. Histopathology 2015; 66(6):824-835.
15. Lotfollahzadeh S, Taherian M, Anand S. Hirschsprung Disease. [Updated 2023 Jun 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562142/.
16. Duci M, Magoni A, Santoro L, Dei Tos AP, Gamba P, Uccheddu F, Fascetti-Leon F. Enhancing diagnosis of Hirschsprung's disease using deep learning from histological sections of post pull-through specimens: preliminary results. Pediatr Surg Int 2023;40(1):12.
17. Lukac M, Antunović SS, Vujović D, Petronić I, Nikolić D, Radlović V, Krstajić T, Krstić Z. Effectiveness of various surgical methods in treatment of Hirschsprung's disease in children. Vojnosanit Pregl 2016;73(3):246-50.
18. Byström C, Örtqvist L, Gunnarsdóttir A, Wester T, Löf Granström A. Fertility in patients with Hirschsprung's disease: population-based cohort study. BJS Open 2023;7(3):zrad043.
19. Jankovic R. Analysis of glial cell index and interstitial cells of Cajal in colorectal biopsies of children with Hirschsprung disease and related disorders [doctoral dissertation]. Belgrade: Faculty of Medicine, University of Belgrade; 2016.
20. Feichter S, Meier-Ruge WA, Bruder E. The histopathology of gastrointestinal motility disorders in children. Seminars Ped Surg 2009; 18(4):206-11.
21. Knowles CH, De Giorgio R, Kapur RP, Bruder E, Farrugia G, Geboes K, Gershon MD, Hutson J, Lindberg G, Martin JE, Meier-Ruge WA, Milla PJ, Smith VV, Vandervinden JM, Veress B, Wedel T. Gastrointestinal neuromuscular pathology: guidelines for histological techniques and reporting on behalf of the Gastro 2009 International Working Group. Acta Neuropathol 2009; 118(2):271–301.
22. Kinoshita Y, Ishihara S. Eosinophilic gastroenteritis: epidemiology, diagnosis, and treatment. Curr Opin Allergy Clin Immunol 2020; 20(3):311-315.
23. D'Auria E, Calcaterra V, Maestri L, Meroni M, Selvaggio GGO, Villanacci V, Nebuloni M, Pelizzo G. Case Report: Eosinophilic Myenteric Ganglionitis in a Child With Hirschsprung's Disease: A Challenge in Pseudo-Obstruction. Front Pediatr 2021; 8:617309.
24. Kapur RP, Kennedy AJ. Transitional zone pull through: surgical pathology considerations. Sem Pediatr Surg 2012; 21(4):291-301.
25. Kapur RP, Reyes-Mugica M. Intestinal Neuronal Dysplasia Type B: An Updated Review of a Problematic Diagnosis. Arch Pathol Lab Med 2019;143(2):235-243.
26. Berger S, Linke F, Tiling Sv, Kap-herr SH. The clinical relevance of non-classified dysganglionoses and implications for a new grading system. Pediatr Surg Int 2002; 18(5-6):361-367.
27. Turcotte MC, Faure C. Pediatric Intestinal Pseudo-Obstruction: Progress and Challenges. Front Pediatr 2022; 10:837462.
28. Jankovic R, Sindjic-Antunovic S, Lukac M, Vujovic D, Jevtic J, Skender-Gazibara M. Altered Distribution of Interstitial Cells of Cajal in Normoganglionic and Transitional Zone of Hirschsprung Disease and Their Clinical Significance. Central Eur J Paed 2020; 16(1): 1-9.

29. Nham S, Nguyen ATM, Holland AJA. Paediatric intestinal pseudo-obstruction: a scoping review. Eur J Pediatr 2022;181(7):2619-2632

30. Deftereos SP, Foutzitzi S, Karagiannakis G, Aggelidou M, Cassimos DC, Kambouri K. Constipation and dilated bowel: Hirschsprung's disease is not always the case. Clin Pract 2020; 10(4):1270.

HISTOPATOLOŠKI NALAZ U ENTERIČKIM NERVNIM PLEKSUSIMA KOD DECE SA POREMEĆAJIMA INTESTINALNOG MOTILITETA - ISKUSTVO JEDNOG CENTRA

Radmila Janković[1,3], Sanja Sinđić-Antunović[2,3], Miloš Đuknić[1,3], Dragana Vujović[2,3], Nina Ristić[3,4], Jovan Jevtić[1,3], Đorđe Topličić[2], Milena Backović[3,5], Nenad Zdujić[2], Dunja Putniković[3], Marija Lukač[2,3]

Sažetak

Uvod/cilj: Cilj istraživanja je utvrđivanje frekvencije različitih histopatoloških nalaza u biopsijama dece sa intestinalnim hipomotilitetom i određivanjem učestalosti i karakteristika Hirschsprungove bolesti (HD).

Metode: Retrospektivna analiza histopatoloških izveštaja arhive Instituta za patologiju Medicinskog fakulteta u Beogradu biopsija dece sa hipomotilitetom creva i hroničnom opstipacijom u desetogodišnjem periodu (od 2009. do 2018. godine), lečene u Univerzitetskoj dečjoj klinici "Tiršova" u Beogradu.

Rezultati: Analizom je obuvaćeno 287 pacijenata sa poremećajem crevne pokretljivosti sa ukupno 554 biopsijska uzorka. Više od polovine pacijenata (56%; 161/287) je bilo bez morfoloških promena u enteričkom nervnom sistemu (ENS). Najčešći histopatološki nalazi bili su HD (69/287; 24%) i nezrelost ganglijskih ćelija (29/287; 10%). Izolovana hipoganglionioza ENS identifikovana je u 5 (2%) slučajeva. Heterotopija ganglijskih ćelija bila je jedini nalaz u 8 (3%) slučajeva. Retki uzroci crevnog hipomotiliteta bili su: eozinofilni proktitis/kolitis (EPC) (4/287), intestinalna neuronalna displazija tip B (2/287) i neklasifikovane disganglionoze (3/287). Rektosigmoidna HD-a bila je najčešća HD varijanta (80.3%). Metoda acetilcholinesteraze i imunohistohemijsko bojenje korišćeni su u dijagnostici kod 19,5% slučajeva.

Zaključak: HD i nezrelost ganglijskih ćelija bili su najčešći patološki nalazi kod dece sa hroničnom opstipacijom. Važno je razlikovati EPC od ostalih lezija enteričnog pleksusa zbog različite prirode bolesti i preporučene terapije.

Zaključak: U ovoj studiji, poremećaj penjanja je dokazan samo kod *dFMR1*^{B55} mužjaka, dok su *dFMR1*^{B55} ženke imale slične sposobnosti penjanja sa kontrolnim *w*^{1118}.

Ključne reči: Hirschsprungova bolest, sukciona biopsija, biopsija pune debljine zida creva, poremećaji crevnog motiliteta

Primljen: 21.03.2024. I **Revizija:** 11.05.2024. I **Prihvaćen:** 11.05.2024.

Medicinska istaživanja 2024; 57(3):35-40

Medicinska istaživanja 2024; 57(3):41-47 | DOI: 10.5937/medi57-51008

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Relationship between daily physical activity and quality of sleep in maintenance hemodialysis patients

Trbojević-Stanković Jasna[1,2], Stanimirović Sanja[3], Ristanović Ljubica[4], Nešić Dejan[1,5]

[1]University of Belgrade, Faculty of Medicine, Belgrade, Serbia
[2]Department of Hemodialysis, University Clinical Center "Dr Dragisa Misovic", Belgrade, Serbia
[3]Sports Association "Mia Body Space", Valjevo, Serbia
[4]University of Belgrade, Faculty of Sport and Physical Education, Belgrade, Serbia
[5]University of Belgrade, Faculty of Medicine, Institute for Medical Physiology, Belgrade, Serbia

Recived: 13 May 2024
Revised: 25 May 2024
Accepted: 03 June 2024

Check for updates

Funding information:
The authors report no funding associated with the work reported in this article.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Jasna Trbojević-Stanković
Department of Hemodialysis, University Clinical Center "Dr Dragisa Misovic",
1 Heroja Milana Tepića Street, 11000 Belgrade, Serbia
E-mail: jasna.trbojevic-stankovic@med.bg.ac.rs; ts.jasna@gmail.com

Summary

Introduction/Aim: Hemodialysis patients are often sedentary and experience a high prevalence of sleep disorders. In this study, we aimed to assess the level of physical activity and quality of sleep among patients on maintenance hemodialysis and assess the relationship between these issues.

Material and Methods: Seventy-one hemodialysis patients filled in the International Physical Activity Questionnaire (IPAQ) and the Pittsburgh Sleep Quality Index (PSQI) to assess their level of physical activity and their quality of sleep, respectively. Basic demographic, clinical and treatment-related characteristics were obtained from an electronic medical data system, as well as the results of standard laboratory analyses. The results were analyzed with Student t-test, Pearson chi-square test, linear by linear association, and Spearman correlation.

Results: Nearly half of the patients (46.5%) were poor sleepers with an overall PSQI of 5.7 ± 4.4. Older age was significantly associated with worse sleep quality (p=0.019). Patients reported low, moderate or vigorous levels of daily physical activity in 42.3%, 50.7% and 7% of cases, respectively. No statistically significant associations were noted between demographic characteristics, dialysis vintage, comorbidities and the level of physical activity. Distribution of good and poor sleepers was almost identical among patients with low and moderate physical activity (47% vs 53% and 50% vs 50% respectively), whereas patients with vigorous activity were mostly good sleepers (20% vs 80%), but the difference was not statistically significant (p=0.591).

Conclusion: We observed no statistically significant association between the level of physical activity and quality of sleep in this cohort. Further research with a larger sample might additionally elucidate this issue.

Key words: dialysis, hemodialysis, physical activity, sleep quality

Cite this article as: Trbojević-Stanković J, Stanimirović S, Ristanović Lj, Nešić D. Relationship between daily physical activity and quality of sleep in maintenance hemodialysis patients; Medicinska istaživanja 2024; 57(3):41-47 DOI: 10.5937/medi57-51008

INTRODUCTION

Chronic kidney disease (CKD) is among the leading causes of morbidity and mortality worldwide with an estimated global prevalence between 11% and 15% (1). The terminal stage of CKD, end-stage renal disease (ESRD), is associated with a variety of symptoms affecting patients' physical ability and functioning. Hemodialysis (HD) partially improves certain clinical aspects of the disease but fails to sufficiently recover all aspects of daily functioning. In fact, specific features of this treatment regimen may even contribute to the impairment of certain aspects of daily functioning. Namely, patients' daily physical activity is substantially restricted by being attached to a dialysis machine for at least 12 hours each week in three dialysis sessions. Physical activity is any movement that causes energy expenditure, including daily duties, such as work, household activities, and commuting. HD patients are often sedentary, unrelated to gender, urbanicity and dialysis or nondialysis day, whereas older age is significantly associated with lower mobility and less activity (2). Inactivity even aggravates with time and disease severity. Moderate physical activity in the HD population has been associated with better quality of life and lower mortality, even in patients with comorbidities (3, 4).

Sleep disorders are highly prevalent in HD patients, with studies accounting that sleep quality in this population is compromised both subjectively and objectively (5). Their origin in dialysis patients is not fully elucidated, but it is established to be multi-factorial. Impaired quality of sleep is reported by more than half of patients receiving dialysis and is associated with cardiovascular events, depression, impaired daily functioning, lower quality of life, and increased mortality risk (6, 7). Nevertheless, despite their frequency and importance, sleep disorders are often underdiagnosed in this population, and evidence of effective management remains limited.

Previous studies suggested a positive association between physical activity and sleep in different populations, especially among the elderly (8, 9). Even moderate long-term physical activity appears to have a positive effect on sleep quality, especially on its depth, latency and performance in the general population (10). Nevertheless, data on the relationship between physical activity and sleep quality among patients with ESRD are limited.

This study aimed to assess the level of physical activity and quality of sleep among patients on maintenance HD and assess the relationship between these issues.

MATERIALS AND METHODS

Population

Seventy-one out of 98 patients from a single HD unit were willing and capable of participating in the study. The inclusion criteria included age over 18 years, at least 6 months of maintenance HD with 3 treatment sessions per week, regular HD attendance, and the ability to walk. We excluded patients with acute aggravation of clinical condition, severe mobility impairment, and cognitive incapacity to respond to questionnaires. The subjects agreed to participate in the study by completing questionnaires. The study protocol was approved by the institutional Ethical Committee (Decision ID: 8028/1-2022).

Demographic, clinical and treatment-related data were obtained from medical records. Body Mass Index (BMI) was calculated by dividing an individual's weight in kilograms by the square of height in meters. Values between 18.5 and 24.9 were considered within a healthy weight, whereas BMI ≥25 denoted overweight patients. Venous blood samples for laboratory analyses were drawn at the mid-week session upon routine monthly check-ups according to the standard protocol for HD patients' follow-ups. Dialysis in the center was performed within commonly established regulations and protocols.

Instruments

The patients were administered the International Physical Activity Questionnaire (IPAQ) and the Pittsburgh Sleep Quality Index (PSQI).

The IPAQ-short form consists of 7 questions related to the frequency and time spent performing vigorous, moderate and walking activities, as well as the average time spent sitting in the previous 7 days. Data collected with IPAQ is reported as median MET-minutes, where MET stands for the metabolic equivalent of task, denoting the proportion of energy expenditure in certain activity compared to energy expenditure at rest. One MET corresponds to the energy expenditure of 1 kcal/kg/h, walking corresponds to 3.3 METS, moderate physical activity to 4 METS and vigorous physical activity to 8 METS. Weekly physical activity reported by IPAQ was calculated as the sum of MET-minutes per week and categorized as low (≤600 MET-min/week), moderate (600-3000 MET-min/week) and vigorous (≥3000 MET-min/week). The IPAQ has been validated in different populations and has been previously used to assess physical activity in CKD and HD patients (12, 13).

PSQI consists of 19 self-rated questions to assess the quality of sleep in the previous 4 weeks (14). Each question yielded information related to 7 specific sleep components: subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbance, use of sleep medication, and level of daytime dysfunction. Sleep latency represents the time it takes to transit from full wakefulness to sleep. Recommended sleep latency is less than 20 minutes. Sleep efficiency is the ratio of time spent asleep (total sleep time) to the amount of time spent in bed. The reference range for sleep efficiency is 80 - 95%. Each component was scored from 0 to 3 to form a final

PSQI score between 0 and 21 where higher scores indicated lower quality of sleep. PSQI ≥5 denotes poor sleep quality. The questionnaire has been previously used in the HD population (7, 15).

Statistical Analysis

Data analysis was performed using IBM SPSS statistics for Windows, ver. 26 (Armonk, NY: IBM Corp.). Population characteristics were analyzed with descriptive statistics. Continuous variables were depicted using mean and standard deviations, whilst categorical variables were expressed as percentages. Continuous variables were analyzed by comparison of means with the Student's t-test. Categorical variables were analyzed with Pearson chi-square test or linear by linear association. The Spearman correlation coefficient was used to assess the correlation between IPAQ and PSQI. Differences were considered significant if the *p*-value of the test was less than .05.

RESULTS

Demographic, clinical and dialysis-related characteristics of the study population are presented in **Table 1**. The study population consisted of 41 men and 30 women, the age range was 35 – 94 years, and the dialysis vintage was 6 – 216 months. Most patients were 65 years or older. Only 2 patients (2.8%) were actively employed. None of the patients were malnourished according to BMI values. Underlying renal disease was hypertension in 27 patients (52.1%), diabetes in 12 patients (16.9%), glomerulonephritis in 11 patients (15.5%), polycystic renal disease in 7 patients (9.9%), obstructive nephropathy in 3 patients (4.2%), and one patient (1.4%) had congenital disease. HD was performed according to standard protocols on a thrice- weekly basis, using machinery with controlled ultrafiltration, bicarbonate-based dialysate and high and low flux dialyzers. Exactly half of the patients were dialyzed in the morning shift.

Table 1. Basic demographic, clinical and dialysis-related characteristics of the population

Study sample (n)	71
Gender (n, %)	
Male	41 (57.7%)
Female	30 (42.3%)
Median age (years)	71
Age range (n, %)	
18 – 39	3 (4.2%)
40 – 64	14 (19.7%)
65 years and above	54 (76.1%)
Nutrition status (n, %)	
Healthy weight	33 (46.5%)
Overweight	38 (53.5%)
Smoking habit (n, %)	31 (43.7%)
Comorbidities (n, %)	
Cardiovascular	27 (38.0%)
Cerebrovascular	32 (45.1%)
Hypertension	54 (76.1%)
Diabetes	20 (28.2%)
Respiratory	4 (5.6%)
Renal osteodystrophy	27 (38.0%)
Median dialysis vintage (months)	36
Adequate dialysis (n, %)	47 (66.2%)

Sleep Quality

Nearly half of the patients (33; 46.5%) were poor sleepers with an overall PSQI of 5.7 ± 4.4. Patients slept 6.5 ± 1.6 hours per night on average. Average sleep latency, sleep efficiency, and subjective sleep quality grade were 31.2 ± 36.3 minutes, 71.2 ± 21.5% respectively, and 1.7 ± 0.8 respectively. Older age was significantly associated with poor sleep (p=0.019), while none of the other demographic variables, dialysis vintage and comorbidities were associated with sleep quality **(Table 2)**. Also, no

Table 2. Comparison of demographic data, comorbidity, dialysis-related characteristics and physical activity status in good and poor sleepers

Variable		Good sleepers	Poor sleepers	p
Age (years)	18 - 39	3 (7.9%)	0 (0%)	0.019*
	40 – 64	10 (26.3%)	4 (12.1%)	
	≥65	25 (65.8%)	29 (87.9%)	
Sex (male, %)		24 (63.2%)	17 (51.5%)	0.320
Smoking habit (n, %)		19 (50.0%)	12 (36.4%)	0.248
Nutrition status	Healthy weight	16 (42.1%)	17 (51.5%)	
	Overweight	22 (57.9%)	16 (48.5%)	0.431
Dialysis vintage <36 months (n, %)		20 (52.6%)	15 (45.5%)	0.546
Adequate dialysis (n, %)		26 (68.4%)	21 (63,6%)	0.671
Cardiovascular disease (n, %)		11 (28.9%)	16 (48.5%)	0.091
Cerebrovascular disease (n, %)		18 (47.4%)	14 (42.4%)	0.676
Hypertension (n, %)		29 (76.3%)	25 (75.8%)	0.956
Diabetes (n, %)		12 (31.6%)	8 (24.2%)	0.493
Renal osteodystrophy (n, %)		17 (44.7%)	10 (30.3%)	
Physical activity	Low	16 (42.1%)	14 (42.4%)	0.591
	Moderate	18 (47.4%)	18 (54.5%)	
	Vigorous	4 (10.5%)	1 (3.0%)	

Table 3. Comparison of demographic data, comorbidity, dialysis-related characteristics and quality of sleep related to the level of physical activity

Variable		Physical activity level			p
		Low	Moderate	Vigorous	
Age (years)	18 – 39	2 (6.7%)	1 (2.8%)	0 (0%)	0.284
	40 – 64	8 (26.7%)	4 (11.1%)	2 (40.0%)	
	≥65	20 (66.7%)	31 (86.1%)	3 (60.0%)	
Sex (male, %)		18 (60.0%)	22 (61.1%)	1 (20.0%)	0.314
Smoking habit (n, %)		12 (40.0%)	16 (44.4%)	3 (60.0%)	0.699
Nutrition status	Healthy weight	18 (60.0%)	13 (36.1%)	2 (40.0%)	0.454
	Overweight	12 (40.0%)	23 (63.9%)	3 (60.0%)	
Dialysis vintage <36 months (n, %)		14 (46.7%)	17 (47.2%)	4 (80.0%)	0.367
Adequate dialysis (n, %)		21 (70.0%)	22 (61.1%)	4 (80.0%)	0.853
Cardiovascular disease (n, %)		10 (33.3%)	16 (44.4%)	1 (20.0%)	0.839
Cerebrovascular disease (n, %)		12 (40.0%)	18 (50.0%)	2 (40.0%)	0.621
Hypertension (n, %)		21 (70.0%)	29 (80.6%)	4 (80.0%)	0.360
Diabetes (n, %)		9 (30.0%)	10 (27.8%)	1 (20.0%)	0.680
Renal osteodystrophy (n, %)		14 (46.7%(	10 (27.8%)	3 (60.0%)	0.551

statistically significant association was found between the quality of sleep and the level of reported daily physical activity in our patient's cohort **(Table 2)**.

Physical activity

Half of the patients (36; 50.7%) had moderate levels of physical activity, 30 patients (42.3%) had low physical activity, and only 5 individuals (7.0%) reported vigorous physical activity. No statistically significant associations were noted between patients' demographic characteristics, dialysis vintage, comorbidities and the level of physical activity **(Table 3)**.

Patients with moderate physical activity had the highest subjective sleep quality, those with vigorous daily activity had the highest sleep duration and the lowest sleep latency, while sleep efficiency was optimum among individuals with the lowest physical activity. Nevertheless, there was no statistically significant difference related to the level of physical activity in any of the observed determinants of sleep quality **(Table 4)**.

Distribution of good and poor sleepers was almost identical among patients with low and moderate physical activity (47% vs 53% and 50% vs 50% respectively), whereas patients with reported vigorous levels of physical activity were mostly good sleepers (20% vs 80%), but the difference between the groups was not statistically significant (p=0.591) **(Figure 1)**.

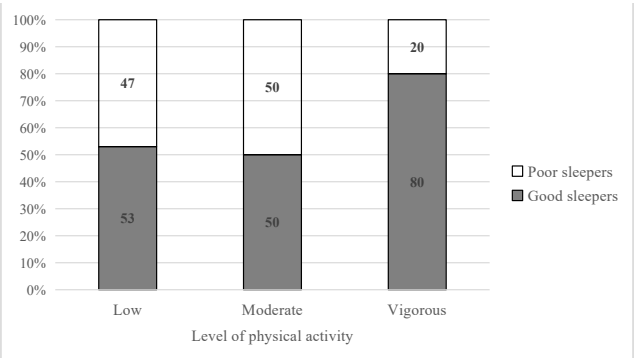


Figure 1. Quality of sleep among maintenance HD patients with different levels of physical activity

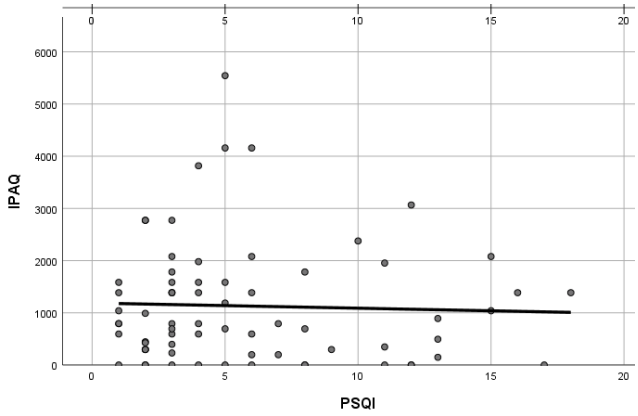


Figure 2. Correlation between PSQI and IPAQ scores

Analysis of the correlation between PSQI and IPAQ scores suggests that higher PSQI, denoting worse sleep quality, is associated with lower IPAQ (Figure 2), denoting the lower level of physical activity, but this correlation was not statistically significant (rho=-0.06; p=0.960).

Table 4. Relationship between sleep determinants and daily physical activity

Sleep determinant	Physical activity level			
	Low (Mean ± SD)	Moderate (Mean ± SD)	Vigorous (Mean ± SD)	P
Subjective sleep quality	1.60 ± 0.62	1.89 ± 0.95	1.40 ± 0.55	0.227
Sleep duration (hours)	6.70 ± 1.51	6.42 ± 1.71	6.80 ± 1.30	0.733
Sleep latency (minutes)	33.50 ± 38.59	29.94 ± 35.15	27.00 ± 37.35	0.894
Sleep efficiency (%)	72.50 ± 20.25	70.28 ± 21.32	70.00 ± 33.73	0.911

DISCUSSION

The present study assessed physical activity and the quality of sleep in the end-stage kidney disease patients treated with maintenance in-center HD. Several methods were used in previous studies to measure the physical activity of dialysis patients: accelerometers, pedometers, and self-reported questionnaires (16, 17). Consumer-grade wearable tracking devices have recently emerged as a less expensive and more practical option than accelerometers, but more data are still needed on their validity and reliability.

Previous research established that HD patients are less active than their healthy counterparts, with the most remarkable difference among the elderly (18). Also, patients with cardiovascular disease, ongoing inflammation, protein-energy wasting, obesity, and diabetes appear predisposed to low physical activity levels, while previous transplantation and higher muscle mass were associated with higher physical activity (19).

Earlier studies have demonstrated a significant relationship between older age and poor sleep quality among patients on maintenance HD (7), while the association between sleep quality and sex in this population yielded conflicting results (7, 20). In our study cohort, older age was also significantly associated with a higher prevalence of poor sleep quality, while other investigated demographic, dialysis-related and clinical parameters were not related to the quality of sleep in our study population.

Data on the relationship between demographic and clinical parameters and physical activity in HD patients are limited. One large study which recruited 1,611 HD patients from 27 dialysis facilities reported a significant association between older age and male sex and a higher level of physical activity in this population, but no association with dialysis vintage and comorbidities (21). However, physical activity in this research was defined as regular exercise, thus not quite corresponding to the definition describing physical activity as any movement that causes energy expenditure, including, but not limited to, daily household activities and commuting. In our study population, we observed no significant association between the level of physical activity and demographic, clinical and dialysis-related indicators. One possible explanation is that only a minor share of patients fit the criteria for vigorous physical activity.

The level of physical activity is known to be positively associated with sleep quality in healthy populations of all ages, with the most notable improvement in sleep-related to physical activity and exercise among older adults (22, 23, 24). On the other hand, there are limited data on the relationship between physical activity and sleep quality in the ESRD population. Studies are scarce, sample sizes small, and methodologies highly variable (25, 26). Furthermore, interventional studies assessing the effect of different physical exercise programs on sleep quality in CKD and ESRD patients so far yielded inconclusive results. Preliminary evidence suggests improvements in self-reported sleep quality following exercise interventions, but high heterogeneity and small effect size limit these results (27). In our study population, HD patients with lower levels of physical activity had worse sleep quality, as assessed by the implemented tools, but the correlation was not statistically significant.

We acknowledge certain limitations to our study. The relatively low number of participants might present a statistical bias in our conclusions. Moreover, we did not perform the nocturnal polysomnography as a gold standard for the diagnosis of sleep-disordered breathing that might influence patients' sleep. Nevertheless, given the scarcity of data in this area we believe that inputs from this research might contribute to the insight on the complexity of the subject.

CONCLUSIONS

Hemodialysis has a profound impact on patients' lives, habits, daily routines and functioning. Thus, any intervention that might be beneficial should be thoroughly explored. In this study, we confirmed a significant association between poor sleep quality and older age in the HD population. No statistically significant association was found between the level of physical activity and quality of sleep in this cohort, but additional research with a larger sample should further elucidate this issue.

Conflicts of Interest: The authors declare no conflicts of interest.

Author Contributions: JTS contributed to study conceptualization, design, supervision, data interpretation, and manuscript editing, SS contributed to data acquisition and analysis, and literature search, LJR contributed to data analysis and interpretation, and DN contributed to data interpretation and manuscript preparation. The final version of the manuscript was reviewed and approved by all the authors.

REFERENCES

1. Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. *Kidney Int Suppl (2011)*. 2022;12(1):7-11. doi:10.1016/j.kisu.2021.11.003
2. Malhotra R, Kumar U, Virgen P, et al. Physical activity in hemodialysis patients on nondialysis and dialysis days: Prospective observational study. *Hemodial Int*. 2021;25(2):240-248. doi:10.1111/hdi.12913
3. Wu YH, Hsu YJ, Tzeng WC. Physical Activity and Health-Related Quality of Life of Patients on Hemodialysis with Comorbidities: A Cross-Sectional Study. *Int J Environ Res Public Health*. 2022;19(2):811. doi:10.3390/ijerph19020811
4. Zhang F, Wang H, Wang W, Zhang H. The Role of Physical Activity and Mortality in Hemodialysis Patients: A Review. *Front Public Health*. 2022; 10:818921. doi:10.3389/fpubh.2022.818921

5. Arazi H, Mohabbat M, Saidie P, Falahati A, Suzuki K. Effects of Different Types of Exercise on Kidney Diseases. *Sports (Basel)*. 2022;10(3):42. doi:10.3390/sports10030042
6. Cheng E, Evangelidis N, Guha C, et al. Patient experiences of sleep in dialysis: systematic review of qualitative studies. *Sleep Med*. 2021; 80:66-76. doi:10.1016/j.sleep.2021.01.019.
7. Trbojević-Stanković J, Stojimirović B, Bukumirić Z, Hadzibulic E, Andric B, Djordjevic V, et al. Depression and quality of sleep in maintenance hemodialysis patients. *Srp Arh Celok Lek*. 2014;142(7-8):437-443.
8. De Nys L, Anderson K, Ofosu EF, Ryde GC, Connelly J, Whittaker AC. The effects of physical activity on cortisol and sleep: A systematic review and meta-analysis. Psychoneuroendocrinology. 2022; 143:105843. doi:10.1016/j.psyneuen.2022.105843
9. Sejbuk M, Mirończuk-Chodakowska I, Witkowska AM. Sleep Quality: A Narrative Review on Nutrition, Stimulants, and Physical Activity as Important Factors. Nutrients. 2022;14(9):1912. doi:10.3390/nu14091912
10. Wilckens K.A., Erickson K.I., Wheeler M.E. Physical Activity and Cognition: A Mediating Role of Efficient Sleep. *Behav. Sleep Med*. 2018; 16:569–586. doi: 10.1080/15402002.2016.1253013.
11. Tattersall J, Martin-Malo A, Pedrini L, Basci A, Canaud B, Fouque D, et al. EBPG guideline on dialysis strategies. Nephrol Dial Transplant. 2007; 22(Suppl 2):iii5-21.
12. Lou X, He Q. Validity and Reliability of the International Physical Activity Questionnaire in Chinese Hemodialysis Patients: A Multicenter Study in China. *Med Sci Monit*. 2019; 25:9402-9408. doi:10.12659/MSM.920900
13. Király E, Szőllősi GJ, Jenei Z, Kárpáti I. Association between physical activity and quality of life in haemodialysed and peritoneal dialyzed patients in Hungary. *Ren Fail*. 2024;46(1):2324079. doi:10.1080/0886022X.2024.2324079
14. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index (PSQI): a new instrument for psychiatric research and practice. Psychiatry Res. 1989; 28(2):193-213.
15. Tian H, Wang L, He Q, et al. Association between sleep quality and cardiovascular disease in maintenance hemodialysis patients: a prospective cohort study. *Ren Fail*. 2023;45(2):2278310. doi:10.1080/0886022X.2023.2278310
16. Cupisti A, D'Alessandro C, Finato V, Del Corso C, Catania B, Caselli GM, Egidi MF. Assessment of physical activity, capacity and nutritional status in elderly peritoneal dialysis patients. BMC Nephrol. 2017;18(1):180. doi: 10.1186/s12882-017-0593-7.
17. Zhang H, Wang H, Huang L, Bai Y, Zhang F. Interventions to increase physical activity level in patients with whole spectrum chronic kidney disease: a systematic review and meta-analysis. Ren Fail. 2023;45(2):2255677. doi: 10.1080/0886022X.2023.2255677.
18. Han M, Williams S, Mendoza M, Ye X, Zhang H, Calice-Silva V, Thijssen S, Kotanko P, Meyring-Wösten A. Quantifying Physical Activity Levels and Sleep in Hemodialysis Patients Using a Commercially Available Activity Tracker. Blood Purif. 2016;41(1-3):194-204. doi: 10.1159/000441314.
19. Panaye M, Kolko-Labadens A, Lasseur C, Paillasseur JL, Guillodo MP, Levannier M, Teta D, Fouque D. Phenotypes influencing low physical activity in maintenance dialysis. J Ren Nutr. 2015;25(1):31-9. doi: 10.1053/j.jrn.2014.07.010.
20. Alshammari B, Alkubati SA, Pasay-An E, Alrasheeday A, Alshammari HB, Asiri SM, Alshammari SB, Sayed F, Madkhali N, Laput V, Alshammari F. Sleep Quality and Its Affecting Factors among Hemodialysis Patients: A Multicenter Cross-Sectional Study. Healthcare (Basel). 2023;11(18):2536. doi: 10.3390/healthcare11182536
21. Kang SH, Do JY, Jeong HY, Lee SY, Kim JC. The Clinical Significance of Physical Activity in Maintenance Dialysis Patients. Kidney Blood Press Res. 2017;42(3):575-586. doi: 10.1159/000480674.
22. Sullivan Bisson AN, Robinson SA, Lachman ME. Walk to a better night of sleep: testing the relationship between physical activity and sleep. Sleep Health. 2019;5(5):487-494. doi: 10.1016/j.sleh.2019.06.003.
23. Vanderlinden J, Boen F, van Uffelen JGZ. Effects of physical activity programs on sleep outcomes in older adults: a systematic review. Int J Behav Nutr Phys Act. 2020 5;17(1):11. doi: 10.1186/s12966-020-0913-3.
24. Hasan F, Tu YK, Lin CM, Chuang LP, Jeng C, Yuliana LT, Chen TJ, Chiu HY. Comparative efficacy of exercise regimens on sleep quality in older adults: A systematic review and network meta-analysis. Sleep Med Rev. 2022; 65:101673. doi: 10.1016/j.smrv.2022.101673.
25. Sabbagh R, Iqbal S, Vasilevsky M, Barré P. Correlation between physical functioning and sleep disturbances in hemodialysis patients. Hemodial Int. 2008;12 Suppl 2:S20-4. doi: 10.1111/j.1542-4758.2008.00319.x
26. Yoshikoshi S, Yamamoto S, Suzuki Y, Imamura K, Harada M, Yamabe S, Matsunaga Y, Osada S, Tagaya H, Matsunaga A. Association between Physical Frailty and Sleep Disturbances among Patients on Hemodialysis: A Cross-Sectional Study. Nephron. 2024;148(3):152-159. doi: 10.1159/000533418.
27. Zhang F, Wang H, Huang L, Bai Y, Wang W, Zhang H. Effect of exercise interventions for sleep quality in patients with chronic kidney disease: a systematic review and meta-analysis. Int Urol Nephrol. 2023;55(5):1193-1204. doi: 10.1007/s11255-022-03413-z.

FIZIČKA AKTIVNOST I KVALITET SNA KOD BOLESNIKA LEČENIH HRONIČNIM HEMODIJALIZAMA

Trbojević-Stanković Jasna[1,2], Stanimirović Sanja[3], Ristanović Ljubica[4], Nešić Dejan[1,5]

Sažetak

Uvod/Cilj: Bolesnici na hroničnoj hemodijalizi često imaju smanjenu fizičku aktivnost i loš kvalitet sna. Cilj ovog istraživanja bio je da se proceni nivo uobičajene fizičke aktivnosti i kvalitet sna kod bolesnika na hemodijalizi, kao i da se proceni povezanost između ova dva entiteta.

Materijal i metode: Sedamdeset jedan hemodijalizirani bolesnik je popunio Internacionalni upitnik o fizičkoj aktivnosti i Pitsburški upitnik o kvalitetu sna. Osnovne demografske, kliničke i karakteristike dijalizne terapije, kao i rezultati laboratorijskih analiza, dobijeni su iz elektronskih istorija bolesti. Rezultati su obrađeni Studentovim t-testom, Pirsonovim hi-kvadrat testom, linearnom asocijacijom i Spirmanovom korelacijom.

Rezultati: Gotovo polovina bolesnika (46,5%) ima loš kvalitet sna sa prosečnim PSQI 5,7 ± 4,4. Stariji bolesnici su imali značajno lošiji kvalitet sna (p=0,019). Fizička aktivnost je bila niska kod 42,3% bolesnika, umerena kod 50,7% i visoka kod 7%. Nije uočena značajna udruženost između nivoa fizičke aktivnosti, demografskih karakteristika, dužine dijaliziranja i komorbiditeta. Distrubucija dobrog i lošeg kvaliteta sna bila je gotovo identična kod bolesnika sa niskom i umerenom fizičkom aktivnošću (47% vs 53%, odnosno 50% vs 50%), dok su bolesnici sa visokim nivoom aktivnosti češće dobro spavali (20% vs 80%), ali bez statistički značajne razlike (p=0,591).

Zaključak: Nije uočena značajna povezanost između nivoa fizičke aktivnosti i kvaliteta sna kod hemodijaliziranih bolesnika u ovom istraživanju. Ispitivanje većeg broja bolesnika moglo bi doprineti boljem uvidu u ovaj problem.

Ključne reči: dijaliza, hemodijaliza, fizička aktivnost, kvalitet sna

Primljen: 13.05.2024. I **Revizija:** 25.05.2024. I **Prihvaćen:** 03.06.2024.

Medicinska istaživanja 2024; 57(3):41-47

Medicinska istaživanja 2024; 57(3):49-58 | DOI: 10.5937/medi57-49899

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Differences in the indicators of inflammation between patients with bipolar and unipolar depression

Sara Dodić[1,2], Marija Purić[1,2], Marta Gostiljac[2], Milica Nešić[1,2], Bojana Dunjić-Kostić[1,3], Vanja Mandić-Maravić[1,3], Emilija Erić[2], Maja Ivković[1,2], ✉ Maja Pantović-Stefanović[1,2]

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

[2] Department of Bipolar Disorders, Clinic of Psychiatry, University Clinical Centre of Serbia, Belgrade, Serbia

[3] Institute of Mental Health, Belgrade, Serbia

Recived: 18 March 2024

Revised: 16 May 2024

Accepted: 28 May 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

✉ **Correspondence to:**
Maja Pantović-Stefanović
Clinic of Psychiatry, University Clinical Centre of Serbia,
2 Pasterova Street, Belgrade, Serbia
Email: majapantovic@yahoo.it

Summary

Introduction/Aim: Patients with bipolar disorder, unrecognized and with a delayed onset of manic or hypomanic episodes are often mistakenly diagnosed with unipolar depression (UD) due to shared symptomatology. The two disorders, however, have related but not identical etiopathogenesis. Immune system alterations might play a crucial role in both the onset and manifestation of these conditions. This study aimed to compare immune markers between patients with bipolar depression (BD) and unipolar depression (UD) and explore their associations with acute episode characteristics and disease progression.

Material and Methods: This retrospective study included patients with BD (n=60) and UD (n=242) who were hospitalized within a two-year period and whose sociodemographic information, acute episode and course of illness characteristics, and indicators of inflammation were available.

Results: Patients with BD exhibited elevated mean platelet volume (MPV) compared to those with UD. MPV levels correlated with clinical characteristics in both groups; higher MPV was observed in UD patients with an earlier age of onset and a longer duration of illness. In BD patients, elevated MPV was associated with the severity of psychopathology, particularly in individuals with a history of suicide attempts and a prolonged duration of untreated disorder.

Conclusion: This study indicates the presence of chronic low-grade inflammation in specific subpopulations of patients with affective disorders. Immune changes are distinct in regard to the polarity of the disorder and could be a potential indicator of the severity of psychopathology and illness chronicity.

Keywords: bipolar affective disorder, inflammation, unipolar depression

Cite this article as: Dodić S, Purić M, Gostiljac M, Nešić M, Dunjić-Kostić B, Mandić-Maravić V, Erić E, Ivković M, Pantović-Stefanović M. Differences in the indicators of inflammation between patients with bipolar and unipolar depression; Medicinska istaživanja 2024; 57(3):49-58 DOI: 10.5937/medi57-49899

INTRODUCTION

Bipolar disorder (BD) and major depressive disorder (MDD) are among the most common and severe mood disorders. Due to their overlapping symptomatology and often unrecognized or delayed manifestations of manic or hypomanic episodes, patients with bipolar disorder are frequently misdiagnosed with MDD (1). Consequently, inadequate recognition and treatment of bipolar disorder, along with the absence of mood stabilizing therapy, increases the risk of antidepressant-induced mania and the frequency of affective episodes, facilitating disorder progression and impaired functioning. Despite having a spectrum of shared symptoms, bipolar disorder and MDD are related but do not have an identical etiopathogenesis (2–4). Numerous studies describe the association of alterations in immune mediator levels and immune status in general with the etiopathogenesis of mood disorders, regardless of their polarity. Changes in immune signaling molecules are linked not only to depression within MDD and bipolar disorder diagnosis but also to depressive symptoms in patients with primarily somatic illnesses, sub-threshold depressive symptoms, or different types of affective temperaments in the population of mentally healthy individuals (5–9). The data show that altered levels of C-reactive protein (CRP), as well as the number of immune-active cells such as lymphocytes (Ly), neutrophils (Ne), or platelets (PLT), are present in patients with mood disorders. Additionally, some of these indicators of inflammation, including platelet count or mean platelet volume (MPV), may serve as potential markers of specific affective states, such as depression or mania (10). As potential mediators in the clinical presentation of mood disorders, these parameters are also suggestive of the severity of the disorder itself (11). The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), MPV, and CRP are direct and easily accessible biomarkers of alterations in neuroimmune functioning that could facilitate the differential diagnosis of these two disorders and enable a more precise treatment (11–14). This finding also suggested that they could play a significant role in the etiology of mood disorders, especially symptoms of depression, or even be an additional aid in discriminating between patients with unipolar (UD) and bipolar depressive episodes (BD). It is additionally important to assess whether patients with specific clinical characteristics belong to the group of those with higher inflammation parameters, as this may indicate disease progression and the occurrence of comorbidities (15–17). However, to date, the values of the selected indicators of inflammation have not been systematically investigated in regard to the aforementioned differences.

The aim of this study was to assess differences in indicators of inflammation (NLR, PLR, MLR, MPV, CRP) between patients with acute episodes of bipolar depression and those with acute depressive episodes within major depressive disorder. Our secondary aim was to examine the associations of indicators of inflammation with the clinical characteristics of acute episodes and the disease course.

MATERIALS AND METHODS

Study Design

This study represents a clinical, retrospective, noncommercial assessment of the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), mean platelet volume (MPV), and C-reactive protein (CRP) in the blood/serum of patients with BD and UD. The study was approved by the Ethics Committee of the University Clinical Centre of Serbia (No. 622/1).

Participants

The study included 302 participants of both sexes aged 18 to 79 years. Participants were divided into two groups: the study group of patients with bipolar depression (BD = 60) and the study group of patients with unipolar depression (UD = 242). The sample size necessary for the study with the power of $1 - \beta = 0.80$ at $\alpha = 0.05$ was calculated based on the study by Chang et al. (18) and the values of C-reactive protein levels. The minimum number of participants required to detect differences among groups was determined to be 27 participants per group.

Inclusion criteria

Specific inclusion criteria for the BD and UD patients were as follows: a) age ≥ 18 years; b) patients experiencing an acute exacerbation of the illness, hospitalized at the Clinic of Psychiatry over a two year period, for UD or BD according to the criteria of the 10th International Classification of Diseases (19); and c) completed laboratory work-up, including leukocyte count, neutrophil count, lymphocyte count, platelet count, mean platelet volume, and C-reactive protein, conducted no later than three days following admission.

Exclusion criteria

The exclusion criteria based on available medical records were as follows: a) comorbidity with acute inflammatory, neurodegenerative, or infectious diseases and other severe decompensated conditions; b) pregnancy or lactation; and c) the presence of another psychiatric comorbidity other than personality disorders.

Semi-Structured Psychiatric Questionnaire

At the beginning of the study, data relevant to the research were collected from medical records using a semistructured psychiatric questionnaire, including a) general sociodemographic data (gender, age, marital status, education); b) data related to the clinical characteristics of the acute episode (time elapsed until initial remission, current psychotic symptoms, current suicide risk); c) data related to the clinical characteristics of the disease course (age at the onset of initial symptoms, the duration of untreated illness, the duration of illness, a total number and type of previous affective episodes, the number of hospitalizations, the number of previous suicide attempts, the analysis of previous complementary diagnostics – MRI, CT, psychological evaluation); d) personal medical history (history of alcohol and psychoactive substance abuse, the presence of acute or chronic somatic illnesses); e) family medical history; and f) complete blood count and biochemical analysis (absolute leukocyte count, absolute neutrophil count, absolute lymphocyte count, absolute platelet count, mean platelet volume, and C-reactive protein).

Variables such as age at the onset of initial symptoms, a total number of previous episodes, type of previous episode, number of hospitalizations, and number of suicide attempts were obtained from medical records. Regarding the variable "current psychotic symptoms" (binary variable), psychotic features were assessed as present (assigned a value of 1) if the patient was diagnosed with any of the affective disorders with psychotic symptoms (19) upon admission by the attending psychiatrist. The value of 0 was assigned for this variable for other examined diagnoses. Data on current suicide risk, assessed through the current presence of suicidal phenomena (suicidal thoughts, intentions, attempts), were expressed through a single binary variable, "current suicide risk" (without suicidal phenomena - 0, with suicidal phenomena - thoughts or intentions currently and attempt within the period of up to one week before admission) - 1), based on data from the mental status examination and medical history.

Certain clinical characteristics for which there is no consensus (duration of untreated disorder, stage of the disorder) were defined based on current research findings. The duration of untreated disorder was defined as the time elapsed from the onset of initial symptoms to the initiation of appropriate treatment. In the context of UD, "appropriate treatment" involves the use of antidepressants, while mood stabilizers are regarded as adequate pharmacotherapy for bipolar disorder (20). To determine differences in immune indicators related to the progression of the disorder, the patients were categorized into early (up to 5 years after the onset of initial symptoms), middle (from 5 to 10 years after the onset of initial symptoms), and late stages of illness development (over 10 years after the onset of initial symptoms), using the criteria of Pedrini et al. (21).

Laboratory analyses

The analysis of absolute neutrophil, lymphocyte, monocyte, and platelet counts and MPV was performed on Sysmex XN-1000 and Beckman Coulter LH 750 machines, while the analysis of C-reactive protein levels was carried out on the Abbott Alinity C instrument. The neutrophil-to-lymphocyte ratio was calculated as the quotient of the absolute neutrophil count and the absolute lymphocyte count. The monocyte-to-lymphocyte ratio was calculated as the quotient of the absolute monocyte count and the absolute lymphocyte count. The platelet-to-lymphocyte ratio was calculated as the quotient of the platelet count and the absolute lymphocyte count.

Statistical data analysis

The database was created in Microsoft Office Excel for Windows 2007, and data analysis was conducted using the Statistical Package for Social Sciences (SPSS) for Windows v. 21.0 (SPSS Inc., Chicago, IL). Descriptive statistical methods, including measures of central tendency and variability, were used to illustrate the sample characteristics.

Bivariate analytical statistical methods included tests examining the association between indicators of inflammation and other variables (independent samples Student's t test for independent samples, $\chi 2$ test with Yates's correction, Fisher's exact probability test, and correlation using Pearson's correlation coefficient). Multivariate statistical methods involved partial correlation and analysis of covariance (ANCOVA) with a post hoc least significant difference (LSD) test. Sex and age were used as covariates in all multivariate analyses. The choice of the covariates was based on previous studies finding an association between inflammatory parameters and these sociodemographic variables (22).

The Kolmogorov-Smirnov test with Lilliefors correction revealed a non-normal distribution for some variables (NLR, TLR, MLR, CRP). Parametric method results were considered statistically significant if significance was confirmed by the "bootstrapping" procedure on 1000 subsamples, as the population did not exhibit a normal distribution for most inflammatory parameters. The test values were considered statistically significant if $p < 0.05$. The confidence interval value in the "bootstrapping" method was regarded as more informative and superior to the p value in cases with borderline p values. Therefore, the confidence intervals are presented in the text for variables that showed statistical significance in the analyses (23).

RESULTS

The sociodemographic characteristics of the participants are presented in **Table 1**. Apart from statistically

Table 1. Sociodemographic and clinical characteristics of the patients

Sociodemographic characteristics	BD (n = 60)	UD (n=242)	Test	p value	CI
Gender (male/female)	6/54	106/136	-	0.000 [a]	
Age (years)	48.46 ± 12.36	51.67 ± 11.10	t=-1.948	0.071 [b]	-6.90 – 0.36
Marital status (has a partner/does not have a partner, N)	33/27	149/93	χ^2=0.678	0.447 [c]	
Education (years)	12.18 ± 2.46	12.59 ± 3.34	t=-0,873	0.383[b]	-1.15 – 0.37
Clinical characteristics					
Age at the onset (years)	30.47 ± 11.09	37.52 ± 13.78	t = -3.676	0.000[b]	-10.38 – -3.79*
Duration of untreated disorder (months)	107.67 ± 101.04	38.53 ± 70.03	t = 6.044	0.001[b]	42.06 – 97.13*
Duration of illness (years)	18.01 ± 11.52	14.84 ± 12.27	t = 1.810	0.071[b]	0.01 – 6.69*
Total number of previous episodes	10.57 ± 7.09	4.73 ± 3.30	t = 1.810	0.071[b]	0.01 – 6.69*
Number of depressive episodes	6.51 ± 4.70	4.73 ± 3.30	t = 3.258	0.013[b]	0.47 – 3.33*
Number of manic episodes	2.81 ± 2.37	–	–	–	–
Number of mixed episodes	1.91 ± 3.03	–	–	–	–
Time elapsed until remission (days)	37.96 ± 15.06	43.16 ± 28.30	t = -1.374	0.042[b]	-9.84 – 0.12
Current psychotic symptoms (yes/no, N)	21/39	44/198	χ^2=7.086	0.008[c]	–
Number of hospitalizations	7.77±7.22	4.41±3.95	t = 4.869	0.001[b]	1.61 – 5.46
Stage of disorder (<5 years, 5-10, ≥10 years, N)	12/8/40	65/48/129	χ^2=3.571	0.168[d]	
Heredity (yes/no, N)	32/28	105/137	χ^2=1.538	0.215[c]	
Current suicide risk (yes/no, N)	36/24	78/164	χ^2=13.203	0.000[c]	–
Previous suicide attempt (yes/no, N)	30/30	100/202	χ^2=8.713	0.003	–
Number of suicide attempts (yes/no, N)	1	0	t = 2.848	0.017	0.11– 0.89

The values are presented as the means ± standard deviations unless otherwise stated. [a]Fisher's exact probability test, [b]Independent samples t test, [c]chi-square test with Yates' correction; d Pearson's chi-square test, *Statistical significance, relative to confidence intervals. Abbreviations: BD = bipolar depression, UD = unipolar depression, CI = confidence interval

significant differences in gender (10.0% vs. 43.8%, $p < 0.001$), individuals with BD and UD did not differ in terms of age, marital status, or education. When comparing clinical characteristics, BD patients had a significantly longer duration of untreated illness (107,67 ± 101,04 vs. 38.,53 ± 70,03 months, $p = 0.001$), higher number of depressive episodes (6,51 ± 4,70 vs. 4,73 ± 3,30, $p = 0.013$), a shorter time to remission (37,96 vs. 43.16, p = 0.042), and a higher number of prior hospitalizations (7.77 ± 7,22 vs. 4.41 ± 3,95, p = 0.001). In addition, BD patients more frequently exhibited psychotic symptoms at the time of admission (53.8% vs. 22.2%, $p = 0.008$) and suicidal behavior (100% vs. 49.5%, p = 0.003).

Values of the indicators of inflammation in the blood and serum of the participants in our study are shown in **Table 2**. Apart from significant differences in mean levels of monocytes (0,55 ± 0,25 vs. 0,46 ± 0.20, $p = 0.002$) and lymphocytes (2.28 ± 0.70 vs. 2.48 ± 2.08, $p = 0.042$), there were no differences in other indicators of inflammation that we assessed. The MLR was higher in patients with bipolar depression than in patients with unipolar depression (0.25 ± 0.12 vs. 0.22 ± 0.14, p = 0.017). Additionally,

Table 2. Blood cell count and indicators of inflammation

The blood cell count	BD (n = 60)	UD (n=242)	Test	p value	CI
Leukocyte count 10^9/L	6.22 ± 1.93	8.05 ± 1.38	t=-1.484	0.142 [a]	-6.61– 0.96
Neutrophil count 10^9/L	4.19 ± 1.59	4.21 ± 1.73	t=-0.273	0.786 [a]	-6.54 – 2.65
Lymphocyte count 10^9/L	2.28 ± 0.70	2.48 ± 2.08	t=-2.114	0.042 [a]	-1.17 – -0.02*
Monocyte count 10^9/L	0.55 ± 0.25	0.46 ± 0.20	t=3.029	0.002 [a]	0.10 – 0.46*
Platelet count 10^9/L	238.34 ± 65.11	226.88 ± 65.05	t=1.148	0.252 [a]	-8.18 – 31.10
Indicators of inflammation					
NLR	2.04 ± 1.12	2.05 ± 1.51	F = 0.028	0.867[b]	-0.415 – 0.493
MLR	0.25± 0.12	0.22 ± 0.14	F = 5.750	0.017[b]	0.15 – 0.90*
TLR	113.12 ± 41.96	107.37 ± 48.93	F = 0.396	0.530 [b]	-9.55 – 18.75
MPV (fL)	9.49 ± 1.37	8.97 ± 1.20	F = 6.739	0.010 [b]	-0.93 – -0.18*
CRP (mg/L)	4.62 ± 5.93	6.62 ± 3.38	F = 0.523	0.471 [b]	-8.82 – 1.54

The values are presented as the means ± standard deviations unless otherwise stated. [a]Independent samples t test with the bootstrapping method, [b]Analysis of covariance (ANCOVA) with bootstrapping methods (covariates: sex and age). *statistically significant relative to the confidence interval. Abbreviations: BD = bipolar depression, UD = unipolar depression, CI = confidence interval, NLR = ratio of neutrophils to lymphocytes, MLR = ratio of monocytes to lymphocytes, PLR = ratio of platelets to lymphocytes, MPV = mean platelet volume, CRP = C-reactive protein

Table 3. Associations between indicators of inflammation and clinical characteristics of acute episodes and disease course in patients with BD

Clinical characteristics	NLR	MLR	PLR	MPV(fL)	CRP(mg/L)
Age at the onset of initial symptoms (years)	r=-0.197, p=0.171	r=-0.147, p=0.310	r=-0.111, p=0.443	r=0.042, p=0.774	r=-0.096, p=0.641
Duration of untreated disorder (months)	r=0.126, p=0.385	r=-0.200, p=0.888	r=0.100, p=0.489	r=0.277, p=0.047*	r=0.192, p=0.146
Duration of disorder (years)	r=0.217, p=0.130	r=0.163, p=0.259	r=0.116, p=0.432	r=0.047, p=0.747	r=0.087, p=0.671
Total number of previous episodes	r=0.093, p=0.520	r=0.042, p=0.770	r=-0.065, p=0.659	r=-0.070, p=0.629	r=-0.091, p=0.658
Number of depressive episodes	r=-0.044, p=0.763	r=-0.074, p=0.611	r=-0.115, p=0.426	r=-0.229, p=0.109	r=-0.122, p=0.552
Number of manic episodes	r=-0.042, p=0.773	r=-0.111, p=0.443	r=0.020, p=0.889	r=0.010, p=0.945	r=-0.091, p=0.657
Number of mixed episodes	r=0.187, p=0.500	r=0.175, p=0.701	r=-0.002, p=0.998	r=0.287, p=0.430	r=0.056, p=0.785
Time elapsed until remission (days)	r=-0.247, p=0.084	r=-0.239, p=0.094	r=-0.022, p=0.879	r=0.029, p=0.842	r=0.149, p=0.468
Number of hospitalizations	r=0.067, p=0.645	r=0.078, p=0.592	r=-0.117, p=0.420	r=0.022, p=0.878	r=-0.162, p=0.428
Number of suicide attempts	r=-0.006, p=0.968	r=0.165, p=0.253	r=0.007, p=0.959	r=0.308, p=0.029*	r=-0.108, p=0.598

The values are presented as correlation coefficients and p values (covariates: sex and age). Abbreviations: NLR = ratio of neutrophils to lymphocytes, MLR = ratio of monocytes to lymphocytes, PLR = ratio of platelets to lymphocytes, MPV = mean platelet volume, CRP = C-reactive protein. *p < 0.05

MPV was higher in those with bipolar depression (9,49 ± 1,37 vs. 8,97 ± 1,20, $p = 0.010$).

Indicators of inflammation in patients with bipolar depression

In BD patients with acute suicidality, MPV was higher than in their nonsuicidal counterparts (9,71 ± 1,16 vs. 8,47 ± 0.96, $p < 0.001$). Additionally, within the bipolar depression group, patients with a history of suicide attempts had higher MPV compared to those who had not attempted suicide during their lifetime (9,32 ± 1,23 vs. 8,59 ± 1,20, $p = 0.03$). Moreover, in the same group of patients, MPV was higher in those with a higher number of suicide attempts and in those with a longer duration of untreated illness. All analyses were conducted with the control of sex and age covariates **(Table 3 and Table 4)**.

Table 4. Differences in the values of the indicators of inflammation in relation to the clinical characteristics of acute episodes and the disease course in patients with BD

Clinical characteristic	NLR	MLR	PLR	MPV(fL)	CRP(mg/L)
Current psychotic symptoms	F=1.195, p=0.322	F=0.747, p=0.529	F=0.860, p=0.468	F=0.273, p=0.884	F=0.750, p=0.553
Yes	2.39±1.44	0.26±0.10	113.58±50.45	9.18±0.90	3.73±4.07
No	1.86±0.88	0.25±0.13	112.90±37.55	8.86±1.33	4.98±5.59
Stage of disorder	F=0.439, p=0.649	F=0.683, p=0.515	F=0.183, p=0.833	F=0.336, p=0.717	F=0.310, p=0.737
< 5 years	2.36±2.24	0.27±0.10	101.43±59.81	8.99±1.13	4.94±5.01
5-10	1.72±1.83	0.21±0.11	109.82±41.48	8.33±1.01	4.16±3.75
>10 years	2.25±0.90	0.29±0.12	119.43±43.74	8.66±1.16	4.79±6.51
Heredity	F=0.742, p=0.393	F=0.018, p=0.892	F=0.036, p=0.851	F=2.550, p=0.160	F=0.894, p=0.354
yes	2.20±1.34	0.26±0.12	114.35±39.06	9.24±1.30	5.54±4.53
no	1.88±0.85	0.25±0.11	111.92±45.43	8.70±1.05	3.93±4.54
Current suicidal risk	F=0.060, p=0.800	F=0.733, p=0.527	F=0.010, p=0.919	F=16.950, p=0.000*	F=0.811, p=0.377
yes	2.00±1.20	0.26±0.13	112.13±40.09	9.71±1.16	5.52±5.07
no	2.11±1.01	0.26±0.11	114.63±45.55	8.47±0.96	3.58±4.33
Previous suicidal attempt	F=0.757, p=0.525	F=0.806, p=0.497	F=0.888, p=0.454	F=1.700, p=0.028*	F=0.707, p=0.757
yes	2.13±1.24	0.26±0.12	113.85±36.30	9.32±1.23	5.21±5.20
no	1.96±1.01	0.25±0.12	112.48±47.29	8.59±1.20	3.94±5.78

The values are presented as F (ANCOVA) and p values (covariates: sex and age). Abbreviations: NLR = ratio of neutrophils to lymphocytes, MLR = ratio of monocytes to lymphocytes, PLR = ratio of platelets to lymphocytes, MPV = mean platelet volume, CRP = C-reactive protein. *p < 0.05

Table 5. Associations between the indicators of inflammation and clinical characteristics of acute episodes and disease course in patients with UD

Clinical characteristics	NLR	MLR	PLR	MPV(fL)	CRP(mg/L)
Age at the onset of initial symptoms (years)	r=-0.020, p=0.766	r=0.021, p=0.751	r=0.119, p=0.073	r=-0.172, p=0.009*	r=-0.100, p=0.354
Duration of untreated disorder (months)	r=-0.015, p=0.834	r=-0.004, p=0.950	r=0.008, p=0.909	r=0.094, p=0.190	r=0.116, p=0.326
Duration of disorder (years)	r=0.014, p=0.832	r=-0.032, p=0.627	r=-0.113, p=0.088	r=0.155, p=0.019*	r=0.094, p=0.384
The total number of previous episodes	r=-0.063, p=0.400	r=0.046, p=0.540	r=-0.011, p=0.883	r=0.052, p=0.493	r=0.081, p=0.506
Time elapsed until remission (days)	r=0.024, p=0.713	r=0.001, p=0.994	r=-0.068, p=0.302	r=0.126, p=0.056	r=0.139, p=0.189
Number of hospitalizations	r=-0.046, p=0.488	r=-0.006, p=0.931	r=-0.098, p=0.137	r=0.057, p=0.395	r=0.067, p=0.532
Number of suicide attempts	r=0.102, p=0.120	r=0.111, p=0.093	r=0.012, p=0.858	r=-0.060, p=0.361	r=-0.027, p=0.798

The values are presented as correlation coefficients and p values (covariates: sex and age). Abbreviations: NLR = ratio of neutrophils to lymphocytes, MLR = ratio of monocytes to lymphocytes, PLR = ratio of platelets to lymphocytes, MPV = mean platelet volume, CRP = C-reactive protein. *p < 0.05

Indicators of inflammation in patients with unipolar depression

In UD patients, a statistically significant positive correlation was found for MPV in those with an earlier age of onset (r = 0.172, p = 0.009) and in patients with a longer duration of illness (r = 0.155, p = 0.019). All analyses were conducted with the control of sex and age covariates **(Table 5 and Table 6)**.

DISCUSSION

The current study, involving a substantial number of patients with bipolar and unipolar depression and including multiple readily available indicators of inflammation in the clinical setting, is among the rare delving into the examination of low-grade inflammation indicators, specifically comparing these two patient groups during the acute phase.

In our research, patients with bipolar depression had higher MLRs and MPVs than did those with unipolar depression. The mean platelet volume also differed in regard to the clinical characteristics of both groups of participants. Namely, for patients with unipolar depression, MPV was higher in those with an earlier age of onset of the disorder and in those with a longer duration of illness. However, in patients with bipolar depression, it was also associated with more severe clinical presentations throughout their lifetime, as well as currently. Patients with a history of suicide attempts or those who were currently at risk for suicide had higher values of this indicator of inflammation. Interestingly, the same parameter was higher in BD patients with a longer duration of untreated disorder.

Changes in the immune system are considered significant factors potentially contributing to the onset and clinical manifestations of mood disorders. Previous studies examining similar indicators of inflammation have highlighted MLR as an indicator of manic episodes in bi-

Table 6. Differences in the values of indicators of inflammation in relation to the clinical characteristics of acute episodes and the disease course in patients with UD

Clinical characteristic	NLR	MLR	PLR	MPV(fL)	CRP(mg/L)
Current psychotics symptoms	F=0.614, p=0.434	F=0.782, p=0.378	F=0.876, p=0.350	F=0.252, p=0.616	F=1.023, p=0.315
yes	1.88±1.42	0.21±0.13	100.93±51.37	9.39±1.42	12.80±36.13
no	2.09±1.53	0.23±0.15	108.83±48.39	9.51±1.36	6.70±16.33
Stage of disorder	F=0.827, p=0.439	F=0.325, p=0.723	F=1.626, p=0.199	F=0.837, p=0.434	F=0.632, p=0.534
< 5 years	2.17±1.51	0.25±0.13	109.76±44.34	9.30±1.67	10.06±30.38
5-10	2.28±2.27	0.22±0.23	117.92±60.71	9.43±1.26	2.57±2.49
>10 years	1.93±1.19	0.21±0.10	102.77±46.96	9.60±1.28	8.53±19.39
Heredity	F=1.818, p=0.179	F=2.176, p=0.142	F=0.013, p=0.908	F=0.026, p=0.871	F=0.025, p=0.874
yes	2.19±1.83	0.23±0.17	108.48±53.51	9.52±1.31	7.99±3.15
no	1.95±1.20	0.22±0.11	106.50±45.25	9.47±1.42	7.29±2.98
Current suicidal risk	F=0.250, p=0.617	F=0.061, p=0.805	F=0.095, p=0.758	F=0.027, p=0.870	F=0.988, p=0.321
yes	2.11±1.87	0.22±0.18	106.91±51.60	9.49±1.24	4.09±5.08
no	2.02±1.31	0.22±0.11	107.93±46.37	9.50±1.45	8.78±24.14
Previous suicide attempt	F=0.225, p=0.636	F=0.041, p=0.839	F=0.947, p=0.332	F=0.742, p=0.390	F=0.062, p=0.804
yes	2.13±2.01	0.22±0.19	103.34±56.28	9.37±1.17	6.84±14.72
no	2.02±1.26	0.22±0.11	109.07±45.58	9.54±1.46	7.94±83.08

Values are presented as F (analysis of covariance, ANCOVA) and p values (covariates: sex and age). Abbreviations: NLR = ratio of neutrophils to lymphocytes, MLR = ratio of monocytes to lymphocytes, PLR = ratio of platelets to lymphocytes, MPV = mean platelet volume, CRP = C-reactive protein.

polar disorder patients (12,13,24,25). While few studies comparing the MLR in patients with bipolar and unipolar depression have shown no differences in the values of this indicator of inflammation, our results show that patients with BD have a higher MLR than those with UD. The discrepancies in the findings may be attributed to the fact that our study included a significantly larger number of participants compared to the study by Mazza et al. (12). Additionally, the same study used different confounding variables compared to our study (12). However, the same study only revealed a difference in the number of lymphocytes in individuals with BD and UD, whereas our study documented differences in both the absolute lymphocyte count and the absolute monocyte count. This discrepancy potentially indicates immune reactivity in both cell groups in our patient population, especially in those with BD. It is important to note that the studies by other authors included younger patients, potentially with less advanced immune changes or fewer functional changes within the cardiovascular system (26, 27).

Research indicates that platelet size, measured as the mean platelet volume, is indeed an indicator of platelet immune reactivity (28). Previous studies comparing MPV in individuals with unipolar and bipolar depression found no difference in the values of this marker (10). However, the majority of studies analyzing individual diagnostic entities have reported increased MPV in individuals with unipolar and bipolar depression (28–30). In our study, patients with bipolar depression had higher MPV than patients with unipolar depression, suggesting increased immune activity in patients with BD. Documented immune activity may represent a risk factor, particularly for cardiovascular diseases, which are more prevalent or more pronounced in individuals with bipolar disorder than in those with major depressive disorder (31). The differences between our findings and those of previous research may stem from different participant selection, including individuals older than 65 years, or from the use of different statistical data processing methods.

Moreover, our study identified low-grade inflammation, with MPV as an indicator, in individual patient groups. Previous studies revealed an association between NLR and suicide risk in individuals with bipolar disorder (32). However, our study did not yield similar results. It is essential to acknowledge that previous studies with positive results were prospective, measured symptomatology during acute episodes using psychometric instruments, and found an association between NLR and suicide risk only in patients with a positive family history of suicide (32), which our study did not assess. Interestingly, our study found an association between other inflammatory parameters and suicide risk in patients with BD. MPV was higher in patients with a history of suicide attempts and those who were acutely suicidal, but it was also associated with a higher number of previous suicide attempts. Moreover, similar NLR values in individuals with unipolar and bipolar depression in our sample could be explained by their association with the type of acute affective symptomatology. Previous research has identified NLR as an indicator of manic states; thus, comparable levels of NLR in individuals with BD and UD in our sample could be expected (13,27). Our study did not find an association between the presence of psychotic symptoms and inflammatory parameters, somewhat in line with previous research. Similarly, Kayhan et al. (33) did not find an association between NLR and psychotic symptoms in individuals with unipolar depression. However, the same authors found an association between psychotic symptoms and PLR in individuals with major depressive disorder, regardless of its severity. The difference in our results may be due to the retrospective nature of our study, which made precise quantification of psychotic symptoms and grading patients in terms of disease severity unattainable.

An observation stemming from our work relates specifically to platelet reactivity, measured by MPV, which was increased in patients with bipolar depression with a longer duration of untreated disorder. Such data further underline the significance of timely treatment and align with previous research on the immunomodulatory effects of psychopharmacological agents (34). Elevated MPV values have also been documented in individuals with UD compared to healthy participants (30). Although our study did not include a healthy control group, increased MPV values were found in individuals with UD with an earlier onset and longer duration of illness. Prior research specifically indicates increased chronic low-grade inflammation in individuals with affective disorders, which may be associated with the severity and chronicity of the illness (28). Although our study did not directly investigate the association between stressful life events and inflammatory parameters, some studies have suggested that altered immune status, particularly increased inflammation, may result from the cumulative effects of stressors, contributing synergistically to a proinflammatory status and the chronic course of the disorders (35).

This study needs to be viewed in light of its strengths as well as potential limitations that may affect the results and their further interpretation. The most significant disadvantage of this study is its retrospective nature, which prevented a comprehensive assessment of acute psychopathology through psychometric instruments and thus the evaluation of the intensity of acute depressive symptoms in relation to indicators of inflammatory status. Another important limitation is that the association between applied pharmacological treatment and indicators of inflammation was not analyzed, which could impact the interpretation of the results. Nevertheless, bearing in mind that this study has overcome the limitations of previous studies that included a smaller number of participants and lack of control for covariates (10, 28, 32), we believe it provides additional, novel information regarding the

role of inflammation indicators in patients with affective disorders of both polarities. It is important to emphasize that one of the greatest qualities of the current study is its naturalistic, clinical sample, which has yielded additional insights. In practical and clinical terms, the results from such a sample could indicate the possibility that certain subsets of our patients are exposed to increased inflammation and require special attention concerning the prevention of somatic comorbidities (17) that further complicate the disease course and hinder treatment.

CONCLUSION

Our study highlights the presence of chronic low-grade inflammation in specific subcategories of patients with affective disorders of both polarities. Immune changes differ according to the polarity of the disorder and may serve as indicators of the severity of psychopathology and the absence of timely treatment in patients with bipolar depression. However, in patients with unipolar depression, these immune dysregulations could be mediated by the chronicity of the disease and the potential accumulation of environmental stressors. The results of our study highlight the importance of monitoring particular populations of patients who may be at risk of developing somatic comorbidities due to increased inflammation.

Acknowledgments: None

Conflicts of interest: The authors declare that there are no conflicts of interest associated with this work.

Author contributions: Conceptualization, M.P.S., S.D.; methodology, M.P.S., M.I., M.N., V.M.M. and B.D.K.; software, S.D., M.G., and M.P.; formal analysis, S.D., M.G., E.E., and M.P.; writing—original draft preparation, S.D., M.P.S.; writing—editing and review, B.D.K., V.M.M., and M.I.; visualization, B.D.K., V.M.M., E.E., M.N.; and supervision, M.P.S., M.I. All authors have read and agreed to the published version of the manuscript.

Ethical approval: Ethics Committee of the University Clinical Centre of Serbia (No. 622/1).

REFERENCES

1. Hashimoto K. Brain-derived neurotrophic factor (BDNF) and its precursor proBDNF as diagnostic biomarkers for major depressive disorder and bipolar disorder. Eur Arch Psychiatry Clin Neurosci. 2015 Feb;265(1):83–4. https://doi.org/10.1007/s00406-014-0557-x PMID: 25362578
2. Hashimoto K. Chapter Three - Metabolomics of Major Depressive Disorder and Bipolar Disorder: Overview and Future Perspective. In: Makowski GS, editor. Advances in Clinical Chemistry [Internet]. Elsevier; 2018 [cited 2024 Feb 17]. p. 81–99. https://doi.org/10.1016/bs.acc.2017.12.005
3. McIntyre RS, Zimmerman M, Goldberg JF, First MB. Differential Diagnosis of Major Depressive Disorder Versus Bipolar Disorder: Current Status and Best Clinical Practices. J Clin Psychiatry. 2019 May 14;80(3):ot18043ah2. doi: 10.4088/JCP.ot18043ah2. PMID: 31091027.
4. Menezes IC, von Werne Baes C, Fígaro-Drumond FV, Dias Macedo BB, Bueno AC, Lacchini R, Feijó de Mello M, de Castro M, Juruena MF. Differential Diagnosis of Major Depressive Disorder and Bipolar Disorder: Genetic and Hormonal Assessment and the Influence of Early-Life Stress. Brain Sci. 2022 Oct;12(11):1476. doi: 10.3390/brainsci12111476. PMID: 36358401; PMCID: PMC9688727.
5. Hori H, Teraishi T, Sasayama D, Hattori K, Hashikura M, Higuchi T, et al. Relationship of temperament and character with cortisol reactivity to the combined dexamethasone/CRH test in depressed outpatients. J Affect Disord. 2013 May;147(1–3):128–36. https://doi.org/10.1016/j.jad.2012.10.022 PMID: 23178186
6. Ruiz NAL, Del Ángel DS, Brizuela NO, Peraza AV, Olguín HJ, Soto MP, Guzmán DC. Inflammatory Process and Immune System in Major Depressive Disorder. Int J Neuropsychopharmacol. 2022 Jan;25(1):46-53. doi: 10.1093/ijnp/pyab072. PMID: 34724041; PMCID: PMC8756095.
7. Jones GH, Vecera CM, Pinjari OF, Machado-Vieira R. Inflammatory signaling mechanisms in bipolar disorder. J Biomed Sci. 2021 Jun;28(1):45. doi: 10.1186/s12929-021-00742-6. PMID: 34112182; PMCID: PMC8194019.
8. van Sloten TT, Schram MT, Adriaanse MC, Dekker JM, Nijpels G, Teerlink T, et al. Endothelial dysfunction is associated with a greater depressive symptom score in a general elderly population: the Hoorn Study. Psychol Med. 2014 May;44(7):1403–16. https://doi.org/10.1017/S0033291713002043 PMID: 23942242
9. Ivković M, Pantović-Stefanović M, Petronijević N, Dunjić-Kostić B, Velimirović M, Nikolić T, et al. Predictive value of sICAM-1 and sVCAM-1 as biomarkers of affective temperaments in healthy young adults. J Affect Disord. 2017 Jan 1;207:47–52. https://doi.org/10.1016/j.jad.2016.09.017 PMID: 27693464
10. Wysokiński A, Szczepocka E. Platelet parameters (PLT, MPV, P-LCR) in patients with schizophrenia, unipolar depression and bipolar disorder. Psychiatry Res. 2016 Mar 30;237:238–45. https://doi.org/10.1016/j.psychres.2016.01.034 PMID: 26805563
11. Arabska J, Łucka A, Magierski R, Sobów T, Wysokiński A. Neutrophil-lymphocyte ratio is increased in elderly patients with first episode depression, but not in recurrent depression. Psychiatry Res. 2018 May;263:35–40. https://doi.org/10.1016/j.psychres.2018.02.043 PMID: 29490259
12. Mazza MG, Tringali AGM, Rossetti A, Botti RE, Clerici M. Cross-sectional study of neutrophil-lymphocyte, platelet-lymphocyte and monocyte-lymphocyte ratios in mood disorders. Gen Hosp Psychiatry. 2019;58:7–12. https://doi.org/10.1016/j.genhosppsych.2019.02.003 PMID: 30818102
13. Inanli I, Aydin M, Çaliskan AM, Eren I. Neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio, and mean platelet volume as systemic inflammatory markers in different states of bipolar disorder. Nord J Psychiatry. 2019 Aug;73(6):372–9. https://doi.org/10.1080/08039488.2019.1640789 PMID: 31304832
14. Adhikari A, Dikshit R, Karia S, Sonavane S, Shah N, De Sousa A. Neutrophil-lymphocyte Ratio and C-reactive Protein Level in Patients with Major Depressive Disorder Before and After Pharmacotherapy. East Asian Arch Psychiatry. 2018 Jun;28(2):53–8. PMID: 29921741
15. Muneer A. Staging Models in Bipolar Disorder: A Systematic Review of the Literature. Clin Psychopharmacol Neurosci. 2016 May 31;14(2):117–30. https://doi.org/10.9758/cpn.2016.14.2.117 PMID: 27121423
16. Stoyanov DS. Endophenotypes and Pathway Phenotypes in Neuro-psychiatry: Crossdisciplinary Implications for Diagnosis. CNS Neurol Disord Drug Targets. 2023;22(2):150-151. doi: 10.2174/1871527322022091412553O. PMID: 36482720.
17. Huczek Z, Kochman J, Filipiak KJ, Horszczaruk GJ, Grabowski M, Piatkowski R, et al. Mean platelet volume on admission predicts impaired reperfusion and long-term mortality in acute myocardial infarction treated with primary percutaneous coronary intervention. J

Am Coll Cardiol. 2005 Jul 19;46(2):284–90. https://doi.org/10.1016/j.jacc.2005.03.065 PMID: 16022956

18. Chang HH, Wang T-Y, Lee IH, Lee S-Y, Chen KC, Huang S-Y, et al. C-reactive protein: A differential biomarker for major depressive disorder and bipolar II disorder. World J Biol Psychiatry. 2017 Feb;18(1):63–70. https://doi.org/10.3109/15622975.2016.1155746 PMID: 26895280
19. World Health Organization, editor. The ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines. Geneva: World Health Organization; 1992. 362 p.
20. Drancourt N, Etain B, Lajnef M, Henry C, Raust A, Cochet B, et al. Duration of untreated bipolar disorder: missed opportunities on the long road to optimal treatment. Acta Psychiatr Scand. 2013 Feb;127(2):136–44. https://doi.org/10.1111/j.1600-0447.2012.01917.x PMID: 22901015
21. Pedrini M, Massuda R, Fries GR, de Bittencourt Pasquali MA, Schnorr CE, Moreira JCF, et al. Similarities in serum oxidative stress markers and inflammatory cytokines in patients with overt schizophrenia at early and late stages of chronicity. J Psychiatr Res. 2012 Jun;46(6):819–24. https://doi.org/10.1016/j.jpsychires.2012.03.019 PMID: 22520512
22. Wener MH, Daum PR, McQuillan GM. The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. J Rheumatol. 2000 Oct;27(10):2351–9. PMID: 11036829
23. Haukoos JS, Lewis RJ. Advanced statistics: bootstrapping confidence intervals for statistics with "difficult" distributions. Acad Emerg Med. 2005 Apr;12(4):360–5. https://doi.org/10.1197/j.aem.2004.11.018 PMID: 15805329
24. Özdin S, Sarisoy G, Böke Ö. A comparison of the neutrophil-lymphocyte, platelet-lymphocyte and monocyte-lymphocyte ratios in schizophrenia and bipolar disorder patients - a retrospective file review. Nord J Psychiatry. 2017 Oct;71(7):509–12. https://doi.org/10.1080/08039488.2017.1340517 PMID: 28644753
25. Kirlioglu SS, Balcioglu YH, Kalelioglu T, Erten E, Karamustafalioglu N. Comparison of the complete blood count-derived inflammatory markers in bipolar patients with manic and mixed episodes. Bratisl Lek Listy. 2019;120(3):195–9. https://doi.org/10.4149/BLL_2019_051 PMID: 31023037
26. Kim J-H, Lee Y-J, Park B. Higher monocyte count with normal white blood cell count is positively associated with 10-year cardiovascular disease risk determined by Framingham risk score among community-dwelling Korean individuals. Medicine (Baltimore). 2019 Apr;98(17):e15340. https://doi.org/10.1097/MD.0000000000015340 PMID: 31027108
27. Kalelioglu T, Akkus M, Karamustafalioglu N, Genc A, Genc ES, Cansiz A, et al. Neutrophil-lymphocyte and platelet-lymphocyte ratios as inflammation markers for bipolar disorder. Psychiatry Res. 2015 Aug 30;228(3):925–7. https://doi.org/10.1016/j.psychres.2015.05.110 PMID: 26154814
28. Chen Z, Wang J, Carru C, Sedda S, Nivoli AM, Li Z. Meta-analysis of peripheral mean platelet volume in patients with mental disorders: Comparisons in depression, anxiety, bipolar disorder, and schizophrenia. Brain Behav. 2023 Nov;13(11):e3240. doi: 10.1002/brb3.3240. Epub 2023 Aug 29. PMID: 37644555; PMCID: PMC10636414.
29. Mert DG, Terzi H. Mean platelet volume in bipolar disorder: the search for an ideal biomarker. Neuropsychiatr Dis Treat. 2016; 12:2057–62. https://doi.org/10.2147/NDT.S112374 PMID: 27578978
30. Cai L, Xu L, Wei L, Chen W. Relationship of Mean Platelet Volume To MDD: A Retrospective Study. Shanghai Arch Psychiatry. 2017 Feb 25;29(1):21–9. https://doi.org/10.11919/j.issn.1002-0829.216082 PMID: 28769542
31. Xie F, Zhou L, Hu Q, Zeng L, Wei Y, Tang X, Gao Y, Hu Y, Xu L, Chen T, Liu H, Wang J, Lu Z, Chen Y, Zhang T. Cardiovascular variations in patients with major depressive disorder versus bipolar disorder. J Affect Disord. 2023 Nov 15;341:219-227. doi: 10.1016/j.jad.2023.08.128. Epub 2023 Aug 30. PMID: 37657620.
32. Ivković M, Pantović-Stefanović M, Dunjić-Kostić B, Jurišić V, Lačković M, Totić-Poznanović S, et al. Neutrophil-to-lymphocyte ratio predicting suicide risk in euthymic patients with bipolar disorder: Moderatory effect of family history. Compr Psychiatry. 2016 Apr;66:87–95. https://doi.org/10.1016/j.comppsych.2016.01.005 PMID: 26995241
33. Kayhan F, Gündüz Ş, Ersoy SA, Kandeğer A, Annagür BB. Relationships of neutrophil-lymphocyte and platelet-lymphocyte ratios with the severity of major depression. Psychiatry Res. 2017 Jan;247:332–5. https://doi.org/10.1016/j.psychres.2016.11.016 PMID: 27978453
34. Bhatt S, Dhar AK, Samanta MK, Suttee A. Effects of Current Psychotropic Drugs on Inflammation and Immune System. Adv Exp Med Biol. 2023; 1411:407-434. doi: 10.1007/978-981-19-7376-5_18. PMID: 36949320.
35. Anderson G, Berk M, Dean O, Moylan S, Maes M. Role of immune-inflammatory and oxidative and nitrosative stress pathways in the etiology of depression: therapeutic implications. CNS Drugs. 2014 Jan; 28(1):1–10. https://doi.org/10.1007/s40263-013-0119-1 PMID: 24150993

RAZLIKE U INDIKATORIMA INFLAMACIJE OBOLELIH OD BIPOLARNE I UNIPOLARNE DEPRESIJE

Sara Dodić[1,2], Marija Purić[1,2], Marta Gostiljac[2], Milica Nešić[1,2], Bojana Dunjić-Kostić[1,3], Vanja Mandić-Maravić[1,3], Emilija Erić[2], Maja Ivković[1,2], Maja Pantović-Stefanović[1,2]

Sažetak

Uvod/Cilj: Usled preklapajuće simptomatologije, a često i neprepoznate ili odložene pojave manične ili hipomanične epizode u bipolarnom poremećaju, pacijenti sa ovim oboljenjem često se nepravilno dijagnostikuju kao oboleli od unipolarne depresije. Ova dva poremećaja imaju srodnu, ali ne i istu etiopatogenezu. Izmene u imunskom sistemu potencijalno značajno doprinose nastanku i kliničkoj ekspresiji poremećaja raspoloženja. Studija ispituje razlike u indikatorima inflamacije kod pacijenata sa bipolarnom (BD) i unipolarnom depresijom (UD) i njihovu povezanost sa kliničkim karakteristikama akutne epizode i toka bolesti oba poremećaja.

Materijali i metode: Istraživanje predstavlja retrospektivnu studiju u koju su uključeni pacijenti oboleli od BD (n=60) i UD (n=242), koji su hospitalno lečeni u dvogodišnjem periodu i za koje su evidentirane socio-demografske informacije i karakteristike akutne epizode i toka bolesti i ispitivani indikatori inflamacije.

Rezultati: Oboleli od BD imaju viši srednji volumen trombocita u odnosu na obolele od UD. Isti indikator inflamacije je izmenjen i u odnosu na kliničke karakteristike obe grupe ispitanika. Tako je kod pacijenata sa UD njegova vrednost bila viša ukoliko je bolest ranije počela i duže trajala. Kod obolelih od BD, srednji volumen trombocita je povezan sa težinom psihopatologije tokom života, pa su pacijenti sa istorijom pokušaja suicida i oni koji su duže čekali na započinjanje terapije pri prvoj epizodi tokom života takodje imali više vrednosti ovog indikatora inflamacije.

Zaključak: Naše istraživanje ukazuje na postojanje hronične inflamacije niskog stepena kod specifičnih subpopulacija obolelih od afektivnih poremećaja. Prisutne imunske izmene razlikuju se u odnosu na polaritet oboljenja i mogu biti potencijalni indikator težine psihopatologije i hroniciteta bolesti.

Ključne reči: bipolarni afektivni poremećaj, inflamacija, unipolarna depresija

Primljen: 18.03.2024. I **Revizija:** 16.05.2024. I **Prihvaćen:** 28.05.2024.

Medicinska istaživanja 2024; 57(3):49-58

Medicinska istaživanja 2024; 57(3):59-63 | DOI 10.5937/medi57-50470

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Epidemiology of spinal column injuries before, during, and after the COVID-19 pandemic – is there any difference?

Slavisa Zagorac[1,2], Milos Vasic[2], Uros Novakovic[1,2], Milos Mladenovic[2], Ivan Tulic[1,2], ✉Valerija Teodosic[1,2]

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

[2] Clinic of Orthopedic Surgery and Traumatology, University Clinical Center of Serbia, Belgrade, Serbia

Recived: 15 April 2024

Revised: 23 June 2024

Accepted: 27 June 2024

Check for updates

Funding information:
The authors did not receive support from any organization for the submitted work. No funding was received to assist with the preparation of this manuscript. No funding was received for conducting this study.

Competing interests:
The authors have declared that no competing interests exist

✉ **Correspondence to:**
Valerija Teodosić

Clinic of Orthopedic Surgery and Traumatology, University Clinical Center of Serbia,

2 Pasterova Street, 11 000 Belgrade, Serbia

E-mail: valerija.teodosic996@gmail.com

Summary

Introduction: The lack of information in international literature regarding the impact of the introduction and the subsequent termination of epidemiological restrictions during the COVID-19 pandemic on the epidemiology of spinal injuries has led to the constant monitoring and recording of all relevant data on patients with spinal column injuries since the beginning of the pandemic.

Study Aim: To analyze and compare epidemiological data on patients with spinal column injuries treated at a tertiary healthcare facility in Serbia, before, during, and after the COVID-19 pandemic.

Materials and methods: This is a retrospective study spanning three observation periods analyzing patients with spinal column injuries.

Results: The average age of patients in the first observation period was 47 years, 68 years in the second, and 39 years in the third period. In the first two observation periods, the dominant mechanism of injury was same-level fall, and most of the patients were treated conservatively. When comparing the data on the three groups of respondents, statistically significant differences in the age of the patients and the mechanism of injury can be observed.

Conclusion: The COVID-19 pandemic contributed to spinal column injuries being more common among the elderly, due to falls occurring at home. However, after people returned to an active lifestyle, which was especially true of the younger population, there was a sudden increase in the number of spinal column injuries due to road traffic accidents, while due to the frequent occurrence of spinal cord injuries, there was also an increase in the number of emergency surgeries.

Keywords: COVID-19, spinal column injuries, mechanism of injury, surgical treatment

Cite this article as: Zagorac S, Vasić M, Novaković U, Mladenović M, Tulić I, Teodosić V. Epidemiology of spinal column injuries before, during, and after the covid-19 pandemic – is there any difference?; Medicinska istaživanja 2024; 57(3):59-63 DOI: 10.5937/medi57-50470

INTRODUCTION

The COVID-19 pandemic and the resulting epidemiological restrictions limiting free movement, prohibiting large gatherings, and restricting the working hours of most establishments in the entertainment, hospitality, and services industries, brought about certain changes, both in everyday life and in the functioning of the healthcare system (1-3). The overburdening of the healthcare system with COVID patients on the one hand, and the reduced movement and activity of people on the other hand, led to certain changes in the epidemiology of spinal column injuries and other bone-joint injuries, as compared to the period before the pandemic (4-8). After the pandemic restrictions were lifted, people resumed an active lifestyle. The health system gradually recovered and began to operate at its full capacity, i.e., like before the pandemic.

Generally, in the epidemiology of spinal column injuries, most studies have shown the ratio of male to female injuries to be 2:1 (9). The most common mechanisms of injury are road traffic trauma in the younger population and falls in the older population (9-11). What makes spinal column injuries both unique and complex is the injury to the spinal cord. The mechanism of injury to the spinal column, i.e. the type of injury, as well as the degree of injury, play a major role in spinal cord damage, and thus in the degree of neurological deficit (12). The prognosis for recovery is uncertain, and the estimate is that people above the age of 60 years with a spinal cord injury have a poorer prognosis for recovery and experience a lower quality of life after the injury (9). Medicamentous therapy with methylprednisolone and early surgical stabilization within the first 24 hours after injury play a role in the degree of recovery after injury to the spinal column and spinal cord (13-16).

The study aims to compare the epidemiological data on spinal column injuries from the period before, during, and after the COVID-19 pandemic, and to determine whether there were differences in the number of spinal column injuries, the demographic characteristics of patients, the mechanisms of injury, and the treatment modalities, in the context of the introduction and then the termination of epidemiological restrictions. The hypothesis is that, during the COVID-19 pandemic, there was a lower rate of patients with spinal column injuries, as compared to the time before and after the pandemic.

MATERIALS AND METHODS

This is an observational, descriptive, retrospective, single-center study. Epidemiological data on patients with spinal column injuries treated at the trauma center of a tertiary healthcare facility in Serbia were collected for three different observation periods. A total of 368 patients with spinal column injuries were recorded in the first observation period, i.e., in the 18 months preceding the COVID-19 pandemic. In the second observation period, i.e. over the duration of the COVID-19 pandemic, 363 patients with spinal column injuries were recorded. Finally, in the third observation period, lasting 18 months after the COVID-19 pandemic, 326 patients with spinal column injuries were recorded. Sex, age, the mechanism of injury, the type of injury, neurological deficit, and the treatment modality were recorded in all patients. The data were obtained from the hospital admission protocol of the University Clinical Center of Serbia Emergency Center, the treatment protocol of the Department of Spinal Surgery of the University Clinical Center of Serbia Emergency Center, and the surgery treatment protocol of the said department. In all patients, the appropriate diagnostic procedures were performed (conventional radiography – X-rays; computed tomography – CT), their neurological status was assessed (the ASIA score – American Spinal Injury Association Impairment Scale was applied; according to ASIA score neurological findings can be without neurological impairment – ASIA E, complete neurological impairment (paraplegia or quadriplegia) – ASIA A, or with preserved sensibility and loss of motor skills in varying degrees – ASIA B, C, D), as was their general health status, upon which a decision was made whether to treat the patient conservatively or surgically.

The findings were processed using methods of descriptive statistics, one-way ANOVA, Student's t-test, Chi-square test and the statistical significance was set at $p < 0.05$. The EZR software was used for statistical data processing.

RESULTS

The values regarding age, gender distribution, mechanism of injury, injury level and treatment modality are presented in Table 1. All conservatively treated patients in all three periods had normal neurological findings – ASIA E (Appendix I, Table 2). During the first observation period two patients died, of whom one patient with a cervical spine injury and quadriplegia, and one patient with a thoracic spine injury and paraplegia. During the second observation period four patients died, of whom three patients with a cervical spine injury and quadriplegia, and one patient with a thoracic spine injury and paraplegia. During the third observation period six patients died, of whom four patients with a cervical spine injury and quadriplegia, and two patients with a thoracic spine injury and paraplegia.

DISCUSSION

The results of this epidemiological study showed that there was an approximately equivalent number of patients with spinal column injury during all three observation

Table 1. Descriptive parameters of spinal column injuries before, during, and after the COVID-19 pandemic

	Before the COVID-19 pandemic (n=368, 100%)	During the COVID-19 pandemic (n=363, 100%)	After the COVID-19 pandemic (n=326, 100%)
Age (years) [median (min-max)]	47 (14-92)	68 (15-92)	39 (14-77)
Male / Female	219 / 149	228 / 135	198 / 128
	(59.5% / 40.5%)	(62.8% / 37.1%)	(60.7% / 39.3%)
Mechanism of injury			
Same-level fall	204 (55.4%)	211 (58.1%)	94 (28.8%)
Road traffic accident	111 (30.2%)	97 (26.7%)	154 (47.3%)
Fall from height above two meters	53 (14.4%)	55 (15.2%)	78 (23.9%)
Injury level			
Thoracic and lumbar spine	249 (67.7%)	305 (84.1%)	193 (59.2%)
Cervical spine	119 (32.3%)	58 (15.9%)	133 (40.8%)
Treatment modality			
Conservative	257 (69.8%)	206 (56.7%)	176 (54%)
Surgical	111 (30.2%)	157 (43.3%)	150 (46%)

periods. Men were dominantly injured in all three periods. Regarding age, the younger population was dominant in the period after COVID-19 pandemic, while older population was dominant in the periods before and during the pandemic. In the periods before and during the pandemic, same-level falls were the most common mechanism of injury, while in the period after the pandemic, road traffic trauma was the dominant mechanism of injury. In the first two observation periods, the most frequently injured segment of the spinal column was the thoracolumbar spine, while in the third observation period, an approximately equivalent number of patients with thoracolumbar and cervical spine injuries was recorded. In the periods before and during the pandemic, most patients were treated conservatively, while after the pandemic a similar number of patients were treated conservatively and surgically **(Appendix I, Table 1)**.

Furthermore, here was recorded that in the period after the COVID-19 pandemic, the drivers who caused road traffic accidents were often inebriated. Also, in our study, an increased number of attempted suicides (Lat. *tentamen suicidi*) was recorded among the younger population, as compared to the period before and during the pandemic.

This study has shown that in a tertiary health institution in Serbia, there was no significant difference in the number of patients with spinal column injuries, the number of surgical procedures, and the injury level, before, during, and after COVID-19 pandemic ($p > 0.05$). However, it was established that there was a significant difference in the age, as well as the mechanism of injury, before, during, and after COVID-19 pandemic ($p < 0.05$), **(Appendix I, Table 1)**.

A comparison of the observation periods for variables where statistical significance was found had shown that, regarding patient age and mechanism of injury, there was a significant difference between the first and the third, as well as between the second and the third observation periods, while between the first and the second observation periods, a significant difference was not found **(Appendix II, Table 3)**.

Studies performed during COVID-19 pandemic worldwide have proved that there was a significantly lower rate of patients with spinal cord injuries admitted to major trauma centers during the pandemic, as well as that a lower rate of both elective and emergency spinal surgeries was performed, as compared to the period before the pandemic (17-19). Restrictions on free movement during the pandemic could be considered as the factor inducing such a situation, as well as the large influx of patients suffering from the new disease. Iyengar K. et al. showed that Coronavirus outbreak had refocused orthopedic minds on managing many injuries (among them spinal column injuries) conservatively, which would have otherwise been managed with operative fixations (20).

Table 2. Neurological findings in the followed periods

	Before the COVID-19 pandemic	During the COVID-19 pandemic	After the COVID-19 pandemic
Preoperative neurological finding	**(n=111, 100%)**	**(n=157, 100%)**	**(n=150, 100%)**
Normal	68 (61.3%)	118 (75.2%)	82 (54.7%)
Paraplegia	33 (29.7%)	31 (19.7%)	45 (30%)
Quadriplegia	10 (9%)	8 (5.1%)	23 (15.3%)
Postoperative neurological finding in the patients with a neurologic deficit found before surgery	**43 (100%)**	**39 (100%)**	**68 (100%)**
Improved as compared to the initial finding	15 (34.9%)	17 (43.6%)	20 (29.4%)
Unchanged as compared to the initial finding	28 (65.1%)	22 (56.4%)	48 (40.6%)

Table 3. The comparison of data for all three observation periods, as well as the comparison between individual periods for variables where a statistically significant difference was found

	Comparing all three observation periods	Comparing the first and the second observation periods	Comparing the second and the third observation periods	Comparing the first and the third observation periods
Number of patients	p = 0.071	p = 0.065	p = 0.074	p = 0.074
Age	**p < 0.001**	p = 0.569	**p < 0.001**	**p < 0.001**
Sex	p = 0.788	p = 0.612	p = 0.743	p = 0.067
Mechanism of injury	**p < 0.001**	p = 0.718	**p < 0.001**	**p < 0.001**
Injury level	p = 0.06838	p = 0.083	p = 0.078	p = 0.061
Treatment modality	p = 0.07861	p = 0.062	p = 0.071	p = 0.079

The data obtained from other studies do not correlate with our study, and the reason for this may be that the Emergency Center of the University Clinical Center of Serbia was the only functioning trauma center during the COVID-19 pandemic, operating at its full capacity before, during, and after the COVID-19. More patients from the entire territory of Serbia than usual were cared for and treated here, while other health facilities throughout Serbia performed less spinal column injuries surgery than before, due to transforming capacities into COVID-19 centers with the primary goal of caring for patients suffering from the new COVID-19 infection.

The limitations of this study are that it included just one trauma center, as well as the fact that the trauma center was operating at its regular capacity even during the pandemic.

CONCLUSION

The COVID-19 pandemic and the resulting epidemiological restrictions contributed to spinal column injuries being more common among the elderly, due to falls occurring at home. Additionally, patients were generally without neurologic deficits and mostly treated conservatively. However, after the restrictions were lifted, people resumed their former lifestyle, using cars and public transportation more frequently, which led to a sudden increase in the number of spinal column injuries caused by road traffic accidents, especially among the younger population. The severity of trauma caused by such a mechanism of injury also led to an increase in the number of emergency surgeries, as compared to the period before and during the pandemic.

REFERENCES

1. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, evolution and treatment of Corona virus (Covid-19). [Updated 2022 Jan 5]. In: StatPerls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan.
2. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet*. 2020; 395:470-743. doi: 10.1016/S0140-6736(20)30185-9.
3. Prasaath Sastha KR, Arul B, Kothai R. Understanding COVID-19 - the pandemic of 2020. *Int J Res Pharm Sci*. 2020; 11:94-102.
4. Tuczynska M, Matthews-Kozanecka M, Baum E. Accessibility to Non-COVID Health Services in the world during the COVID-19 pandemic: Review. *Front Public Health*. 2021; 9:760-795. doi: 10.3389/fpubh.2021.760795.
5. Lim MA, Ridia KGM, Pranata R. Epidemiological pattern of orthopaedic fracture during the COVID-19 pandemic: A systematic review and meta-analysis. *J Clin Orthop Trauma*. 2021; 16:16-23. doi: 10.1016/j.jcot.2020.12.028.
6. Al-Omran AS. COVID-19 pandemic impact on Othopaedic Trauma Practise: A global perspective. *Orthop Res Rev*. 2022; 14:9-15. doi: 10.2147/ORR.S345301
7. Liang ZC, Wang W, Murphy D, Hoi Po Hui J. Novel Coronavirus and Orthopaedic Surgery. *J Bone Joint Surg Am*. 2020; 102:745-749. doi: 10.2106/JBJS.20.00236.
8. Chhabra HS, Bagaraia V, Keny S, Kalidindi KKV, Mallepally A, Dhillon MS, et al. COVID-19: Current Knowledge and Best Practices for Orthopaedic Surgeons. *Indian J Orthop*. 2020; 54(4):411-425. doi: 10.1007/s43465-020-00135-1.
9. Alizadeh A, Dyck SM, Karimi-Abdolrezaee S. Traumatic spinal cord injury: An overview of pathophysiology, models and acute injury mechanisms. *Front Neurol*. 2019; 10:282. doi: 10.3389/fneur.2019.00282.
10. Kumar R, Lim J, Mekary RA, Rattani A, Dewan MC, Sharif SY, et al. Traumatic spinal injury: Global epidemiology and worldwide volume. *World Neurosurgery*. 2018; 113:e345-e363. doi: 10.1016/j.wneu.2018.02.033.
11. Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, et al. Acute spinal cord injury, part I: Pathophysiologic mechanisms. *Clin Neuropharmacol*. 2001; 24(5):254-264. doi: 10.1097/00002826-200109000-00002.
12. Reinold M, Knop C, Beisse R, Audige L, Kandziora F, Pizanis A, et al. Operative treatment of 733 patients with acute thoracolumbar spinal injuries: comprehensive results from the second, prospective, internet-based multicenter study of the Spine Study Group of the German Association of Trauma Surgery. *Eur Spine J*. 2010; 19(10):1657–1676. doi: 10.1007/s00586-010-1451-5.
13. Zagorac S. Methylprednisolone therapy in acute spinal cord injuries. *Serbia Journal of the Medical Chamber*. 2021; 2(4):407-413. doi:10.5937/smclk2-34472.
14. Wang TY, Park C, Zhang H, Rahimpour S, Murphy KR, Goodwin CR, et al. Management of Acute Traumatic Spinal Cord Injury: A Review of the Literature. *Front Surg*. 2021; 8:698-736. doi: 10.3389/fsurg.2021.698736.
15. Kanna RM, Shetty AP, Rajasekaran S. Timing of intervention for spinal injury in patients with polytrauma. *J Clin Orthop Trauma*. 2021; 12(1):96–100. doi:10.1016/j.jcot.2020.10.003.
16. Hager S, Eberbach H, Lefering R, Hammer TO, Kubosch D, Jäger C, et al. Possible advantages of early stabilization of spinal fractures in multiply injured patients with leading thoracic trauma - analysis based on the TraumaRegister DGU®. *Scand J Trauma Resusc Emerg Med*. 2020; 28:42. doi: 10.1186/s13049-020-00737-6. PMID: 32448190.

17. Dolci A, Marongiu G, Leinardi L, Lombardo M, Dessi G, Capone A. The epidemiology of fractures and musculo-skeletal traumas during Covid-19 lockdown: A detailed survey of 17.591 patients in a wide Italian metropolitan area. *Geriatr Orthop Surg Rehabil*. 2020; 11:1-8. doi:10.1177/2151459320972673. PMID: 33240557.

18. Zou J, Yu H, Song D, Niu J, Yang H. Advice on Standardized Diagnosis and Treatment for Spinal Diseases during the Coronavirus Disease 2019 Pandemic. *Asian Spine J*. 2020; 14(2):258–263. doi:10.31616/asj.2020.0122. PMID: 32222720.

19. Wong SHJ, Cheung SCK. Impact of Covid-19 on orthopaedic and trauma service: An epidemiological study. *J Bone Joint Surg Am*. 2020; 102(14):1-9. doi:10.2106/JBJS.20.00775.

20. Iyengar K, Vaish A, Vaishya R. Revisiting conservative orthopaedics management of fractures during COVID-19 pandemic. *J Clin Orthop Trauma*. 2020; 11(4):718-720. doi:10.1016/j.jcot.2020.05.010.

EPIDEMIOLOGIJA POVREDA KIČMENOG STUBA PRE, ZA VREME I NAKON PANDEMIJE IZAZVANE KOVIDOM 19 – IMA LI RAZLIKE?

Slaviša Zagorac[1,2], Miloš Vasić[2], Uroš Novaković[1,2], Miloš Mladenović[2], Ivan Tulić[1,2], Valerija Teodosić[1,2]

Sažetak

Uvod: Nedostatak informacija u svetskoj literaturi o uticaju uvođenja, a potom i ukidanja epidemioloških mera na epidemiologiju povreda kičmenog stuba nave nas je na konstantno praćenje i beleženje svih relevantnih podataka o pacijentima sa povredama kičmenog stuba od početka pandemije.

Cilj rada: Sagledavanje i upoređivanje epidemioloških podataka u vezi sa povredama kičmenog stuba lečenih u tercijernoj zdravstvenoj ustanovi u Srbiji, pre, za vreme i nakon pandemije izazvane kovidom 19.

Materijal i metode: Retrospektivna studija koja obuhvata tri vremenska perioda praćenja pacijenata sa povredom kičmenog stuba.

Rezultati: Prosečne godine starosti pacijenata u prvom periodu praćenja su iznosile 47, u drugom 68, a u trećem 39 godina. U prva dva perioda praćenja dominantan mehanizam povređivanja je bio pad na ravnom, a pacijenti su najčešće lečeni konzervativno. Poređenjem podataka tri grupe ispitanika, uočavaju se statistički značajne razlike u godinama starosti pacijenata i mehanizmu povređivanja.

Zaključak: Pandemija izazvana kovidom 19 doprinela je većoj učestalosti povreda kičmenog stuba među starijim osobama, usled padova u kućnim uslovima. Međutim, nakon vraćanja aktivnom načinu života, posebno među mlađom populacijom, došlo je do naglog povećanja broja povreda kičmenog stuba usled saobraćajnog traumatizma, a zbog čestog postojanja povrede kičmene moždine, i do povećanja broja urgentnih operativnih zahvata.

Ključne reči: kovid19, povrede kičmenog stuba, mehanizam povređivanja, operativno lečenje

Primljen: 15.04.2024. I **Revizija:** 23.06.2024. I **Prihvaćen:** 27.07.2024.

Medicinska istaživanja 2024; 57(3):59-63

Medicinska istaživanja 2024; 57(3):65-73 | DOI 10.5937/medi57-49293

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

COVID-19 vaccine hesitancy in Serbia

Vida Jeremic Stojković[1,2], Smiljana Cvjetković[1,2], Vesna Bjegović-Mikanović[1, 2], Tatjana Gazibara[1], Adrijan Sarajlija[1]

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

[2] School of Public Health and Health Management, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Recived: 15 February 2024

Revised: 26 June 2024

Accepted: 01 July 2024

Check for updates

Funding information:
This study did not receive funding.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Smiljana Cvjetković

Department of Humanities, Faculty of Medicine, University of Belgrade

2 Pasterova Street, 11000 Belgrade, Serbia

Email: smi13smi@yahoo.com, smiljana.cvjetkovic@med.bg.ac.rs

Summary

Introduction/Aim: Vaccine hesitancy is recognized as important determinant of routine immunization coverage, but also as a factor of inadequate acceptance of the COVID-19 vaccine. The World Health Organization proposed a "3C" model, with confidence, complacency and convenience as the core components of vaccine hesitancy. The purpose of this study was to assess the intention to receive COVID-19 vaccine through the 3C framework in Serbia.

Materials and Methods: This cross-sectional study was based on the data collected from 1,435 adult respondents from the general population using an online questionnaire in the period December 2020-January 2021. Convenience, non-probability sampling was applied. Participants were reached through the existing social networks and mailing lists. The main outcome variable was the intention to get vaccinated against COVID-19, with three categories: vaccine refusal, vaccine indecisiveness, and vaccine acceptance. To explore associations of predictor variables (socio-demographics, source credibility, general vaccine attitudes and COVID-19 vaccine hesitancy measured through three scales – Confidence, Convenience and Complacency) with the outcome variable, binary logistic regression models were conducted.

Results: Less than one third of respondents (28.6%) were vaccine refusing, 33.7% were vaccine accepting, while 37.7% were undecided. Increased likelihood of being both vaccine undecided and vaccine refusing was significantly associated with lower scores on Confidence and Convenience scales, and a higher score on the Complacency scale.

Conclusion: Confidence in health authorities and government, confidence in COVID-19 vaccine safety and efficacy, perception of ease of access to vaccination and complacency (perceived lack of need for vaccination) were the most important factors driving the intention to get vaccinated, confirming relevance of the "3C" model.

Keywords: COVID-19, vaccination intention, vaccine hesitancy, World Health Organization.

Cite this article as: Zagorac S, Vasić M, Novaković U, Mladenović M, Tulić I, Teodosić V. Epidemiology of spinal column injuries before, during, and after the covid-19 pandemic – is there any difference?; Medicinska istaživanja 2024; 57(3):65-73 DOI 10.5937/medi57-49293

INTRODUCTION

Less than a year before the COVID-19 pandemic was declared, the World Health Organization (WHO) identified vaccine hesitancy as one of the 10 most important global health threats (1). The first conceptualization of the global phenomenon of vaccine hesitancy was provided by the WHO in 2014, which defined the concept of vaccine hesitancy as a delay in the acceptance or refusal of vaccines despite the availability of vaccination services (2). In fact, the WHO proposed a so-called "3C" model which has three core components of vaccine hesitancy: confidence, complacency and convenience.

Confidence has been defined as trust in the effectiveness and safety of vaccines as well as in the system that provides the vaccines and the policymakers who decide on target populations for vaccination. Complacency is related to the perceived lack of need for vaccinations, where the risks of vaccine-preventable disease are perceived as low, and vaccines are perceived as unnecessary. Convenience accounts for the perceived constraints to access vaccinations such as physical availability, affordability, willingness to pay, geographical accessibility, ability to understand, and quality of the immunization services.

During the COVID-19 pandemic, it was apparent that all three components were prominent as the drivers of vaccine hesitancy. Specifically, confidence in vaccines was largely hampered because of the information overload and delivery of inaccurate information through various communication channels (3, 4). This gave rise to the infodemic, which resulted in low coverage rates. In addition, complacency was in place largely because of the rapid spread of information giving way to conspiracy theories (5). Indeed, convenience seen through access and global allocation of vaccines and vaccine deployment (6) reflected larger structural issues that were country- and/or region-specific (7).

Bearing all mentioned above in mind, the vaccine hesitancy concept is important now more than ever before, because in the post-COVID-19 pandemic period, the propensity toward the decrease in vaccination coverage of vaccines in the childhood immunization programs is high. In fact, in the Republic of Serbia there has been a downward trend in immunization coverage in children over the past two decades (8). Vaccine hesitancy is especially prominent in Serbia, resulting in a major measles outbreak just before the onset of the COVID-19 pandemic (9). This means that systematic efforts need to be made to combat vaccine hesitancy in order to keep the optimum coverage and maintain herd immunity in the population (10).

Applying a "3C lens" to the issue of intention to receive the COVID-19 vaccine could help to calibrate the public health response to vaccination in the post COVID-19 pandemic period and provide necessary clues on how to frame future health policies. The aim of this study was to evaluate 1) the intention to receive COVID-19 vaccine, 2) components of the 3C model in the COVID-19 vaccine hesitancy context, and 3) psycho-social factors associated with COVID-19 vaccination intention.

MATERIAL AND METHODS

Participants and procedure

A cross-sectional online survey was conducted in the period December 2020-January 2021 among adult general population in Serbia. Convenience non-probability sampling was applied. Respondents were reached through the existing social networks and mailing lists. The self-administered questionnaire was disseminated through Google Forms platform, and it required approximately 15 minutes to be completed. The introductory part of the questionnaire included information for respondents including the purpose of the study and their rights as participants. Informed consent of respondents was assumed by their competition of the survey. The questionnaire was anonymous, no personally identifying data were collected. Participation in the study was voluntary, no incentives were provided to respondents. The study protocol was approved by The Ethics Commission of the Faculty of Medicine, the University of Belgrade (approval number: 1322/ XII-7).

Study instrument and measures

The questionnaire consisted of six parts:

1. The primary outcome – *intention to get vaccinated against COVID-19*. was measured by a single item assessing the likelihood of getting vaccinated on a 10-point scale (ranging from 1 – extremely unlikely to 10 – extremely likely). This variable was compressed into three categories: vaccine refusal, vaccine indecisiveness, and vaccine acceptance.
2. *Socio-demographic* questionnaire included: gender, age, region, type of settlement, education level, employment, income, marital status, religiosity, having children and the number of household members.
3. *Health-related characteristics* included three binary (yes/no) items: having had COVID-19 infection, pre-existing health conditions in the respondent, and pre-existing health conditions in household members. In addition, health self-assessment was measured by a single 5-point scale item ranging from 1 "very poor "to 5 "very good".
4. The *source credibility* was assessed by the list of various sources of information concerning the COVID 19 vaccines: domestic scientific literature, international scientific literature, national TV channels, internet portals, You Tube channels, social networks (Facebook, Viber, WhatsApp), and family/friends. The

respondents were asked to indicate the credibility of each source on the 5-point scale (ranging from 1 "does not represent a useful source at all" to 5 "represents an entirely useful source").

5. *General vaccine attitudes* were measured by two five-point items (ranging from 1 "strongly disagree" to 5 "strongly agree"): 1) In general, I believe vaccines are safe, and 2) In general, I believe vaccines are efficacious.
6. *COVID-19 Vaccine Hesitancy Questionnaire (COVID-19 VHQ)* included eight statements. Factor analysis confirmed three-factor structure based on the "3C" vaccine hesitancy model, with the factors being Confidence, Complacency and Convenience. Four items represented the Confidence aspect of vaccine hesitancy (items 1-4), two items represented Complacency (items 5-6), and two items represented Convenience (items 7-8). Responses to each item were graded on a 5-point Likert scale ranging from 1 "strongly disagree" to 5 "strongly agree". This questionnaire was previously developed within the 3C framework and showed excellent psychometric properties (11).

Statistical analysis

Descriptive statistics were used to characterize the sample and study variables. To explore factors associated with the intention to get vaccinated against COVID-19, the binary logistic regressions were conducted. Two regression models were run. The outcome variable in the first model was coded so that those who were refusing the vaccine were compared with those who were vaccine accepting. The outcome variable in the second model was coded so that those who were undecided about the vaccine were compared with those who were vaccine accepting. Dummies were created for multinomial variables (region, type of settlement, education level, income, marital status). Variables found to be significant in the univariate analysis were entered in the multiple analysis for both models. The hierarchical multiple analysis using consecutive blocks was conducted to assess the respective contributions of the five sets of variables: 1) socio-demographics, 2) health-related variables, 3) general vaccine attitudes, 4) sources credibility, and 5) Confidence, Complacency and Convenience.

All analyses were performed in Statistical Package for Social Sciences (SPSS) for Windows, version 25 (IBM Corp., Armonk, NY) and $p < 0.05$ was considered statistically significant.

RESULTS

The total number of respondents who completed the survey was 1,435. Socio-demographic and health-related characteristics of respondents are presented in **Table 1**. The average age was 40.4±11.9 years. Women accounted for 68.4% of the respondents. Only 12.8% of respondents had laboratory confirmed COVID-19, and 15.8% reported the presence of a chronic disease.

Table 1. Socio-demographic and health-related characteristics of respondents

Socio-demographic variables	N (%)
Age (Mean.±SD)	40.4±11.9
Gender	
Male	454 (31.6)
Female	891 (68.4)
Region	
Belgrade	742 (51.7)
Šumadija and Western Serbia	348 (24.3)
Vojvodina	199 (13.9)
South and Eastern Serbia	146 (10.2)
Type of settlement	
Less than 5000 inhabitants	11.8 (8.2)
5000-100.000 inhabitants	538 (37.5)
100.000-million inhabitants	275 (19.2)
Over million inhabitants	504 (35.1)
Education	
Elementary school	7 (0.5)
Secondary school	246 (17.1)
Bachelor's degree	408 (28.4)
Master's degree	679 (47.3)
Doctoral degree	95 (6.6)
Employment	
Yes	1,132(78.9)
No	303 (21.1)
Marital status	
Unmarried	435 (30.3)
Married	845 (58.9)
Divorced	11 (7.7)
Widowed	44 (3.1)
Having a child	
Yes	802 (55.9)
No	633 (44.1)
No of household members	
1	161 (11.2)
2	374 (26.1)
3	348 (24.3)
4	359 (25.0)
5 and more	175 (12.2)
Income per household member	
< 250 €	283 (19.7)
250-400 €	306 (21.3)
400-550 €	328 (22.9)
> 550 €	518 (36.1)
Religiosity	
Yes	781 (54.4)
No	654 (45.6)
Confirmed COVID-19	
Yes	184 (12.8)
No	1,240(86.4)
Health self-assessment	
Very poor	3 (0.2)
Poor	39 (2.7)
Neither poor nor good	165 (11.5)
Good	714 (49.8)
Very good	514 (35.8)

Table 2. General vaccine attitudes and attitudes towards the credibility of sources of information about COVID-19 vaccines

General vaccine attitudes	Strongly disagree (N(%))	Disagree (N(%))	Neither disagree nor agree (N(%))	Agree (N(%))	Strongly agree (N(%))
1. Generally, I believe vaccines are safe	120 (8.4)	92 (6.4)	262 (18.3)	392 (27.4)	565 (39.5)
2. Generally, I believe vaccines are efficacious	85 (5.9)	71 (4.9)	219 (15.3)	383 (26.7)	676 (47.1)
Sources of information about COVID-19 vaccines perceived as credible					
1.Domestic scientific literature	117 (8.2)	181 (12.6)	458 (31.9)	366 (25.5)	313 (21.8)
2. International scientific literature	56 (3.9)	90 (6.3)	303 (21.1)	432 (30.1)	554 (38.6)
3. National TV channels	643 (44.8)	358 (24.9)	322 (22.4)	77 (5.4)	35 (2.4)
4. Internet portals	246 (17.1)	297 (20.7)	535 (37.3)	247 (17.2)	110 (7.7)
5. You Tube	401 (27.9)	362 (25.2)	459 (32.0)	156 (10.9)	57 (4.0)
6. Social networks (Facebook, Viber, WhatsApp)	477 (33.2)	355 (24.7)	391 (27.2)	150 (10.5)	62 (4.3)
7. Family and friends	281 (19.6)	333 (23.2)	536 (37.4)	194 (13.5)	91 (6.3)

General vaccine attitudes are presented in **Table 2**. A smaller proportion of respondents agreed (27.4%) and strongly agreed (39.5%) that vaccines are generally safe, while 26.7% agreed and 47.1% strongly agreed that vaccines are generally efficacious.

Source credibility concerning COVID-19 vaccines is presented in the **Table 2**. Great majority of respondents agreed (30.1%) and strongly agreed (38.6%) that international scientific literature is the most credible source of information on COVID-19 vaccines.

COVID-19 vaccine hesitancy

The average total score on the Confidence sub-scale was 11.42 ± 4.59 out of a maximum of 20, while on the Convenience sub-scale it was 5.60 ± 2.77 out of a maximum of 10. On the Complacency sub-scale, the average total score was 4.02 ± 2.10 out of a maximum of 10 points. The distribution of responses on each item of the questionnaire is presented in Table 3. Nearly half of respondents agreed (20.7%) or strongly agreed (24.0%) that mass immunization would not have been planned if COVID-19 vaccines were not effective and safe. However, more than half of respondents disagreed (19.9%) or strongly disagreed (37.9%) with the statement expressing trust in health authorities and government when it comes to the decision on the choice of the COVID 19 vaccine that will be procured.

Intention to get vaccinated against COVID-19

Less than one third of respondents (28.6%) were extremely unlikely to get vaccinated against COVID-19 and were therefore labeled as vaccine refusing. Furthermore, 33.7% of respondents were vaccine accepting, while 37.7% were undecided.

Predictors of vaccine indecisiveness

All the variables found to be significant in univariate analyses were subjected to the hierarchical binary logistic regression model predicting risk for vaccine indecisiveness relative to vaccine acceptance. The results are presented in **Table 4**.

The final model explained 63.2% (Nagelkerke R^2) of the variance in vaccine indecisiveness outcome. In-

Table 3. COVID-19 Vaccine Hesitancy Questionnaire score distribution.

Items	1	2	3	4	5
1.If the vaccine against COVID 19 was not safe and effective, mass vaccination would certainly not have been planned.	281 (19.6)	185 (12.9)	328 (22.9)	287 (20.7)	344 (24.0)
2. I trust the health authorities and the state (government) when it comes to the decision on the choice of the COVID 19 vaccine that will be procured.	544 (37.9)	285 (19.9)	336 (23.4)	180 (12.5)	90 (6.3)
3. A vaccine against the coronavirus would enable a return to normal life.	210 (14.6)	119 (8.3)	400 (27.9)	398 (27.7)	308 (21.5)
4. The vaccine against COVID 19 should be mandatory for all citizens.	560 (39.0)	142 (9.9)	286 (19.9)	163 (11.4)	284 (19.8)
5. I believe that I am immune to the corona virus, so there is no need to get vaccinated.	837 (58.3)	214 (14.9)	243 (16.9)	69 (4.8)	72 (5.0)
6. Given that a sufficient number of people will receive the COVID 19 vaccine, I do not think it is necessary for me to be vaccinated.	627 (43.7)	272 (19.0)	317 (22.1)	84 (5.9)	135 (9.4)
7. Even if not enough vaccines against COVID 19 are available, I would try to get one.	455 (31.7)	190 (13.2)	313 (21.8)	175 (12.2)	302 (21.0)
8. Even if the state did not provide a sufficient number of free vaccines against COVID 19, I would be willing to pay for the vaccination.	499 (34.8)	157 (10.9)	246 (17.1)	162 (11.3)	371 (25.9)

creased likelihood of being vaccine undecided was significantly associated with lower scores on Confidence and Convenience scales, and higher score on the Complacency scale. Socio-demographic characteristics **(Table 1)** explained 11.7% of variance. Respondents inhabiting Vojvodina region had higher chances to be vaccine accepting, while respondents living in Šumadija and West Serbia region had higher chances to be vaccine undecided. Respondents holding PhD degree had higher chances to be vaccine accepting, while respondents who identified themself as religious and were younger than 30 years had higher chances to be vaccine undecided. Health-related characteristics **(Table 2)** increased the explained variance of the model to 12.2%. General vaccine attitudes **(Table 3)** explained additional 23.1% of variance. When source credibility **(Table 4)** was added, it resulted in the increase of 3.8% in explained variance. Considering the international scientific literature and social networks groups as less credible, and YouTube channels and family/friends as more credible sources of information were significantly associated with higher likelihood of being vaccine undecided. Finally, when Confidence, Complacency and Convenience scores were included **(Table 5)**, it explained an additional 24.1% of variance.

Predictors of vaccine refusal

The variables exhibiting significant associations in univariate analyses were then used in the hierarchical binary logistic regression model predicting risk for vaccine refusal relative to vaccine acceptance, and the results are showed in **Table 5**.

The final model explained 95% (Nagelkerke R^2) of the variance in the vaccine refusal outcome. Similar to vaccine indecisiveness, the only significant predictors of vaccine refusal in the final model were lower scores on Confidence and Convenience and higher score on Complacency.

Socio-demographic characteristics **(Table 1)** explained 26% of variance, with female gender, age younger than 30 years, larger number of household members and being religious presenting significant predictors of vaccine refusal. Respondents who had master and doctoral level of education had higher chances to be vaccine accepting. Health-related characteristics **(Table 2)** explained additional 2.2% of variance. Not having chronic disease and better health self-assessment were significantly associated with increased likelihood of vaccine refusal. When general attitudes towards vaccines were added **(Table 3)**, they explained additional 48% of variance,

Table 4. Hierarchical regression analysis of factors associated with the vaccine indecisiveness vs. acceptance (as the reference)

Variable	Model 1	Model 2	Model 3	Model 4	Model 5
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Age					
18-29 years	Ref.	Ref.	Ref.	Ref.	Ref.
30-41 years	1.76 (1.14-2.72)*	1.76 (1.14-2.72)*	1.87 (1.15-3.05)*	2.00 (1.21-3.32)	1.75 (0.93-3.32)
42-53 years	2.47 (1.52-4.00)**	2.40 (1.48-3.89)**	2.64 (1.53-4.55)**	2.74 (1.56-4.83)	1.97 (0.97-4.00)
54+ years	1.84 (1.06-3.20)*	1.73 (0.99-3.01)	2.05 (1.10-3.82)*	2.37 (1.23-4.56)	1.53 (0.67-3.49)
Region					
Belgrade	Ref.	Ref.	Ref.	Ref.	Ref.
Šumadija and West Serbia	0.70 (0.49-099)*	0.71 (0.50-1.00)	0.84 (0.57-1.26)	0.78 (0.52-1.18)	0.92 (0.55-1.53)
Vojvodina	1.66 (1.10-2.51)*	1.65 (1.09-2.49)*	1.69 (1.06-2.68)*	1.65 (1.02-2.66)*	1.60 (0.89-2.87)
South and East Serbia	1.42 (0.90-2.25)	1.39 (0.88-2.20)	1.43 (0.85-2.40)	1.35 (0.80-2.28)	1.19 (0.63-2.23)
Education					
Elementary/high school	Ref.	Ref.	Ref.	Ref.	Ref.
Bachelor's degree	1.38 (0.90-2.12)	1.38 (0.90-2.12)	1.32 (0.80-2.16)	1.44 (0.86-2.40)	1.15 (0.62-2.16)
Master's degree	1.28 (0.85-1.92)	1.27 (0.85-1.91)	1.12 (0.71-1.78)	1.13 (0.70-1.83)	1.17 (0.65-2.12)
PhD	2.16 (1.17-3.96)*	2.16 (1.17-3.98)*	1.37 (0.70-2.69)	1.22 (0.60-2.47)	1.75 (0.74-4.18)
Marital status					
Married	Ref.	Ref.	Ref.	Ref.	Ref.
Single	0.88 (0.62-1.25)	0.90 (0.63-1.28)	1.02 (0.68-1.52)	0.99 (0.66-1.50)	0.97 (0.59-1.61)
Divorced	0.74 (0.43-1.26)	0.75 (0.44-1.28)	0.73 (0.41-1.33)	0.74 (0.40-1.36)	0.86 (0.40-1.83)
Widowed	0.63 (0.27-1.48)	0.62 (0.26-1.45)	0.57 (0.22-1.51)	0.60 (0.22-1.60)	0.40 (0.12-1.37)
Revenue					
< 250 €	Ref.	Ref.	Ref.	Ref.	Ref.
250-400 €	1.00 (0.64-1.56)	1.00 (0.63-1.56)	1.05 (0.63-1.75)	1.08 (0.64-1.83)	0.85 (0.44-2.12)
400-550 €	1.07 (0.68-1.66)	1.08 (0.69-1.69)	1.15 (0.69-1.91)	1.15 (0.68-1.92)	1.01 (0.53-1.91)
> 550 €	1.19 (0.77-1.83)	1.20 (0.78-1.85)	1.27 (0.77-2.07)	1.29 (0.78-2.13)	0.90 (0.48-1.67)
No of household members	0.91 (0.80-1.03)	0.91 (0.80-1-03)	0.91 (0.79-1.04)	0.90 (0.78-1.03)	0.89 (0.74-1.05)
Religiosity					
No	Ref.	Ref.	Ref.	Ref.	Ref.
Yes	0.47 (0.36-0.62)**	0.47 (0.36-0.62)**	0.76 (0.56-1.03)	0.88 (0.64-1.21)	0.83 (0.55-1.23)

Chronic disease					
No		Ref.	Ref.	Ref.	Ref.
Yes		1.41 (0.99-2.02)	1.56 (1.04-2.33)*	1.45 (0.96-2.19)	1.21 (0.74-1.99)
Vaccine safety			2.78 (2.05-3.76)**	2.59 (1.90-3.51)**	1.37 (0.96-1.97)
Vaccine efficacy			2.01 (1.42-2.86)**	1.76 (1.22-2.52)**	1.21 (0.80-1.84)
National scientific literature				0.92 (0.78-1.10)	0.89 (0.72-1.11)
International scientific literature				1.67 (1.33-2.10)**	1.03 (0.77-1.37)
You Tube channels				0.86 (0.72-1.03)	1.00 (0.80-1.24)
Social networks				1.32 (1.11-1.57)**	1.11 (0.90-1.37)
Friends and family				0.82 (0.69-0.97)*	0.85 (0.70-1.04)
Confidence					1.42 (1.31-1.54)**
Convenience					0.67 (0.59-0.77)**
Complacency					1.45 (1.31-1.62)**
Nagelkerke R^2	0.117	0.122	0.353	0.391	0.632

*p<0.05; **p<0.001

with more negative attitudes towards general vaccine safety and vaccine efficacy being significant predictors of vaccine refusal. Source credibility (Table 4) explained an additional 2.8% of variance. Respondents who considered the international scientific literature and national TV channels as more credible sources of information had significantly lower chance of vaccine refusal. Finally, when confidence, complacency and convenience scores were added (Table 5), an additional 16% of variance was explained.

DISCUSSION

Our study used the WHO-proposed "3C" model to examine COVID-19 vaccine hesitancy and intention to receive the vaccine in Serbia. The results of this study support previous research findings which suggested that vaccine hesitancy would present a significant obstacle in the effort to reach an adequate COVID-19 vaccine coverage in diverse populations (12-15). Overall, more than one third (33.7%) of the surveyed participants intended

Table 5. Hierarchical regression analysis of factors associated with the vaccine rejection vs. acceptance (as the reference)

Variable	Model 1	Model 2	Model 3	Model 4	Model 5
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Gender					
Male	Ref.	Ref.	Ref.	Ref.	Ref.
Female	0.62 (0.44-0.87)**	0. 59 (0.42-0.82) **	0.56 (0.32-0.96)*	0.66 (0.37-1.18)	1.07 (0.34-3.36)
Age					
18-29 years	Ref.	Ref.	Ref.	Ref.	Ref.
30-41 years	1.99 (1.25-3.17) **	1.89 (1.19-3.03) **	2.77 (1.33-5.77)**	3.06 (1.41-6.62)**	2.78 (0.47-16.63)
42-53 years	2.75 (1.65-4.59) **	2.31 (1.37-3.91) **	4.82 (2.05-11.34)**	5.63 (2.26-14.02)**	3.33 (0.46-24.1809
54+ years	2.22 (1.29-3.83) **	1.75 (0.99-3.07)	4.87 (1.93-12.27)**	7.33 (2.68-20.02)**	4.81 (0.65-35.53)
Region					
Belgrade	Ref.	Ref.	Ref.	Ref.	Ref.
Šumadija and West Serbia	0.79 (0.49-1.29)	0.75 (0.46-1.23)	0.66 (0.31-1.41)	0.62 (0.28-1.39)	1.98 (0.34-11.57)
Vojvodina	1.24 (0.73-2.09)	1.19 (0.70-2.02)	1.79 (0.72-4.46)	2.00 (0.76-5.27)	5.05 (0.61-41.89)
South and East Serbia	1.29 (0.72-2.31)	1.18 (0.66-2.12)	1.25 (0.51-3.10)	1.28 (0.50-3.26)	1.49 (0.20-11.13)
Education					
Elementary/high school	Ref.	Ref.	Ref.	Ref.	Ref.
Bachelor's degree	1.20 (0.75-1.92)	1.23 (0.76-1.98)	1.34 (0.59-3.07)	1.33 (0.54-3.27)	1.18 (0.19-7.35)
Master's degree	1.68 (1.07-2.63)*	1.77 (1.12-2.78) *	1.12 (0.52-2,42)	0.91 (0.38-2.17)	0.58 (0.11-3.10)
PhD	2.93 (1.40-6.12)**	3.06 (1.45-6.48) **	1.28 (0.38-4.29)	0.85 (0.23-3.31)	0.42 (0.05-3.87)
Settlement					
< 5000	Ref.	Ref.	Ref.	Ref.	Ref.
5000-100 000	0.71 (0.40-1.26)	0.72 (0.40-1.27)	0.86 (0.35-2.11)	0.91 (0.36-2.30)	0.08 (0.01-0.53)**
100 000-1 000000000	0.77 (0.42-1.44)	0.78 (0.42-1.45)	0.84 (0.32-2.21)	1.02 (0.37-2.81)	0.18 (0.03-1.22)
>1 000000000	0.89 (0.46-1.74)	0.89 (0.45-1.73)	1.01 (0.35-2.90)	1.34 (0.44-4.03)	0.19 (0.02-1.91)
Employment					
No	Ref.	Ref.	Ref.	Ref.	Ref.
Yes	1.23(0.82-1.86)	1.29 (0.85-1.96)	1.60 (0.81-3.14)	1.48 (0.71-3.08)	1.50 (0.29-7.90)
No of household members	0.84 (0.74-0.95)**	0.84 (0.74-0.95)**	0.97 (0.80-1.18)	0.97 (0.79-1.19)	0.96 (0.61-1.50)
Religiosity					
No	Ref.	Ref.	Ref.	Ref.	Ref.

Yes	0.22 (0.16-0.30)**	0.21 (0.15-0.29)**	0.42 (0.25-0.69)**	0.48 (0.28-0.83)**	0.62 (0.20-1.93)
Health self-assessment		0.76 (0.61-0.96) *	0.75 (0.52-1.10)	0.79 (0.53-1.18)	2.10 (0.91-4.83)
Chronic disease					
No		Ref.	Ref.	Ref.	Ref.
Yes		1.79 (1.12-2.84) *	3.03 (1.38-6.67)**	3.07 (1.33-7.07)**	5.84 (0.87-39.38)
Vaccine safety			4.18 (2.86-6.13)**	4.16 (2.72-6.36)**	1.51 (0.62-3.73)
Vaccine efficacy			3.43 (2.26-5.20)**	2.72 (1.70-4.37)**	1.27 (0.47-3.43)
National scientific literature				0.84 (0.62-1.13)	0.50 (0.25-1.00)
International scientific literature				1.98 (1.39-2.84)**	1.81 (0.75-4.40)
National TV channels				1.83 (1.39-2.41)**	1.28 (0.71-2.31)
You Tube channels				0.79 (0.61-1.02)	0.83 (0.45-1.53)
Friends and family				0.80 (0.63-1.03)	0.86 (0.51-1.43)
Confidence					1.92 (1.52-2.43)**
Complacency					0.64 (0.48-0.87)**
Convenience					2.33 (1.72-3.15)**
Negelkerke R^2	0.260	0.282	0.762	0.790	0.950

*p<0.05; **p<0.001

to get vaccinated against COVID-19. Still, a similar proportion claimed to be undecided (37.7%) while more than one quarter were extremely unlikely to accept the vaccine (28.6%). The proportion of refusing and undecided respondents is larger in our sample compared to other studies (16-18). This means that despite the availability of vaccines against SARS-CoV-2, Serbia faced significant reluctance to vaccination in its population. Therefore, getting a deeper insight into the factors that possibly drive the intention to vaccinate could inform the development of a tailored public intervention to increase COVID-19 vaccine acceptance.

First, we explored the extent of COVID-19 vaccine hesitancy in our sample. Given that the COVID-19 vaccination rollout in Serbia is carried out successfully, with a sufficient supply of free vaccines for all the interested citizens (19), we can conclude that the most significant factor to be considered when addressing COVID-19 vaccine hesitancy in Serbia is confidence. The observed result that a minority of respondents has trust in health authorities and the government when it comes to the choice of the vaccine is not surprising in the context of the global structural crisis of trust in institutions (20). Although the majority of respondents still perceive scientific literature as a credible source of information about COVID-19 vaccines, only 7.8% value national TV channels as credible. This result suggests that in order to enhance the sustainability of vaccination promotion programs public health authorities should employ different approach in educating people about vaccines, relying on scientific authorities rather than state officials and political figures.

Second, our results indicate that significant predictors of both vaccine indecisiveness and vaccine refusal were COVID-19 vaccine hesitancy constructs - confidence, complacency and convenience, confirming the significance of the "3C" vaccine hesitancy model in the COVID-19 context. However, general vaccine attitudes explained far larger proportion of variance in vaccine refusal compared to vaccine indecisiveness. This finding is in line with the results of other studies, where negative attitude towards vaccination and distrust in science were the main factors that differed between undecided and unwilling to get vaccinated (13, 21). These findings imply that adamant refusal of COVID-19 vaccines has its roots in a much wider negative attitude toward vaccines in general, and a general lack of trust in science.

Third, sociodemographic variables explained moderately high level of variance in both vaccine indecisiveness and vaccine refusal. The association between socio-demographic factors and COVID-19 vaccine acceptance is highly heterogeneous between countries, which can be attributed to different cultural, socio-environmental and psychological factors (22). Younger age (below 30 years old) was associated with both vaccine indecisiveness and vaccine refusal, which was found in numerous other studies (12, 13, 15, 16, 18). This finding could be expected, given that older people are more susceptible to serious forms of COVID-19 infection, and expectedly have a greater perceived risk of COVID-19 infection. That higher level of education is strongly associated with general vaccine confidence and vaccine uptake is a well-known and empirically confirmed fact (23), and our study results endorse that education is similarly associated with COVID-19 vaccine acceptance. Our finding that religious study participants were less willing to accept the vaccine is in accordance with the research conducted in Illinois, where significant negative association between the religiosity and COVID-19 vaccination intention was found. It can be assumed that the religious respondents are more prone to rely on the external locus of control (24). Our study also revealed that respondents from some regions in Serbia are particularly vaccine undecided, which is potentially valuable information for vaccination campaigns. In addition, female respondents who did

not have any chronic conditions and who assessed their health as better had higher chances to refuse vaccine, which was expected, given that individual characteristics associated with more serious forms of COVID-19 disease are male gender and co-morbidities, and in line with results of other research (16).

Fourth, trust in sources of information explained a small proportion of variance in both outcomes. However, trust in the international scientific literature was negatively associated with both vaccine indecisiveness and vaccine refusal, confirming that trust in science is the cornerstone of vaccine confidence. Trust in YouTube channels was significantly associated with vaccine indecisiveness, which supports the finding that the rapid spread of information of questionable quality (a phenomenon labeled as "infodemic") further undermines trust in science and institutions, providing space for misconceptions and conspiracy theories (25).

This study had several limitations. First, we employed convenience sampling, which does not offer the same level of representativeness provided by probability sampling. Second, due to the COVID-19 physical distancing measures being active at the time of conducting the survey, the survey was administered using online platforms, which potentially limited our sample to respondents who have internet access and use digital technologies. Third, the cross-sectional design of the study does not allow conclusions about causal relationship between variables.

CONCLUSIONS

To conclude, although Serbia has carried out a successful initial phase of COVID-19 vaccination, a large proportion of people had the intention to skip vaccination. Confidence in health authorities and government, confidence in COVID-19 vaccine safety and efficacy, perception of ease of access to vaccination and complacency were the most important factors driving the intention to accept the vaccine. People who had higher chances of being undecided or vaccine refusing were females, in good health, younger than 30 years, less educated, religious and relying on information from YouTube. Our findings can contribute to the development of sustainable vaccination programs and public campaign tailoring by emphasizing information on necessity, safety and effectiveness of offered vaccines, from the credible scientific source, specifically targeting socio-demographic groups most likely to be vaccine hesitant or vaccine refusing.

Funding: This study did not receive any funding.

Conflicts of interest: None to declare.

Author contributions: VJS and SC contributed to study conception and design, data collection, analysis and interpretation of data and drafting the manuscript. VBM, TG and AS contributed to study design, analysis and interpretation of data and critical revision of the manuscript. All authors approved the final version of the manuscript before submission and agree to be held accountable for all aspects of the manuscript.

References

1. World Health Organization. Ten threats to global health in 2019 [Internet]. Available from: https://www.who.int/ news-room/feature-stories/ten-threats-to-global-health-in-2019/;
2. MacDonald NE. Vaccine hesitancy: Definition, scope and determinants. *Vaccine*. 2015; 33:4161-4.
3. World Health Organization. Infodemic. 2021. Available from: https://www.who.int/health-topics/infodemic
4. Chu L, Fung HH, Tse DCK, Tsang VHL, Zhang H, Mai C. Obtaining Information From Different Sources Matters During the COVID-19 Pandemic. Gerontologist. 2021;61(2):187-195
5. Milošević Đorđević J, Mari S, Vdović M, Milošević A. Links between conspiracy beliefs, vaccine knowledge, and trust: Anti-vaccine behavior of Serbian adults. Soc Sci Med. 2021;277:113930.
6. Wouters OJ, Shadlen KC, Salcher-Konrad M, et al. Challenges in ensuring global access to COVID-19 vaccines: production, affordability, allocation, and deployment. *Lancet*. 2021;397:1023-34.
7. Bayati M, Noroozi R, Ghanbari-Jahromi M, Jalali FS. Inequality in the distribution of Covid-19 vaccine: a systematic review. Int J Equity Health. 2022;21(1):122.
8. Veljkovic M, Loncarevic G, Kanazir M, Kisic-Tepavcevic D, Gazibara T. Trend in mandatory immunisation coverage: linear and joinpoint regression approach, Serbia, 2000 to 2017. Euro Surveill. 2021;26(26):2000417.
9. Aritonovic Pribakovic J, Katanic N, Ilic A, Stojanovic Tasic M, Mitic NR, Mirkovic M, et al. The measles epidemic in northern Kosovo and Metohija, Serbia, October 2017-August 2019. J Infect Dev Ctries. 2022;16(5):850-856.
10. Lazić A, Žeželj I. Negativity in online news coverage of vaccination rates in Serbia: a content analysis. Psychol Health. 2022, in press. doi: 10.1080/08870446.2022.2121962
11. Cvjetković S, Jeremić Stojković V, Piperac P, Djurdjević O, Bjegović-Mikanović V. Determinants of COVID-19 vaccine hesitancy: questionnaire development and validation. Central European J Public Health 2022;30(2):99-106.
12. Lazarus JV, Ratzan SC, Palayew A, et al. A global survey of potential acceptance of a COVID-19 vaccine. Nat Med. 2020; 20:1-4.
13. Neumann-Böhme S, Varghese NE, Sabat I, et al. Once we have it, will we use it? A European survey on willingness to be vaccinated against COVID-19. *Eur J Health Econ*. 2020;21:977–82.
14. Rhodes A, Hoq M, Measey MA, Danchin M. Intention to vaccinate against COVID-19 in Australia. *Lancet Infect Dis*. 2020;21(5):e110.
15. Paul E, Steptoe A, Fancourt D. Attitudes towards vaccines and intention to vaccinate against COVID-19: Implications for public health communications. *The Lancet Regional Health-Europe* 2021;1:100016.
16. Ruiz JB, Bell RA. Predictors of intention to vaccinate against COVID-19: Results of a nationwide survey. *Vaccine*. 2021; 39:1080-6.
17. Sherman SM, Smith LE, Sim J, et al. COVID-19 vaccination intention in the UK: results from the COVID-19 vaccination acceptability study (CoVAccS), a nationally representative cross-sectional survey. *Hum Vaccin Immunother*. 2020; 25:1-10.
18. Detoc M, Bruel S, Frappe P, Tardy B, Botelho-Nevers E, Gagneux-Brunon A. Intention to participate in a COVID-19 vaccine clinical trial and to get vaccinated against COVID-19 in France during the pandemic. *Vaccine* 2020;38:7002-6.

19. Higgins A. As Vaccinations Speed Along in Serbia, the Country Basks in the Glow of a Successful Campaign. The New York Times. https://www.nytimes.com/2021/03/17/world/europe/as-vaccinations-speed-along-in-serbia-the-country-basks-in-the-glow-of-a-successful-campaign.html/; 2021 [accessed 08 April 2021].
20. Goldenberg M. Public misunderstanding of science? Reframing the problem of vaccine hesitancy. *Perspect Sci*. 2016;24:552-81.
21. Attwell K, Lake J, Sneddon J, Gerrans P, Blyth C, Lee J. Converting the maybes: Crucial for a successful COVID-19 vaccination strategy. *PloS one*. 2021;16:e0245907.
22. Lazarus JV, Wyka K, Rauh L, et al. Hesitant or Not? The Association of Age, Gender, and Education with Potential Acceptance of a COVID-19 Vaccine: A Country-level Analysis. *J Health Commun*. 2020; 25:799-807.
23. de Figueiredo A, Simas C, Karafillakis E, Paterson P, Larson HJ. Mapping global trends in vaccine confidence and investigating barriers to vaccine uptake: a large-scale retrospective temporal modelling study. *Lancet*. 2020;396: P898-908.
24. Olagoke AA, Olagoke OO, Hughes AM. Intention to vaccinate against the novel 2019 coronavirus disease: The role of health locus of control and religiosity. *J Relig Health*. 2021;60**:**65–80.
25. The Lancet Infectious Diseases. The COVID-19 infodemic. *Lancet Infect Dis*. 2020;20:875.

NEODLUČNOST U VEZI SA VAKCINACIJOM PROTIV COVID-19 U SRBIJI

Vida Jeremic Stojković[1,2], Smiljana Cvjetković[1,2], Vesna Bjegović-Mikanović[1,2], Tatjana Gazibara[1], Adrijan Sarajlija[1]

Sažetak

Uvod/Cilj: Vakcinalna neodlučnost prepoznata je kao značajan faktor smanjenog obuhvata rutinskom imunizacijom u svetu, ali i kao faktor koji je uticao na nedovoljnu prihvaćenost vakcine protiv kovida 19 tokom pandemije. Svetska zdravstvena organizacija predložila je „3C" model koji obuhvata tri ključne komponente vakcinalne neodlučnosti: poverenje (*confidence*), komotnost (*complacency*) i pogodnost (*convenience*). Cilj ovog rada je procena namere da se primi vakcina protiv kovida 19 u okviru „3C" modela u Srbiji.

Materijal i metode: Studija preseka sprovedena je u uzorku od 1.435 odraslih stanovnika Srbije, u periodu od decembra 2020. do januara 2021. godine. Primenjeno je prigodno uzorkovanje, a upitnik je diseminovan onlajn putem društvenih mreža i mejling lista. Glavna ishodna varijabla bila je namera da se primi vakcina, sa tri kategorije: odbijanje vakcinacije, vakcinalna neodlučnost i prihvatanje vakcinacije. Za utvrđivanje povezanosti prediktornih varijabli (socio-demografskih činilaca, kredibilnosti izvora informisanja, opštih stavova prema vakcinaciji i vakcinalne neodlučnosti merene putem tri skale – poverenja, komotnosti i pogodnosti) sa ishodnom varijablom primenjena je logistička regresiona analiza.

Rezultati: Manje od jedne trećine ispitanika (28,6%) je odbilo vakcinaciju protiv kovida 19, 33,7% je nameravalo da se vakciniše, dok je 37,7% bilo neodlučno u vezi sa vakcinacijom. Povećana verovatnoća i neodlučnosti u vezi sa vakcinacijom i odbijanja vakcinacije bila je značajno povezana sa nižim skorovima na skalama poverenja i pogodnosti i višim rezultatom na skali komotnosti.

Zaključak: Poverenje u zdravstvene autoritete i vladu, poverenje u bezbednost i efikasnost vakcine protiv kovida 19, doživljaj adekvatnog pristupa i dostupnosti vakcinacije i komotnost (doživljaj vakcinacije kao nepotrebne) imali su najveći uticaj na nameru ispitanika da se vakacinišu protiv kovida 19, potvrđujući relevantnost „3C" modela.

Ključne reči: kovid 19, namera vakcinacije, neodlučnost u vezi sa vakcinacijom, Svetska zdravstvena organizacija

Primljen: 15.04.2024. I **Revizija:** 23.06.2024. I **Prihvaćen:** 27.07.2024.

Medicinska istaživanja 2024; 57(3):65-73

Medicinska istaživanja 2024; 57(3):75-80 | DOI 10.5937/medi57-50191

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Association of body mass index with disease severity, phenotypes, and clinical presentation in patients with bronchiectasis

Jelena Janković[1,2], Zlatan Bojić[1], Dragan Vukosavljević[1], Andrej Zečević[1]

[1] Clinic for Pulmonology, University Clinical Center of Serbia, Belgrade, Serbia

[2] University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Recived: 01 April 2024

Revised: 30 June 2024

Accepted: 09 July 2024

Check for updates

Funding information:
No funding was received to assist with the preparation of this manuscript.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Jelena Janković

Clinic for Pulmonology, Univerity Clinical Center of Serbia

26 Koste Todorovića Street, 11000 Belgrade, Serbia

E mail: jjelena1984@gmail.com

Summary

Introduction/Aim: Bronchiectasis is a chronic respiratory condition characterized by permanent dilatation of the bronchi with chronic respiratory symptoms. Some studies have found association between malnutrition and bronchiectasis. However, research on obesity remains insufficient and further studies are needed. The aim was to evaluate the association between BMI (body mass index) and phenotypes, endotypes, clinico-radiological presentation and severity.

Methods: A retrospective study included 120 patients with bronchiectasis. The socio-epidemiological, clinical, radiographic and laboratory characteristics were compared using statistical analyzes, depending on BMI.

Results: The mean age was 61.3 ± 7.6 years. Underweight, normal, overweight, and obese accounted for 6.7%, 48.3%, 27.5%, and 17.5% of all patients. There were statistically significant differences in BACI score ($p = 0.01$), normal sputum finding ($p = 0.03$), lower hemoglobin level ($p = 0.02$) in the underweight group and eosinophil endotype in all groups except underweight ($p = 0.04$). The mean BACI (Bronchiectasis Aetiology and Co-morbidity Index) score had a rising trend from overweight and obese patients to normal weight the followed by the underweight category. Chronical colonization of Haemophilus was dominant in the underweight whereas Pseudomonas predominated in the overweight and obese. Asthma was most common in overweight and obese patients. We did not find differences between the groups in spirometry findings (but, the majority of all study patients with restriction belonged to the underweight group), Reiff score and radiological phenotype.

Conclusion: Underweight patients were females and they were younger than overweight patients, they had lower diffusion capacity, systemic inflammation and higher BACI score, post-infective phenotype and predominantly normal sputum bacterial analysis for colonization screening. On the other hand, overweight and obese patients had chronic colonization by P. aeruginosa, asthma comorbidity, and eosinophil endotype. Those differences are very important for future specific treatment.

Keywords: bronchiectasis, BMI, lung function, phenotype, endotype

Cite this article as: Zagorac S, Vasić M, Novaković U, Mladenović M, Tulić I, Teodosić V. Epidemiology of spinal column injuries before, during, and after the covid-19 pandemic – is there any difference?; Medicinska istaživanja 2024; 57(3):75-80 DOI 10.5937/medi57-50191

INTRODUCTION

Bronchiectasis is a chronic respiratory condition characterized by permanent dilatation of the bronchi and bronchioles caused by repeated inflammation and infections (1). This facilitates the collection of purulent secretions in airways, which further deteriorates symptoms and bacterial colonization (2). The most frequent symptoms are coughing, which can persist for a long time, expectoration of colored purulent sputum sometimes with hemoptysis, fever, weight loss, dyspnea and fatigue (3). Bronchiectasis can overlap with other chronic respiratory conditions such as asthma, chronic obstructive pulmonary disease (COPD), tuberculosis, pneumonia, cystic fibrosis and primary ciliary dyskinesia (4,5). For many years, bronchiectasis was an underrecognized disease, largely due to the lack of advanced diagnostic methods like computed tomography (CT) and its non-specific symptoms (5). Its symptoms are hidden under the already mentioned other diagnoses and conditions and they were just an incidental radiographic finding. In the past decade, there has been a significant increase in literature on bronchiectasis.

Some studies have found association between malnutrition and bronchiectasis. Patients with bronchiectasis had a lower body mass index (BMI) compared to the population without bronchiectasis (6). Also, because of mechanisms underlying the pathology of cystic fibrosis (CF) and its implications on nutrient absorption, weight loss is the dominant symptom in patients with CF bronchiectasis, which manifests as underweight (7). A poor nutritional status was related to mortality, skeletal muscle dysfunction with poor activity, decreased pulmonary function and it had a significant impact on patients' quality of life (8,9). On the contrary, a gain in weight can improve pulmonary function, mostly in CF bronchiectasis patients (7). There is lack of studies investigating obesity and bronchiectasis. A Turkish study by Onen and colleges suggested that a higher BMI had benefits in survival in patients with bronchiectasis (10). More studies are needed that examine the association of BMI with the phenotypes and characteristics of bronchiectasis.

The aim of this study was to evaluate the association between BMI and phenotypes, endotypes, clinico-radiological presentation, and severity of bronchiectasis.

MATERIAL AND METHODS

This is a retrospective study conducted at the Clinic for Pulmonology, University Clinical Center of Serbia. This study is in accordance with the Helsinki Declaration, and it has been approved by the Institutional Committee (841/2).

Study group and data collection

The study enrolled 120 patients diagnosed with bronchiectasis. The diagnosis of bronchiectasis was determined by chest CT scan and respiratory symptoms according to the European Respiratory Society guidelines (11). The data included the following: basic demographics data, clinical presentation and symptoms, radiological phenotypes, endotypes according to cell predominance in blood sample, sputum sample for bacteriological examination, BMI, hematological results of blood analysis, bronchiectasis etiology co-morbidity index (BACI), Reiff score, and pulmonary tests.

The modified Reiff scores indicating radiological severity of bronchiectasis were score calculated number of lobe involvement and dilatation degree (12). For quantitative assessment of comorbidities, BACI has been calculated. The BACI calculator is available online at http://www. ronchiectasisseverity.com. The spirometry function was classified according to the American Thoracic Society guidelines (13). Patients were categorized into four groups according to the World Health Organization expert consultation on BMI criteria: underweight (BMI<18.5 kg/m2), normal weight (18.5 ≤BMI<25.0 kg/m2), overweight (25.0 ≤BMI<30.0 kg/m2), and obese (BMI≥30.0 kg/m2) (14).

Endotypes of bronchiectasis were defined according to the types of inflammation (blood sample) and they were divided in three main groups - the first one with type 2 inflammation- eosinophilic endotype, the second one with neutrophilic inflammation and the third one with systemic inflammation.

Statistical analysis

Complete statistical analysis was performed with the SPSS software version 23 (Chicago). Numerical variables are presented as mean values with standard deviation, attribute variable as the frequency for continuous variables and were compared using the ANOVA. Chi-squared test was used to compare categorical variables, and data were presented as numbers with percentage. All analyses were assessed with the $p<0.05$ level of statistical significance. Results are presented as graphics and tables.

RESULTS

A total of 120 patients with bronchiectasis treated at the Clinic for Pulmonology from 2020 to 2022 were included. The mean age was 61.3 ± 7.6 years, and there were not statistically significant differences in age between the groups. Over 78% of the population group were smokers, with a statistically significant higher prevalence among patients with a BMI over 25 kg/m^2 ($p <0.03$).

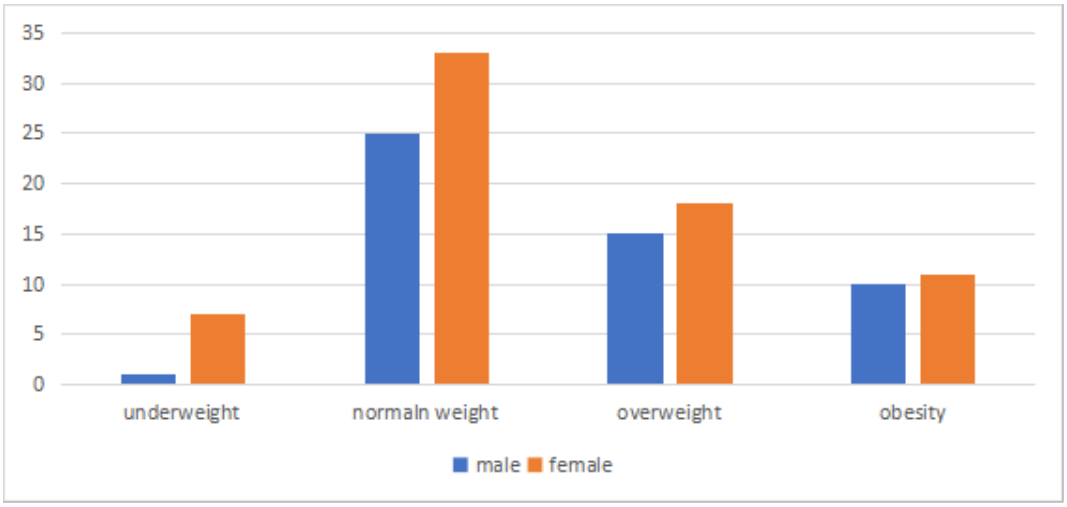


Figure 1. The frequency of patients with bronchiectasis according to gender in four groups

The mean BMI score of patients with bronchiectasis was 24.3 ± 2.9 kg/m2. Underweight, normal, overweight, and obese accounted for 6.7%, 48.3%, 27.5%, and 17.5% of all patients, respectively.

More than half of all patients (57.5%) were female, but there were no statistically significant frequency differences between groups. The frequency of patients according to gender in four groups is presented in **Figure 1**.

Table 1. Most frequent comorbidities in study group

COMORBIDITY	N (%)
Arterial hypertension	22 (18.3)
COPD	13 (10.8)
Asthma	11 (9.2)
Immunodeficiency	10 (8.3)
Hypothyroidism	4 (3.3)
Diabetes mellitus	3 (2.5)

Among all study subjects, the major comorbidities were arterial hypertension (18.6%), COPD (10.2%) asthma (9.4%), immunodeficiency (8.9%), hypothyroidism (3.7%) and diabetes mellitus (2.6%) **(Table 1)**. Regarding comorbidities, the overweight and obese group had the highest proportion of arterial hypertension, COPD and asthma, and the lowest proportion of DM and hypothyroidism compared with the other two BMI groups. A BACI score of over 6 was observed in 17 patients (14.1%).

According to spirometry tests, obstructive finding was the most common dysfunction in 61 (50.8%) patients, followed by normal in 43 (35.8%) and restrictive findings in 16 (13.4%) patients. In 99 (82.5%) patients, cylindrical type was diagnosed, varicose type was found in 8 (6.7%) and cystic type in 13 (10.8%). According to modified Reiff score, mild bronchiectasis was observed in 105 patients (87.5%), moderate was found in 9 patients (7.5%) and severe in 6 patients (5%).

All patients underwent sputum sample collection and analysis. In the study group, 78 patients' sputum specimens tested positive (65%). The most common isolated pathogen was P. aeruginosa, Haemophilus influenzae, Escherichia coli, Staphylococcus haemolyticus, and Aspergillus species.

Differences between groups are presented in **Table 2** and **Table 3**. There were statistically significant differences in BACI score (p = 0.01), normal sputum finding (p = 0.03), anemia and lower hemoglobin level (p = 0.02) in the underweight group and eosinophil endotype in all groups except for the underweight (p = 0.04). The

Table 2. Comparison of data and clinical variables among the four groups

BMI	Underweight	Normal weight	Overweight	Obesity	p
N (%)	8 (6.7)	58 (48.3)	33 (27.5)	21 (17.5)	
Male N (%) Female N (%)	1 (12.5) 7 (87.5)	25 (43.1) 33 (56.9)	15 (45.5) 18 (54.5)	10 (47.7) 11 (52.3)	0.25
Age years	59.5	60.2	65.7	59.8	0.406
BACI ≥6 N (%)	4 (50)	13 (22.4)	3 (9.1)	2 (9.5)	0.044
Sputum N (%) -normal -pathological	 7 (87.5) 1 (12.5)	 12 (20.7) 46 (79.3)	 11 (33.3) 22 (66.7)	 7 (33.3) 14 (66.7)	0.003
Microbiology	Haemophilus	Staphylococcus	Pseudomonas	Pseudomonas	
Clinical phenotype	Post-infectious	Post-infectious immunodeficiency	Asthma	Asthma COPD	
Endotype	systemic inflammation	eosinophilic	eosinophilic	eosinophilic	0.04
Thrombocytes 10^9/ml Hemoglobin 10^9/ml	256 117	224 132	226 134	243 133	0.583 0.002

Table 3. Comparison of data and radiological and functional findings among the four groups

BMI	Underweight	Normal weight	Overweight	Obesity	p
N (%)	8 (6.7)	58 (48.3)	33 (27.5)	21 (17.5)	
Radiological phenotype					
-cystic	1 (12.5)	4 (6.9)	3 (9.1)	2 (9.5)	0.287
-cylindrical	7 (87.5)	52 (89.6)	28 (84.8)	17 (81)	
-varicose	0	2 (3.5)	2 (6.1)	2 (9.5)	
Reiff score N (%)					
-mild	5 (62.5)	52 (89.6)	29 (87.9)	19 (90.6)	0.124
-moderate	3 (37.5)	4 (6.9)	1 (3)	1 (4.7)	
-severe	0	2 (3.5)	3 (9.1)	1 (4.7)	
Spirometry N (%)					
-normal	2 (25)	22 (37.9)	12 (36.4)	7 (33.3)	0.77
-obstruction	4 (50)	29 (50)	17 (51.5)	11 (52.4)	
-restriction	2 (25)	7 (12.1)	4 (12.1)	3 (14.3)	
Decreased diffusion capacity N (%)	5 (62.5)	21 (36.2)	20 (60.6)	15 (71.4)	0.81

mean BACI score had a rising trend from overweight and obese patients to normal weight followed by the underweight category, but the majority were in the group with a low to intermediate risk for mortality. The chronical colonization of Haemophilus was dominant in the underweight and Pseudomonas in overweight and obese group. Underlying causes were identified and the most frequent was post-infection in all groups except for the obese, but those associated with asthma were most common in overweight and obese patients. We did not find statistically significant differences between groups in spirometry findings, Reiff score and radiological phenotype. About half of all four groups had obstructive spirometry finding, as expected. Diffusion capacity was decreased in all groups except in groups with normal BMI, in more than half of patients. **Tables 2 and 3** revealed that underweight individuals were the youngest, predominantly female, and had the highest number of patients with a BACI score over 6, as well as the highest incidence of decreased diffusion capacity and anemia.

DISCUSSION

The purpose of this bronchiectasis population study was to determine whether any subtype (endotype, clinical phenotype, radiological phenotype), pulmonary test findings or severity scores of bronchiectasis were correlated with the patients' BMI. To the best of our knowledge, this is the first study in our country to evaluate factors associated with bronchiectasis.

Numerous studies have highlighted that non-CF bronchiectasis predominantly affects older adults, while CF bronchiectasis is more common in younger individuals. Most patients are over 65 years old, and that predomination has a correlation with comorbidities (15). In several bronchiectasis studies, patients with bronchiectasis were predominantly elderly, with a higher prevalence in females than males (16, 17). Our results are in accordance with these data. The average age was 60 years and females were predominant in all four groups unrelated to BMI. As previously mentioned, the elderly had more comorbidities, with cardiovascular conditions being the most prevalent, consistent with our findings. The association of bronchiectasis with asthma and COPD has been clearly demonstrated in previous studies. There are two main ways in which COPD and bronchiectasis can be associated: COPD can lead to the development of bronchiectasis through persistent airway inflammation and repeated infections; bronchiectasis resulting from other causes in non-smokers can lead to irreversible airway obstruction and subsequent COPD (18, 19). Following arterial hypertension, asthma and COPD presented the most dominant comorbidity in the study population. The higher frequency of COPD in obese individuals may be attributed to associations with metabolic syndrome and the use of corticosteroid therapy (20).

Basically, bronchiectasis is a neutrophilic disorder in about more than 50% of cases according to literature data (21). Contrary to this data, our bronchiectasis participants demonstrated a higher blood eosinophil count and eosinophilic endotype. The reason is the predominance of asthma and COPD underlying disease in overweight and obese patients with this clinical phenotype. Asthma is dominantly eosinophilic disease and COPD has an association with eosinophils in some phenotypes (22). This is important because patients with the eosinophilic endotype benefit from inhaled corticosteroids, while those with the neutrophilic endotype respond better to antibiotics (21,22). Malnutrition is a frequent finding in chronic inflammatory respiratory diseases, so patients have increased mortality risk, exacerbations, chronic colonization of P. aeruginosa and systemic inflammatory amplification (1, 23). This is partly in accordance with our results for underweight patients, systemic inflammation is the dominant endotype and post-infectious is the dominant clinical\etiology phenotype. However, P. aeruginosa was the most frequent microbiology in other groups, not in the underweight. The reason are probably scarce results. Only one had positive sputum bacteriological finding.

BMI has no influence or correlation with structural radiological phenotype. There was no difference between groups in localization and frequency of the three types. The predomination was in the lower lobes and more than 60% of our entire study population had a post-infectious phenotype. That is in accordance with literature data and with etiology, because lower lobe distribution is most often seen in post-infectious bronchiectasis (24). One Chinese study from 2016 showed the absence of correlation between the extent of bronchiectasis and BMI and spirometry tests (25). In contrast, a Korean study found a negative correlation between the extent of bronchiectasis and BMI in moderate to severe disease (26). We did not find this correlation with Reiff 's score for severity.

We hypothesized that being underweight would be associated with worse lung function. Our results confirmed that 75% of them had abnormal spirometry findings. A high percentage of obstruction was in all four BMI groups. The majority of all study patients with restrictions were in the underweight group. The reason for the restriction finding in low BMI group is probably a loss of muscle mass, low physical activity and strength in those patients (27, 28). In a large multicenter study, BMI was found to be positively associated with the forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) in patients who had bronchiectasis and were on mechanical ventilation (28, 29). Additionally, numerous studies on COPD and bronchiectasis patients have examined the relationship between BMI and pulmonary function tests, demonstrating a positive correlation between lower BMI and decreased values in FVC, FEV1, and diffusion capacity (29). The results of our study are in line with those previous studies, except for diffusion capacity where there were no differences between groups. Long-term studies are needed to better identify the effect of a low BMI on the reduction of pulmonary function and diffusion capacity.

The association between BMI and anemia is controversial due to few studies reporting direct association with increasing BMI increases the chance of anemia, while others reported the opposite (30, 31). Inadequate daily iron or dietary nutrient intake is the reason for lower BMI and anemia and this can be the reason for the correlation between underweight and anemia. Lower values of hemoglobin are statistically significant in the underweight group of our study population, which is in line with literature data.

CONCLUSIONS

This study shows that the majority of patients with bronchiectasis in our study population have a normal BMI. Underweight patients were females and younger than overweight patients, they had lower lung function, amplified systemic inflammation, higher BACI score, post-infective phenotype and predominantly normal sputum bacterial analysis for colonization screening. On the other hand, overweight and obese had chronic colonization by P. aeruginosa, asthma comorbidity and eosinophil endotype. Those differences are very important for future decisions about a specific treatment. Improving nutritional status might help improve the disease outlook. Larger sample sizes are needed to measure nutritional status and body composition as an important role of nutrition in bronchiectasis.

Acknowledgements: All authors have contributed equally towards the conception of the manuscript.

Author contributions: Conception and design JJ, data collection DV and ZB, writing the article JJ and ZB, statistical analysis AZ, critical revision of the article AZ and DV.

REFERENCES

1. Qi Q, Li T, Li JC, Li Y. Association of body mass index with disease severity and prognosis in patients with non-cystic fibrosis bronchiectasis. Braz J Med Biol Res. 2015 Aug;48(8):715-24
2. Bird K, Memon J. Bronchiectasis. 2023 May 22. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. PMID: 28613561.
3. Abo-Leyah H, Chalmers JD. Managing and preventing exacerbation of bronchiectasis. Curr Opin Infect Dis. 2020 Apr;33(2):189-196
4. Amati F, Simonetta E, Pilocane T, Gramegna A, Goeminne P, Oriano M. et al. Diagnosis and Initial Investigation of Bronchiectasis. Semin Respir Crit Care Med. 2021 Aug;42(4):513-524
5. Marsland I, Sobala R, De Soyza A, Witham M. Multimorbidity in bronchiectasis: a systematic scoping review. ERJ Open Res. 2023 Jan 16;9(1):00296-2022.
6. Yang B, Jang HJ, Chung SJ, Yoo SJ, Kim T, Kim SH et al. Factors associated with bronchiectasis in Korea: a national database study. Ann Transl Med. 2020 Nov;8(21):1350.
7. Brownell JN, Bashaw H, Stallings VA. Growth and Nutrition in Cystic Fibrosis. Semin Respir Crit Care Med. 2019 Dec;40(6):775-791
8. Lee JM, Lee SA, Han CH, Lee SM, Kim CJ, Lee SC et al. Body mass index as a predictor of mortality in bronchiectasis: A nationwide population-based study. Respir Med. 2021 Apr-May; 180:106370.
9. McDonnell MJ, Aliberti S, Goeminne PC, Restrepo MI, Finch S, Pesci A, et al. Comorbidities and the risk of mortality in patients with bronchiectasis: An international multicentre cohort study. Lancet Respir Med 2016; 4:969-79.
10. Onen ZP, Gulbay BE, Sen E, Yildiz OA, Saryal S, Acican T, et al. Analysis of the factors related to mortality in patients with bronchiectasis. Respir Med 2007; 101: 1390-1397
11. Polverino E, Goeminne PC, McDonnell MJ, Aliberti S, Marshall SE, Loebinger MR et al. European Respiratory Society guidelines for the management of adult bronchiectasis. Eur Respir J. 2017 Sep 9;50(3):1700629.
12. Reiff DB, Wells AU, Carr DH, Cole PJ, Hansell DM. CT findings in bronchiectasis: Limited value in distinguishing between idiopathic and specific types. AJR Am J Roentgenol 1995;165:261-7
13. Celli BR, MacNee W. ATS/ERS Task Force. Standards for the diagnosis and treatment of patients with COPD: A summary of the ATS/ERS position paper. Eur Respir J. 2004; 23:932–46.

14. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004; 363:157–63.
15. Bellelli G, Chalmers JD, Sotgiu G, Dore S, McDonnell MJ, Goeminne PC et al. Characterization of bronchiectasis in the elderly. Respir Med. 2016 Oct; 119:13-19.
16. Aksamit TR, O'Donnell AE, Barker A, et al. Adult Patients With Bronchiectasis: A First Look at the US Bronchiectasis Research Registry. Chest 2017; 151:982-92.
17. Yang B, Jang HJ, Chung SJ, Yoo SJ, Kim T, Kim SH et al. Factors associated with bronchiectasis in Korea: a national database study. Ann Transl Med. 2020 Nov;8(21):1350.
18. Blasi F, Chalmers JD, Aliberti S. COPD and bronchiectasis: phenotype, endotype or co-morbidity? COPD. 2014 Dec;11(6):603-4.
19. Gatheral T, Kumar N, Sansom B, Lai D, Nair A, Vlahos J et al. Copd-related bronchiectasis; independent impact on disease course and outcomes. J COPD 2014; 11:1–10.
20. James BD, Jones AV, Trethewey RE, Evans RA. Obesity and metabolic syndrome in COPD: Is exercise the answer? Chron Respir Dis. 2018 May;15(2):173-181.
21. Fraser CS, José RJ. Insights into Personalised Medicine in Bronchiectasis. J Pers Med. 2023 Jan 10;13(1):133.
22. George L, Brightling CE. Eosinophilic airway inflammation: Role in asthma and chronic obstructive pulmonary disease. Ther Adv Chronic Dis 2016;7:34-51
23. Gupta A, Verma UP, Verma AK, Chaudhary SC, Lal N, Singh N et al. A retrospective correlative profiling of lung functions, microbiological, radiological, periodontal, hematological parameters in noncystic fibrosis bronchiectasis patients of North India. Natl J Maxillofac Surg. 2022 Jan-Apr;13(1):44-53.
24. Giacon V, Sanduzzi Zamparelli S, Sanduzzi Zamparelli A, Bruzzese D, Bocchino M. Correlation between clinical-functional parameters and number of lobes involved in non-cystic fibrosis bronchiectasis. Multidiscip Respir Med. 2021 Dec 3;16(1):791.
25. Jin J, Yu W, Li S, Lu L, Liu X, Sun Y. Factors associated with bronchiectasis in patients with moderate-severe chronic obstructive pulmonary disease. Medicine (Baltimore) 2016;95:e4219.
26. Bak SH, Kim S, Hong Y, Heo J, Lim MN, Kim WJ. Quantitative computed tomography features and clinical manifestations associated with the extent of bronchiectasis in patients with moderate-to-severe COPD. Int J Chron Obstruct Pulmon Dis 2018;13:1421-31
27. Kwan HY, Maddocks M, Nolan CM, et al. The prognostic significance of weight loss in chronic obstructive pulmonary disease-related cachexia: a prospective cohort study. J Cachexia Sarcopenia Muscle. 2019;10(6):1330-1338
28. Despotes KA, Choate R, Addrizzo-Harris D, Aksamit TR, Barker A, Basavaraj A et al. Nutrition and Markers of Disease Severity in Patients With Bronchiectasis. Chronic Obstr Pulm Dis. 2020 Oct;7(4):390-403.
29. Chakrabarti B, Purkait S, Gun P, Moore VC, Choudhuri S, Zaman MJ, et al. Chronic airflow limitation in a rural Indian population: etiology and relationship to body mass index. Int J Chron Obstruct Pulmon Dis 2011; 6: 543-549,
30. Kamruzzaman M. Is BMI associated with anemia and hemoglobin level of women and children in Bangladesh: A study with multiple statistical approaches. PLoS One. 2021 Oct 28;16(10):e0259116
31. Zou Y, Zhang RH, Xia SC, Huang LC, Fang YQ, Meng J et al. The Rural-Urban Difference in BMI and Anemia among Children and Adolescents. Int J Environ Res Public Health. 2016 Oct 18;13(10):1020.

POVEZANOST INDEKSA TELESNE MASE SA TEŽINOM BOLESTI, FENOTIPOVIMA I KLINIČKOM PREZENTACIJOM KOD BOLESNIKA SA BRONHIEKTAZIJAMA

Jelena Janković[1,2], Zlatan Bojić[1], Dragan Vukosavljević[1], Andrej Zečević[1]

Sažetak

Uvod/cilj: Bronhiektazije su hronično respiratorno stanje koje karakteriše trajna dilatacija bronhija se hroničnim respiratornim simptomima. Neke studije su otkrile povezanost između pothranjenosti i bronhiektazija ali je nedovoljno studija na temu gojaznosti. Cilj studije bio je da se proceni povezanost BMI (body mass index) sa fenotipom, endotipom, kliničko-radiološkom prezentacijom i težinom bolesti.

Metode: Retrospektivnom studijom obuhvaćeno je 120 pacijenata sa bronhiektazijama. Socio-epidemiološke, kliničke, radiografske i laboratorijske karakteristike upoređene su sa BMI.

Rezultati: Prosečna starost bila je 61,3 ± 7,6 godina. Pothranjenost, normalna težina, prekomerna težina i gojaznost činili su 6,7%, 48,3%, 27,5% i 17,5% svih pacijenata. Postojale su statistički značajne razlike u BACI (Bronchiectasis Aetiology and Co-morbidity Index) skoru (p = 0,01), normalnom nalazu sputuma (p = 0,03), nižem nivou hemoglobina (p = 0,02) u grupi pothranjenih i endotipu eozinofila u svim grupama osim u grupi pothranjenih (p = 0,04). BACI skor je imao trend rasta od pacijenata sa prekomernom težinom i gojaznih do normalne težine praćene kategorijom pothranjenih. Hronična kolonizacija Haemophilus vrstom bila je dominantna kod pothranjenih, a Pseudomonas je bio zastupljeniji kod onih sa prekomernom težinom i gojaznih. Astma je bila najčešća kod pacijenata sa prekomernom težinom i gojaznih. Nije bilo razlike u nalazu spirometrije (ali većina svih ispitanika sa restrikcijom bila je u grupi pothranjenih), Reiff skoru i radiološkom fenotipu.

Zaključak: Pothranjeni pacijenti bili su ženskog pola i mlađi od pacijenata sa prekomernom težinom (imali su niže vrednosti difuzijskog kapaciteta pluća), sistemsku inflamaciju, viši BACI skor, postinfektivni fenotip i pretežno normalnu analizu sputuma za skrining kolonizacije. Nasuprot tome, pacijenti sa prekomernom težinom i gojazni su imali hroničnu kolonizaciju P. aeruginosa, komorbiditet astme i eozinofilni endotip. Ove razlike su važne za odluku o specifičnom lečenju.

Ključne reči: bronhiektazije, BMI, plućna funkcija, fenotip, endotip

Primljen: 01.04.2024. I **Revizija:** 30.06.2024. I **Prihvaćen:** 09.07.2024.

Medicinska istaživanja 2024; 57(3):75-80

Medicinska istaživanja 2024; 57(3):81-89 | DOI 10.5937/medi57-50986

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Patterns of neuropsychiatric symptoms in primary and secondary tauopathies: caregiver and patient perspectives

Milica Ječmenica Lukić[1], Gorana Mandić[1], Tanja Stojković[1], Aleksandra Tomić[1], Vladana Marković[1], Iva Stanković[1], Nikola Kresojević[1], Igor Petrović[1], Aleksandra Kačar[1], Nataša Dragašević[1], Vladimir Kostić[1], Marina Svetel[1]

[1] Clinic of Neurology, University Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Serbia

Recived: 15 May 2024

Revised:13 July 2024

Accepted: 25 July 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Marina Svetel

Clinic of Neurology, University Clinical Center of Serbia,

6 dr Subotića Street, 11000 Belgrade, Serbia

E-mail: marinasvetel@gmail.com

Summary

Introduction/Aims: Understanding the differences in neuropsychiatric symptoms (NPSs) across tauopathies, particularly in the early stages of the disease, may aid in differential diagnosis. The aims of the research are as follows**:** a) to examine the patterns of NPSs in primary (frontotemporal dementia – FTD and progressive supranuclear palsy – PSP) and secondary (Alzheimer's disease – AD) tauopathies; b) to examine the differences in NPSs reported by patients and caregivers.

Methods: The study included 312 patients, 176 of whom had a disease duration of ≤3 years. The presence of NPSs based on caregiver's report was assessed by neuropsychiatric questionnaire (NPI). Patient's assessment of NPSs was examined by Hamilton's Depression and Anxiety Scales and the Apathy Scale.

Results: In AD, the most common and severe neuropsychiatric symptoms are mood disorders and apathy. In contrast, agitation-related symptoms are also prominent in FTD and PSP. The profile of NPSs in FTD and PSP is similar, but irritability and aberrant motor behavior are more pronounced in FTD, while sleep disturbances are dominant in PSP. The prevalence of NPSs reported by caregivers on NPI was higher than that reported by patients.

Conclusions: FTD and PSP are characterized by more frequent and more severe NPSs and have distinct psychiatric patterns compared to AD, even in the early disease course. Caregiver's observations of the patient's behavior could be of key importance in distinguishing these tauopathies, particularly in the absence of hard motor and cognitive symptoms in early disease course. Assessments of depression, anxiety, and apathy by patients themselves and their caregivers differ significantly, and data from these two sources cannot be considered interchangeable and comparable.

Keywords: tauopathies, Alzheimer's disease, frontotemporal dementia, progressive supranuclear palsy, neuropsychiatric symptoms

Cite this article as: Ječmenica Lukić M, Mandić G, Stojković T, Tomić A, Marković V, Stanković I, Kresojević N, Petrović I, Kačar A, Dragašević N, Kostić V, Svetel M. Patterns of neuropsychiatric symptoms in primary and secondary tauopathies: caregiver and patient perspectives; Medicinska istaživanja 2024; 57(3):81-89 DOI 10.5937/medi57-50986

INTRODUCTION

Tauopathies are neurodegenerative diseases defined histopathologically by the presence of intracellular inclusions, composed of aggregates of pathologically altered tau protein (1). The most common diseases from the group of primary tauopathies include frontotemporal dementia (FTD), progressive supranuclear palsy (PSP), and corticobasal syndrome. On the other hand, Alzheimer's disease (AD) is generally the most common tauopathy, although this disease is now classified as a secondary tauopathy, where in addition to a significant accumulation of pathological tau protein there is also an accumulation of beta-amyloid (2). Despite clearly defined clinical criteria, behind which there are unique pathohistological substrates, these diseases are often difficult to distinguish from each other during life. This especially applies to the early phases of the disease, when a significant overlap of cognitive, motor, and neuropsychiatric symptoms (NPSs) is observed, which diverge toward separate clinical presentations only in the later stages when the clinical diagnosis is easier to establish. There is increasing evidence in the literature that NPSs can even precede the first symptoms and signs of the disease, but these manifestations rarely capture the attention of doctors to the extent that cognitive and motor symptoms do (3–5). Furthermore, NPSs are increasingly integrated into research diagnostic criteria for neurodegenerative disorders (6) and are recognized as an important determinant of impaired quality of life (7, 8), which makes their evaluation even more important. A better understanding of the differences in NPS profiles manifested in the early stages of these diseases could potentially contribute to an easier differential diagnosis.

Equally important is the fact that current assessments of NPSs rely on methodologically diverse scales. Some are based on caregiver evaluations, while others rely on patient self-assessments, making it difficult to accurately understand the true severity and frequency of these symptoms.

Bearing in mind the above-mentioned data, we set the main aims of our study: 1) examination of NPSs patterns in patients with AD, FTD, and PSP, with an emphasis on the early stages of the disease, and 2) examination of differences in the profile of neuropsychiatric symptoms based on caregivers' and patients' reports.

METHODS

Our study included 312 patients with the most common forms of primary and secondary tauopathies: AD (160 patients), FTD (93 patients), and PSP (59 patients), who met the valid criteria for the diagnosis of these diseases (9-11). Patients with a history of neurological, psychiatric, or systemic diseases, as well as those with previous or current substance abuse, were excluded from the study.

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade. The informed consent was signed by patients and/or caregivers.

Detailed demographic and clinical data were collected through a structured questionnaire, based on an interview with the patient and/or caregiver. Age at disease onset was defined as the age at which cognitive and/or motor symptoms attributable to these diseases first appeared. Disease duration of up to 3 years is classified as the early phase, while duration exceeding 3 years is considered the late phase. The Mini-Mental Test (MMSE) (12), the Frontal Assessment Battery (FAB) (13), and the Mattis Dementia Rating Scale (DRS) (14) were used to examine patients' cognitive status.

The presence of NPSs was assessed by the neuropsychiatric inventory (NPI), which is based on an interview with the patient's caregivers (15). It includes the frequency (from 0 to 4) and severity (from 0 to 3) of 12 neuropsychiatric domains: delusions, hallucinations, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behavior, sleep disorder, appetite/eating habits. A score of 0-12 was obtained for each domain by multiplying the frequency with the severity of complaints. Composite scores for each domain were used further in the analysis. Bearing in mind that low composite scores may be trivial in the clinical context, we also analyzed patients who had a composite score ≥4 in the respective NPI domains. The total NPI score is the sum of all domain scores, ranging from 0 to 144, with higher values indicating more severe neuropsychiatric symptoms. In addition to the NPI, symptoms of depression, anxiety, and apathy were also examined with additional scales based on the patient's assessment of symptoms: the Hamilton Depression Rating Scale (HAMD) (16), the Hamilton Anxiety Rating Scale (HAMA) (17), and the Apathy Scale (AS) (18). To define the presence/absence of depression, anxiety, and apathy, previously defined *cut-off* values for these scales were used (HAMD≥8, HAMA≥13, AS≥14) (19, 20, 21).

Statistical analysis

The following software package was used for all statistical analyses: SPSS, version 23.0; SPSS Inc.; Chicago, USA.

To identify differences in the mean values of the respective variables between the three groups of patients, the ANOVA test was used (corrected for multiple testing using the Games-Hovell post-hoc test). The chi-square test and Fischer's exact probability test were used to compare categorical variables, where appropriate. Statistical significance was defined as the value of $p < 0.05$.

RESULTS

Demographic and clinical characteristics of investigated cohorts

The study included 312 patients (176 with disease duration ≤ 3 years (early phase) and 136 with disease duration >3 years (late phase)) diagnosed with AD, FTD, and PSP, whose demographic and clinical characteristics, as well as the basic differences in cognitive profiles, are shown in **Table 1**.

Patients with PSP had a later disease onset and were older at the time of evaluation. In terms of global cognitive status, measured by the DRS scale, as well as in terms of frontal cognitive dysfunction, measured by the FAB scale, patients with AD showed the lowest achievements consistently during the disease course, while PSP and FTD did not differ from each other, both in early and late phases of the disease **(Table 1)**.

The neuropsychiatric profile in early and late phases across different tauopathies

At the time of evaluation, all PSP patients had at least one neuropsychiatric symptom present (NPI>0) even in the early phase of the disease, followed by 85% of FTD and 77% of AD patients. A similar percentage of NPSs was reported by caregivers in late phases in all patient groups **(Table 2)**.

In the early stage of the disease in the group of AD patients, the highest NPI composite scores, reflecting the severity of NPSs, were recorded in the domains of apathy, depression, and anxiety **(Table 2, Figure 1)**. In primary tauopathies, both PSP and FTD, the highest scores were also recorded in the domain of apathy, even significantly more pronounced than in AD. However, the symptoms that showed a statistically significant difference in severity between Alzheimer's disease (AD) and frontotemporal dementia (FTD) or progressive supranuclear palsy (PSP) mainly fall within the agitation subsyndrome (irritability, agitation, disinhibition, euphoria, and aberrant motor behavior), as well as changes in eating habits and sleep patterns. The neuropsychiatric symptom profile in the early stages of FTD is largely similar to that of progressive supranuclear palsy PSP. However, patients with FTD exhibit more pronounced irritability and aberrant motor behavior compared to those with PSP, while sleep disturbances are more characteristic of PSP **(Table 2, Figure 1)**.

The differences in the severity of apathy, disinhibition, irritability, and sleep domains were maintained even in **the late stages** of the disease between primary and secondary tauopathies. Differences in symptoms from the domain of agitation, euphoria, aberrant motor behavior, and changes in eating habits that were observed in the early stages are lost in the late disease course. No differences were observed in the neuropsychiatric profiles between PSP and FTD in the late stages of the disease **(Table 2, Figure 1)**.

Some of these NPSs, in addition to being characterized as the most severe, were also found to be **the most frequent**, such as apathy and depression (Table 2, Figure 1). However, the symptoms found to be mostly characteristics of primary tauopathies (FTD/PSP) (those belonging to agitation subsyndrome), occurred in less than 50% of patients **(Table 2, Figure 1)**.

Table 1. Demographic and clinical characteristics of early and late phases of primary and secondary tauopathies

	Early phase (≤3 years) N=176				**Late phase (>3 years) N=136**			
Characteristics	**AD** N=95	**FTD** N=54	**PSP** N=27	**p-value**	**AD** N=65	**FTD** N=39	**PSP** N=32	**p-value**
female: male ratio	56:39	24:30	8:19	**AD vs. PSP***	42:23	19:20	15:17	ns*
Age (years)	58.81±5.66 (38-67)	58.78±5.93	65.77±7.08	**AD vs. PSP FTD vs. PSP**	61.80±4.01	61.87±6.51	67.97±6.60	**AD vs. PSP FTD vs. PSP**
Education (years)	11.90±2.50 (4-18)	12.74±2.83	12.37±2.51	ns	11.23±3.156	11.69±3.28	11.44±3.78	ns
Disease duration (years)	2.29±0.61 (1-3)	2.14±0.68	2.32±0.66	ns	4.92±1.02	5.36±2.08	5.22±1.42	ns
Age at onset (years)	56.52±5.52 (37-65)	56.64±5.89	63.44±7.17	**AD vs. FTD AD vs. PSP PSP vs. FTD**	56.98±3.93	56.56±7.69	62.73±6.52	**AD vs. PSP PSP vs. FTD**
MMSE	17.61±5.42 (5-28)	21.87±6.45	25.07±4.44	**AD vs. FTD AD vs. PSP**	12.56±5.59	18.03±8.19	23.90±3.42	**AD vs. PSP AD vs. FTD FTD vs. PSP**
FAB total	7.59±4.07 (2-15)	9.84±5.05	10.04±3.81	ns	4.96±3.57	6.52±5.87	9.19±3.66	**AD vs. PSP**
DRS total	95.50±25.88 (22-134)	106.34±27.99	112.52±15.53	**AD vs. PSP**	73.94±33.84	92.23±35.24	103.63±23.01	**AD. vs PSP**

The figures in the table present mean values ± standard deviations with a range in the brackets. The groups of patients with a statistically significant difference were indicated in bold (ANOVA test with post hoc Games Howell, except for * where chi-square test was applied; $p<0.05$); ns: non-significant.
AD: Alzheimer's disease; FTD: frontotemporal dementia; PSP: progressive supranuclear palsy; MMSE: Mini-Mental State Examination test; FAB: Frontal Assessment Battery; DRS: Dementia Rating Scale (Mattis).

Table 2. Differences in severity and frequency of neuropsychiatric symptoms in primary and secondary tauopathies, both in early and late phases

	Early phase (≤3 years) N=176				**Late phase (>3 years) N=136**			
	AD N=95	**FTD** N=54	**PSP** N=27	**p-value**	**AD** N=65	**FTD** N=39	**PSP** N=32	**p-value**
NPI-Delusions								
Mean value ±SD	0.24±1.16	0.37±1.74	0.93±2.56	ns	0.58±1.64	0.56±1.65	0.50±1.74	ns
Patients with symptoms present (No (%))	6 (6)	4 (7)	5 (19)	ns	10 (15)	5 (13)	4 (13)	ns
Patients with score ≥4 (No (%))	4 (4)	2 (4)	4 (15)	ns	6 (9)	3 (8)	2 (6)	ns
NPI-Hallucinations								
Mean value ±SD	0.36±1.64	0.13±0.67	0.41±1.12	ns	0.42±1.51	0.36±1.94	0.09±0.30	ns
Patients with symptoms present (No (%))	8 (8)	2 (4)	4 (15)	ns	6 (9)	2 (5)	3 (9)	ns
Patients with score ≥4 (No (%))	4 (4)	1 (2)	2 (7)	ns	4 (6)	1 (3)	0 (0)	ns
NPI-Agitation								
Mean value ±SD	0.33±1.53	2.17±3.48	1.48±3.04	**AD vs. FTD**	1.22±2.49	1.46±3.36	2.34±2.97	ns
Patients with symptoms present (No (%))	6 (6)	18 (33)	9 (33)	**AD vs. FTD AD vs. PSP**	15 (23)	7 (18)	21 (66)	**AD vs. FTD AD vs. PSP**
Patients with score ≥4 (No (%))	5 (5)	17 (32)	5 (19)	**AD vs. FTD**	13 (20)	7 (18)	9 (28)	ns
NPI-Depression								
Mean value ±SD	3.19±3.44	3.02±3.91	2.22±2.68	ns	2.85±3.35	2.85±3.93	3.22±2.90	ns
Patients with symptoms present (No (%))	52 (55)	25 (46)	18 (67)	ns	32 (49)	15 (39)	27 (84)	**AD vs. FTD AD vs. PSP**
Patients with score ≥4 (No (%))	47 (50)	23 (43)	8 (30)	ns	31 (48)	15 (39)	15 (47)	ns
NPI-Anxiety								
Mean value ±SD	1.27±2.49	1.58±2.58	1.56±2.28	ns	1.15±2.51	1.49±2.41	1.97±2.60	ns
Patients with symptoms present (No (%))	25 (26)	18 (33)	12 (44)	ns	14 (22)	14 (36)	23 (72)	**AD vs. PSP FTD vs. PSP**
Patients with score ≥4 (No (%))	21 (22)	14 (26)	7 (26)	ns	13 (20)	10 (26)	6 (19)	ns
NPI-Euphoria								
Mean value ±SD	0.24±1.54	1.42±3.47	1.44±2.79	ns	0.00±0.00	1.36±3.41	0.50±1.02	ns
Patients with symptoms present (No (%))	3 (3)	9 (17)	8 (30)	**AD vs. FTD AD vs. PSP**	0 (0)	7 (18)	8 (25)	**AD vs. FTD AD vs. PSP**
Patients with score ≥4 (No (%))	2 (2)	8 (15)	5 (19)	**AD vs. FTD AD vs. PSP**	0 (0)	6 (15)	1 (3)	**AD vs. FTD**
NPI-Apathy								
Mean value ±SD	3.31±4.06	6.80±4.35	6.41±4.25	**AD vs. FTD AD vs. PSP**	3.12±3.53	7.54±4.30	7.53±4.27	**AD vs. FTD AD vs. PSP**
Patients with symptoms present (No (%))	46 (48)	42 (78)	24 (89)	**AD vs. FTD AD vs. PSP**	38 (59)	32 (82)	30 (94)	**AD vs. FTD AD vs. PSP**
Patients with score ≥4 (No (%))	41 (43)	41 (76)	21 (78)	**AD vs. FTD AD vs. PSP**	30 (46)	32 (82)	25 (78)	**AD vs. FTD AD vs. PSP**
NPI-Disinhibition								
Mean value ±SD	0.21±1.32	3.35±4.02	2.48±3.97	**AD vs. FTD AD vs. PSP**	0.12±0.70	2.26±4.16	3.31±3.35	**AD vs. FTD AD vs. PSP**
Patients with symptoms present (No (%))	3 (3)	26 (48)	15 (56)	**AD vs. FTD AD vs. PSP**	2 (3)	10 (26)	23 (72)	**AD vs. FTD AD vs. PSP FTD vs. PSP**
Patients with score ≥4 (No (%))	2 (2)	24 (44)	7 (26)	**AD vs. FTD AD vs. PSP**	2 (3)	9 (23)	14 (44)	**AD vs. FTD AD vs. PSP**
NPI-Irritability								
Mean value ±SD	0.59±2.10	3.24±4.10	1.59±3.21	**AD vs. FTD**	0.85±2.22	1.69±3.18	2.25±2.87	ns
Patients with symptoms present (No (%))	11 (12)	26 (48)	10 (37)	**AD vs. FTD AD vs. PSP**	13 (20)	12 (31)	21 (66)	**AD vs. FTD AD vs. PSP**
Patients with score ≥4 (No (%))	7 (7)	23 (43)	4 (15)	**AD vs. FTD FTD vs. PSP**	7 (11)	9 (23)	10 (31)	**AD vs. PSP**
NPI-Aberrant motor behavior								
Mean value ±SD	0.56±2.13	2.81±4.36	1.30±2.79	**AD vs. FTD**	1.06±2.62	3.13±3.90	1.75±2.53	ns
Patients with symptoms present (No (%))	8 (8)	19 (35)	12 (44)	**AD vs. FTD AD vs. PSP**	12 (19)	18 (46)	18 (56)	**AD vs. FTD AD vs. PSP**
Patients with score ≥4 (No (%))	7 (7)	18 (33)	2 (7)	**AD vs. FTD FTD vs. PSP**	10 (15)	17 (44)	8 (25)	**AD vs. FTD**
NPI-Sleep								
Mean value ±SD	1.04±2.63	1.31±3.24	2.07±2.80	ns	0.62±1.40	1.56±3.56	3.22±3.62	**AD vs. PSP**

Patients with symptoms present (No (%))	17 (18)	11 (20)	13 (48)	**AD vs. PSP FTD vs. PSP**	13 (20)	9 (23)	20 (62)	**AD vs. PSP FTD vs. PSP**
Patients with score ≥4 (No (%))	14 (15)	8 (15)	8 (30)	ns	7 (11)	6 (15)	14 (44)	**AD vs. PSP FTD vs. PSP**
NPI-Appetite/eating habits								
Mean value ±SD	0.75±2.39	2.76±3.86	1.48±2.78	**AD vs. FTD**	0.34±1.34	2.44±4.18	1.25±2.11	**AD vs. FTD**
Patients with symptoms present (No (%))	10 (11)	23 (43)	11 (41)	**AD vs. FTD AD vs. PSP**	6 (9)	11 (28)	13 (40)	**AD vs. FTD AD vs. PSP**
Patients with score ≥4 (No (%))	10 (11)	22 (41)	5 (19)	**AD vs. FTD**	3 (5)	10 (26)	5 (16)	**AD vs. FTD**
NPI total								
Mean value ±SD	11.56±12.77	28.81±21.70	24.46±29.33	**AD vs. FTD AD vs. PSP**	12.14±10.94	26.05±20.21	27.94±17.60	**AD vs. FTD AD vs. PSP**
Patients with symptoms present (No (%))	73 (77)	48 (85)	27 (100)	**AD vs. PSP**	56 (86)	33 (85)	32 (100)	ns
HAMA								
Mean value ±SD	3.70±3.15	4.66±3.90	5.78±4.36	ns	4.16±3.45	3.00±3.13	9.16±5.19	**AD vs. PSP FTD vs. PSP**
Patients with score ≥13 (No (%))	1 (1)	2 (6)	2 (7)	ns	40 (62)	18 (46)	6 (19)	**AD vs. PSP FTD vs. PSP**
HAMD								
Mean value ±SD	2.67±2.61	4.66±4.36	10.41±6.33	**AD vs. PSP FTD vs. PSP**	4.00±3.97	3.24±3.65	13.03±6.55	**AD vs. PSP FTD vs. PSP**
Patients with score ≥8 (No (%))	5 (9)	6 (19)	19 (70)	**AD vs. FTD FTD vs. PSP**	6 (24)	4 (19)	27 (84)	**AD vs. PSP FTD vs. PSP**
AS								
Mean value ±SD	9.96±6.47	16.44±10.22	21.07±8.29	**AD vs. FTD AD vs. PSP**	11.33±8.00	11.33±7.78	22.38±8.72	**AD vs. PSP FTD vs. PSP**
Patients with score ≥14 (No (%))	18 (32)	18 (56)	23 (85)	**AD vs. FTD FTD vs. PSP**	11 (46)	7 (35)	28 (88)	**AD vs. PSP FTD vs. PSP**

The scores on different neuropsychiatric scales are presented as mean values ± standard deviations. The distribution of patients with symptoms and those with clinically significant scores (≥4) is presented as absolute numbers, with percentages shown in the brackets. The groups of patients with a statistically significant difference were indicated in bold (ANOVA test with post hoc Games Howell and chi-square test were applied as appropriate; $p<0.05$); ns: non-significant.
AD: Alzheimer's disease; FTD: frontotemporal dementia; PSP: progressive supranuclear palsy; NPI: neuropsychiatric inventory; HAMD: Hamilton's depression scale; HAMA: Hamilton's anxiety scale; AS: apathy scale.

Differences in the prevalence of neuropsychiatric symptoms based on the caregiver's and patient's reports

The data on differences in the prevalence of depression, anxiety, and apathy obtained by caregivers and patient reports are presented in Table 3. Disconcordance between the caregiver's and patient's observations was present in all 3 mood disturbances, but it was most obvious in reporting apathy symptoms.

DISCUSSION

The study results show that NPSs occupy a significant place in the clinical presentation of various forms of tauopathies, even in the early disease course. Primary tauopathies, such as FTD and PSP, exhibit a higher frequency and intensity of neuropsychiatric symptoms even in the early stages of the disease, along with a distinct profile of neuropsychiatric characteristics. This is in contrast to AD, which represents secondary tauopathies. The most prominent and frequent symptoms of AD belong to mood disorders (depression/anxiety), together with apathy. In addition to these symptoms, NPSs from the agitation subsyndrome on NPI play an important role in FTD and PSP, especially in the early stages of the disease, making a distinction toward AD easier. The profile of NPSs within primary tauopathies is very similar, but more pronounced irritability and aberrant motor behavior is a dominant feature of FTD, while sleep disturbances are noted to be mainly characteristic of PSP. Estimates of the prevalence of depression and anxiety, and especially apathy, obtained from caregivers and the patients themselves differ significantly, and the reports from these two sources cannot be considered interchangeable and comparable.

The general patterns of NPSs in the examined tauopathies obtained using the NPI in this study are comparable to the results of previous research. The most common neuropsychiatric symptoms in AD were apathy, followed by depression and anxiety (22–25), which aligns with the symptom profile observed in our patients. In line with previous reports, our data indicate a specific pattern of neuropsychiatric disorders of primary tauopathies, in contrast to AD (24, 26, 27). Namely, we showed that apathy, together with symptoms from the subdomain of agitation, as well as appetite/eating and sleep disorders, constitute a specific behavioral construct in FTD and

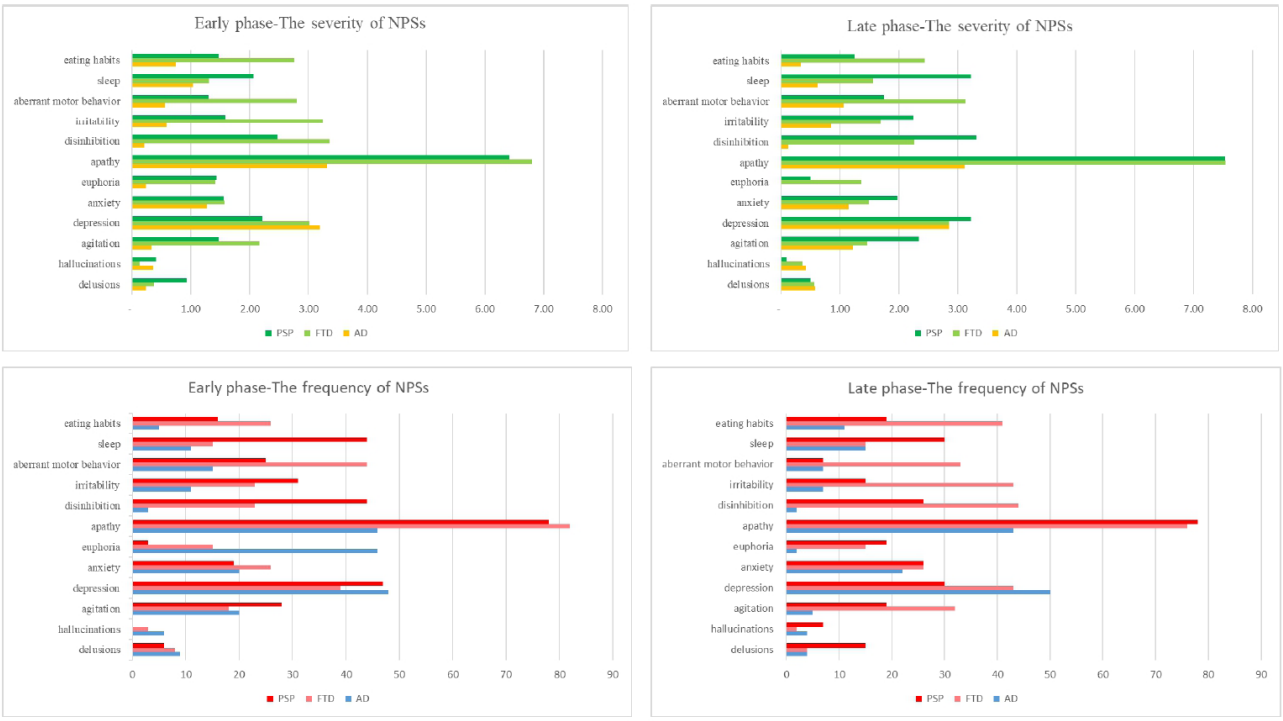


Figure 1. Neuropsychiatric inventory composite scores reflect the severity of symptoms (upper graphs) and the percentage of patients with clinically significant scores ≥4 for each symptom (lower graphs) in early and late phases across different tauopathies.
PSP: progressive supranuclear palsy; FTD: frontotemporal dementia; AD: Alzheimer's disease; NPSs-neuropsychiatric symptoms

Table 3. The differences in the prevalence of depression, anxiety, and apathy between caregivers' and patients' reports in patients with AD, FTD, and PS

		Depression-HAMD			Anxiety-HAMA			Apathy-AS		
		Significant ≥8	**Trivial** <8	**p**	**Significant** ≥13	**Trivial**<13	**p**	**Significant** ≥14	**Trivial** <14	**p**
AD	**Significant NPI**	9	27	**0.009**	0	1	ns	**19**	**15**	**0.002**
	Trivial NPI	2	43		18	63		**10**	**35**	
FTD	**Significant NPI**	6	19	ns	2	0	ns	**24**	**15**	**0.001**
	Trivial NPI	4	24		11	40		**1**	**12**	
PSP	**Significant NPI**	23	0	**0.001**	6	7	**<0.001**	**45**	**1**	**0.001**
	Trivial NPI	23	13		2	44		**6**	**7**	

The figures in the table present absolute numbers of patients. P-value, Fischer exact test ($p<0.05$); ns= non-significant.
AD: Alzheimer's disease; FTD: frontotemporal dementia; PSP: progressive supranuclear palsy; NPI: neuropsychiatric inventory (significant-if composite score for certain domain is ≥4); HAMD: Hamilton's depression scale; HAMA: Hamilton's anxiety scale; AS: apathy scale.

PSP. The different NPS profiles between primary and secondary tauopathies are most noticeable in the early stages of the disease, while with the disease progression, differences are still present but to a lesser extent. This implies that paying attention to the spectrum of NPSs in the early phases of tauopathies is particularly important for the differential diagnosis when early distinction in the absence of hard motor and cognitive signs is not possible.

Although very frequent in all tauopathies, mood changes (depression, anxiety) did not prove to be significant in distinguishing these diseases, as it had been shown in previous research (28-31). Conversely, when apathy occurs with greater intensity and frequency, it is more indicative of FTD and PSP than (AD). Apathy is generally considered to be a consequence of lesions in multiple brain regions, but several imaging studies in degenerative dementias have indicated a significant association of apathy with atrophy and hypometabolism of the medial frontal cortex (predominantly the anterior cingulum) (32, 33). Interestingly, the atrophy of this brain region is also associated with disinhibition and aberrant motor behavior (32), which in our study also stood out as significant symptoms in distinguishing AD from primary tauopathies.

As already mentioned, the profile of NPSs in FTD and PSP in our cohorts turned out to be very similar, both in terms of the total NPI score and in terms of the severity/distribution of the most prevalent symptoms, similar to the study by Yataba et al. (34). However, we need to be cautious in the interpretation of such results, bearing in mind that our FTD cohort is amorphous, without making a distinction between behavioral and language variants of FTD. A recent study comparing different variants of FTD found that delusions, along with symptoms from the agitation subdomain and euphoria, were less prominent in PSP compared to the behavioral variant of FTD (35). In our study, the presence of irritability in early phases was a characteristic mainly of FTD, which is in line with recently published data where irritability was the most common NPS in prodromal FTD (36). On the

other hand, sleep disturbances were the most valuable in differentiating PSP not just from AD, but also from FTD. A very recent study has also shown that the presence of sleep disturbance at the initial visit predicted significantly greater odds of PSP pathologic diagnosis compared to non-PSP tauopathies (37).

Finally, positive psychiatric symptoms, such as hallucinations and delusions, rarely occurred in our patients with tauopathies, a result that corresponds to most previous findings (30, 31, 38). These symptoms are more often part of the clinical spectrum of synucleinopathies and arise as a consequence of the complex interplay of the neurodegenerative process itself and the application of dopaminergic therapy (38).

The prevalence of anxiety, depression, and apathy reported on the NPI by caregivers was higher than reported by patients themselves. This contrasts with the observations in other neurodegenerative diseases, such as Parkinson's disease and multiple system atrophy, where patients report more symptoms than their caregivers can register (39, 40). In the domains of anxiety and depression, the greatest discrepancy was observed in PSP patients, while the discrepancy in apathy reports was consistent in all patients. The key to explaining this inconsistency may lie in the pronounced symptoms of apathy, which include indifference and a lack of insight into the symptoms.

Several limitations of our study should be emphasized. First, our research is primarily based on the NPI questionnaire, which is solely based on caregivers' assessment, and it must be taken into account that the NPS may be underestimated or overestimated to a certain extent. Since the NPI refers to a period no longer than one month before examination, the problem of errors in recalling symptoms cannot be ignored. Second, depression, apathy, and anxiety were also assessed using additional questionnaires but were not diagnosed according to clinically validated criteria, which requires caution when interpreting the data. Third, FTD and PSP include a whole spectrum of clinically heterogeneous diseases, which were not taken into consideration during this research.

CONCLUSION

Neuropsychiatric symptoms are a prominent feature in the clinical presentation of both primary and secondary tauopathies, even in the early stages of the disease. FTD and PSP are characterized by distinct psychiatric constructs in contrast to AD, with more severe and more frequent NPSs. Involving family caregivers through structured interviews on changes in patients' behavioral aspects using the NPI could be crucial for differentiating tauopathies in the early stages of the disease, when clinical symptoms are emerging but motor and cognitive signs are not yet apparent. Estimates of the prevalence of depression and anxiety, and especially apathy, obtained from caregivers and the patients themselves differ significantly, and the reports from these two sources cannot be considered interchangeable and comparable.

Author Contributions:

A. the conception or design of the work;
B. the acquisition, analysis, or interpretation of data;
C. preparing the draft of the manuscript
D. interpretation of the revised version of the manuscript.

MJL, GM, MS: A, B, C, D
TS, AT, NK, VM, IS, AK: B, C, D
IP, ND, VK: A, D

REFERENCES:

1. Devi G. The tauopathies. Handb Clin Neurol [Internet]. 2023 Jan 1 [cited 2024 May 12];196:251–65. Available from: https://pubmed.ncbi.nlm.nih.gov/37620072/
2. Trejo-Lopez JA, Yachnis AT, Prokop S. Neuropathology of Alzheimer's Disease. Neurotherapeutics [Internet]. 2022 Jan 1 [cited 2024 May 12];19(1):173–85. Available from: https://pubmed.ncbi.nlm.nih.gov/34729690/
3. Ferreira DA, Macedo LBC, Foss MP. Neuropsychiatric symptoms as a prodromal factor in Alzheimer's type neurodegenerative disease: a scoping review. Clin Neuropsychol [Internet]. 2023 [cited 2024 May 12]; Available from: https://pubmed.ncbi.nlm.nih.gov/37881945/
4. Russell LL, Rohrer JD. Defining the presymptomatic phase of frontotemporal dementia. Curr Opin Neurol [Internet]. 2023 Aug 1 [cited 2024 May 12];36(4):276–82. Available from: https://pubmed.ncbi.nlm.nih.gov/37340685/
5. Painous C, Martí MJ, Simonet C, Garrido A, Valldeoriola F, Muñoz E, et al. Prediagnostic motor and non-motor symptoms in progressive supranuclear palsy: The step-back PSP study. Parkinsonism Relat Disord [Internet]. 2020 May 1 [cited 2024 May 12];74:67–73. Available from: https://pubmed.ncbi.nlm.nih.gov/32536421/
6. Cummings J. The Role of Neuropsychiatric Symptoms in Research Diagnostic Criteria for Neurodegenerative Diseases. Am J Geriatr Psychiatry [Internet]. 2021 Apr 1 [cited 2024 May 12];29(4):375. Available from: /pmc/articles/PMC7855689/
7. Dixit D, Spreadbury J, Orlando R, Hayward E, Kipps C. Quality of Life Assessments in Individuals With Young-Onset Dementia and Their Caregivers. J Geriatr Psychiatry Neurol. 2021 Sep;34(5):426-433. doi: 10.1177/0891988720933348.
8. Musa Salech G, Lillo P, van der Hiele K, Méndez-Orellana C, Ibáñez A, Slachevsky A. Apathy, Executive Function, and Emotion Recognition Are the Main Drivers of Functional Impairment in Behavioral Variant of Frontotemporal Dementia. Front Neurol. 2022 Jan 13;12:734251.
9. Chare L, Hodges JR, Leyton CE, McGinley C, Tan RH, Kril JJ, et al. New criteria for frontotemporal dementia syndromes: clinical and pathological diagnostic implications. J Neurol Neurosurg Psychiatry [Internet]. 2014 Aug 1 [cited 2024 May 12];85(8):865–70. Available from: https://jnnp.bmj.com/content/85/8/865
10. Höglinger GU, Respondek G, Stamelou M, Kurz C, Josephs KA, Lang AE, et al. Clinical diagnosis of progressive supranuclear palsy: The movement disorder society criteria. Mov Disord. 2017;32(6):853–64.

11. Dubois B, Feldman HH, Jacova C, DeKosky ST, Barberger-Gateau P, Cummings J, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurology. 2007.
12. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189–98.
13. Dubois B, Slachevsky a, Litvan I, Pillon B. The FAB: a Frontal Assessment Battery at bedside. Neurology. 2000;55(11):1621–6.
14. Mattis S. Dementia Rating Scale. Resour Inc Odessa, FL Psychol Assess. 1988;
15. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: Comprehensive assessment of psychopathology in dementia. Neurology. 1994;44:2308–14.
16. Hamilton MC. Hamilton Depression Rating Scale (HAM-D). Redloc. 1960;23:56–62.
17. Hamilton M. Hamilton Anxiety Rating Scale (HAM-A). J Med. 1959;61(4):81–2.
18. Starkstein SE, Mayberg HS, Preziosi TJ, Andrezejewski P, Leiguarda R, Robinson RG. Reliability, validity, and clinical correlates of apathy in Parkinson's disease. J Neuropsychiatry Clin Neurosci [Internet]. 1992;4(2):134–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1627973
19. Zimmerman M, Martinez JH, Young D, Chelminski I, Dalrymple K. Severity classification on the Hamilton depression rating scale. J Affect Disord. 2013.
20. Leentjens AFG, Dujardin K, Marsh L, Martinez-Martin P, Richard IH, Starkstein SE, et al. Apathy and anhedonia rating scales in Parkinson's disease: Critique and recommendations. Mov Disord. 2008;23(14):2004–14.
21. Leentjens AFG, Dujardin K, Marsh L, Richard IH, Starkstein SE, Martinez-Martin P. Anxiety rating scales in Parkinson's disease: A validation study of the Hamilton anxiety rating scale, the Beck anxiety inventory, and the hospital anxiety and depression scale. Mov Disord. 2011.
22. Zhu CW, Schneider LS, Elder GA, Soleimani L, Grossman HT, Aloysi A, et al. Neuropsychiatric Symptom Profile in Alzheimer's Disease and Their Relationship With Functional Decline. Am J Geriatr Psychiatry. 2024 Jun 29:S1064-7481(24)00375-0. doi: 10.1016/j.jagp.2024.06.005.
23. Leung DKY, Chan WC, Spector A, Wong GHY. Prevalence of depression, anxiety, and apathy symptoms across dementia stages: A systematic review and meta-analysis. Int J Geriatr Psychiatry. 2021 Sep 1;36(9):1330–44.
24. Collins JD, Henley SMD, Suárez-González A. A systematic review of the prevalence of depression, anxiety, and apathy in frontotemporal dementia, atypical and young-onset Alzheimer's disease, and inherited dementia. Int psychogeriatrics [Internet]. 2023 Sep 20 [cited 2024 May 12];35(9):457–76. Available from: https://pubmed.ncbi.nlm.nih.gov/32684177/
25. Eikelboom WS, van den Berg E, Singleton EH, Baart SJ, Coesmans M, Leeuwis AE, et al. Neuropsychiatric and Cognitive Symptoms Across the Alzheimer Disease Clinical Spectrum. Neurology [Internet]. 2021 [cited 2024 May 12];97(13). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8480405/
26. Mollah SA, Nayak A, Barhai S, Maity U. A comprehensive review on frontotemporal dementia: its impact on language, speech and behavior. Dement Neuropsychol [Internet]. 2024 [cited 2024 May 12];18. Available from: https://pubmed.ncbi.nlm.nih.gov/38659629/
27. Schwertner E, Pereira JB, Xu H, Secnik J, Winblad B, Eriksdotter M, et al. Behavioral and Psychological Symptoms of Dementia in Different Dementia Disorders: A Large-Scale Study of 10,000 Individuals. J Alzheimers Dis. 2022;87(3):1307-1318. doi:0.3233/JAD-215198. PMID: 35491774; PMCID: PMC9198804.
28. Cerami C, Perdixi E, Meli C, Marcone A, Zamboni M, Iannaccone S, et al. Early Identification of Different Behavioral Phenotypes in the Behavioral Variant of Frontotemporal Dementia with the Aid of the Mini-Frontal Behavioral Inventory (mini-FBI). J Alzheimers Dis. 2022;89(1):299-308. doi: 10.3233/JAD-220173. PMID: 35871334.
29. Collins JD, Henley SMD, Suárez-González A. A systematic review of the prevalence of depression, anxiety, and apathy in frontotemporal dementia, atypical and young-onset Alzheimer's disease, and inherited dementia. Int Psychogeriatr. 2023 Sep;35(9):457-476. doi: 10.1017/S1041610220001118. Epub 2020 Jul 20. PMID: 32684177.
30. Jiskoot LC, Russell LL, Greaves CV, van Schaik E, van den Berg E, Poos JM, et al. Addition of the FTD Module to the Neuropsychiatric Inventory improves classification of frontotemporal dementia spectrum disorders. J Neurol. 2023 May;270(5):2674-2687. doi: 10.1007/s00415-023-11596-3.
31. Younes K, Miller BL. Neuropsychiatric Aspects of Frontotemporal Dementia. Psychiatr Clin North Am [Internet]. 2020 Jun 1 [cited 2024 May 12];43(2):345–60. Available from: https://pubmed.ncbi.nlm.nih.gov/32439026/
32. Rosen HJ, Allison SC, Schauer GF, Gorno-Tempini ML, Weiner MW, Miller BL. Neuroanatomical correlates of behavioural disorders in dementia. Brain. 2005;
33. Sarazin M, Michon A, Pillon B, Samson Y, Canuto A, Gold G, et al. Metabolic correlates of behavioral and affective disturbances in frontal lobe pathologies. J Neurol. 2003;
34. Yatabe Y, Hashimoto M, Kaneda K, Honda K, Ogawa Y, Yuuki S, et al. Neuropsychiatric symptoms of progressive supranuclear palsy in a dementia clinic. Psychogeriatrics. 2011;11(1):54–9.
35. Yiannopoulou KG, Papatriantafyllou JD, Ghika A, Tsinia N, Lykou E, Hatziantoniou E, et al. Defining Neuropsychiatric Inventory scale differences across frontotemporal dementia syndromes. Psychogeriatrics. 2019;
36. Altomare D, Rivolta J, Libri I, Mattioli I, Cantoni V, Padovani A, et al. Neuropsychiatric Symptoms in Frontotemporal Dementia: More Than Just Noise? J Alzheimers Dis [Internet]. 2024 Mar 5 [cited 2024 May 12];98(1):133–44. Available from: https://pubmed.ncbi.nlm.nih.gov/38363612/
37. Keszycki R, Kawles A, Minogue G, Zouridakis A, Macomber A, Gill N, et al. Distinct and shared neuropsychiatric phenotypes in FTLD-tauopathies. Front Aging Neurosci [Internet]. 2023 [cited 2024 May 11];15. Available from: https://pubmed.ncbi.nlm.nih.gov/37358954/
38. Morrow CB, Pontone GM. Exploring Psychosis in Neurodegenerative Dementia: Connecting Symptoms to Neurobiology. J Alzheimers Dis [Internet]. 2024 [cited 2024 May 12];99(1):101–3. Available from: https://pubmed.ncbi.nlm.nih.gov/38669552/
39. Jecmenica-Lukic M, Petrovic IN, Pekmezovic T, Tomic A, Stankovic I, Svetel M, et al. The Profile and Evolution of Neuropsychiatric Symptoms in Multiple System Atrophy: Self- and Caregiver Report. J Neuropsychiatry Clin Neurosci. 2021 Spring;33(2):124-131. doi: 10.1176/appi.neuropsych.20030057.
40. McKinlay A, Grace RC, Dalrymple-Alford JC, Anderson TJ, Fink J, Roger D. Neuropsychiatric problems in Parkinson's disease: comparisons between self and caregiver report. Aging Ment Health. 2008 Sep;12(5):647-53. doi: 10.1080/13607860802343225. PMID: 18855181.

OBRASCI NEUROPSIHIJATRIJSKIH SIMPTOMA U PRIMARNIM I SEKUNDARNIM TAUOPATIJAMA IZ UGLA NEGOVATELJA I PACIJENATA

Milica Ječmenica Lukić[1], Gorana Mandić[1], Tanja Stojković[1], Aleksandra Tomić[1], Vladana Marković[1], Iva Stanković[1], Nikola Kresojević[1], Igor Petrović[1], Aleksandra Kačar[1], Nataša Dragašević[1], Vladimir Kostić[1], Marina Svetel[1]

Sažetak

Uvod/ciljevi: Poznavanje razlika u profilima neuropsihijatrijskih simptoma (NPS) u primarnim i sekundarnim tauopatijama, naročito u ranim fazama bolesti, može biti od značaja u njihovoj diferencijalnoj dijagnozi. Ciljevi rada: a) ispitivanje obrazaca NPS kod primarnih (frontotemporalna demencija – FTD i progresivna supranuklearna paraliza – PSP) i sekundarnih tauopatija (Alchajmerova bolest – AD); b) ispitivanja razlika u NPS prijavljenih od strane samih pacijenata i njihovih negovatelja.

Metode: Ispitivanje je obuhvatilo 312 bolesnika, od kojih 176 sa trajanjem bolesti ≤3 godine. Prisustvo NPS bazirano na proceni negovatelja procenjeno je neuropsihijatrijskim upitnikom (NPI). Procena NPS od strane pacijenata ispitivana je Hamiltonovim skalama depresije i anksioznosti i skalom apatije.

Rezultati: Najizraženiji i najčešći NPS u AD su poremećaji raspoloženja/apatija, dok se u FTD i PSP beleži i značajna učestalost simptoma iz podsindroma agitacije. Profil NPS u FTD i PSP je sličan, ali su iritabilnost i poremećaj motornog ponašanja izraženiji u FTD, dok je poremećaj spavanja dominantna karakteristika PSP. Prevalencija anksioznosti, depresije i apatije prijavljena na NPI upitniku od strane negovatelja, bila je veća od one prijavljene od strane samih pacijenata.

Zaključak: FTD i PSP karakterišu različiti psihijatrijski obrasci u odnosu na AD, sa težim i češćim NPS. Procena negovatelja o promenama u ponašanju pacijenata, mogla bi biti od ključnog značaja za diferencijalnu dijagnozu ovih tauopatija u ranom toku bolesti, posebno u odsustvu jasnih motornih i kognitivnih simptoma. S druge strane, procene simptoma depresije i anksioznosti, a posebno apatije, od strane samih pacijenata i njihovih negovatelja se značajno razlikuju, te se podaci iz ova dva izvora ne mogu smatrati zamenljivim i uporedivim.

Ključne reči: tauopatije, Alchajmerova bolest, frontotemporalna demencija, progresivna supranuklearna paraliza, neuropsihijatrijski simptomi

Primljen: 15.05.2024. I **Revizija:** 13.07.2024. I **Prihvaćen:** 25.07.2024.

Medicinska istaživanja 2024; 57(3):81-89

Medicinska istaživanja 2024; 57(3):91-98 | DOI 10.5937/medi57-51924

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Changes in the hemostatic system in severely ill COVID-19 patients

Marija Milenković[1,2], Mirjana Kovač[3], Ivan Rović[1], Đuro Šijan[1], Bogdan Crnokrak[2,4], Marija Zdravković[2,4]

[1]Emergency centre, Center for Anesthesiology and Resuscitation, University Clinical Centre of Serbia, Belgrade, Serbia
[2]University of Belgrade, Faculty of medicine, Belgrade, Serbia
[3]Blood Transfusion Institute of Serbia, Belgrade, Serbia
[4]University Hospital Medical Center "Bežanijska kosa", Belgrade, Serbia

Recived: 01 July 2024
Revised:18 July 2024
Accepted: 25 July 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Marija Milenković
Emergency centre, Center for Anesthesiology and Resuscitation, University Clinical Centre of Serbia,
2 Pasterova Street, 11000 Belgrade, Serbia
E-mail: smgk055@gmail.com

Summary

Introduction/research objective: COVID-19 coagulopathy is a disorder of the hemostatic system that occurs in critically ill patients infected with the SARS-CoV 2 virus and it increases the risk of mortality. The goal of the research is to evaluate changes in hemostatic parameters and determine their prognostic significance in patients with a severe form of the COVID-19 disease.

Methods: The study was designed as a retrospective cohort study, which included 146 patients treated from June to September 2020 in the Intensive Care Unit (ICU) of the Clinical Hospital Center "Bežanijska Kosa" in Belgrade, diagnosed with COVID-19 pneumonia. Inclusion criteria were as follows: the age over 18 years, proven current SARS-CoV2 infection, and admission to ICU.

Results: 82 patients (56.2%) died during the treatment, while 64 (43.8%) were discharged. Significantly higher D-dimer values on admission to the ICU were recorded in subjects who died during treatment 888 (1226.5) ng/ml compared to persons who were discharged from treatment 666 (1207.3) ng/ml ($p = 0.03$). Differences were not demonstrated for INR, aPTT and fibrinogen. D-dimer values on admission to the ICU greater than or equal to 760ng/ml are a statistically significant predictor of death during hospitalization ($p = 0.04$).

Conclusion: COVID coagulopathy is a complication that increases the mortality of people infected with the SARS-CoV2 virus. The main feature is a state of hypercoagulability, which is detected by elevated D-dimer values. D-dimer greater than or equal to 760 ng/ml on admission to the ICU may have prognostic significance for survival during hospitalization.

Keywords: COVID-19, coagulopathy, D-dimer, Intensive Care Unit

Cite this article as: Milenković M,Kovač M, Rović I, Šijan Đ, Crnokrak B, Zdravković M. Changes in the hemostatic system in severely ill COVID-19 patients; Medicinska istaživanja 2024; 57(3):91-98 DOI 10.5937/medi57-51924

INTRODUCTION

In May 2023, the COVID-19 pandemic, which lasted approximately three years and two months, was officially declared over (1). According to the latest data, more than 703 million people have fallen ill worldwide, and about 7 million have died, according to https://www.worldometers.info/coronavirus/ 02/18/2024. The pandemic was controlled due to the development of vaccines and antiviral drugs (2).

Most of those infected with the SARS-CoV2 virus developed an asymptomatic or mild form of the COVID-19 disease, while a smaller number of people developed viral pneumonia, which could later be followed by complications and other multisystem disorders, and finally lead to death, especially in the elderly (3). The severe form of the disease is characterized by an immense immune response with the release of many cytokines, with antiviral and anti-inflammatory role, while leading to the death of endothelial and epithelial cells and immunothrombosis (4).

COVID-19 coagulopathy is a disorder of the hemostatic system that occurs in critically ill patients infected with the SARS-CoV-2 virus. The state of hypercoagulability is the main feature of COVID-19 coagulopathy, which can be manifested by thromboembolic complications or even disseminated intravascular coagulation. Since the beginning of the pandemic, a higher frequency of thromboembolic complications, both venous and arterial, has been reported in more severe forms of the disease, despite the use of anticoagulant therapy (5). A meta-analysis that included 47 studies and a total of 6459 patients led to the conclusion that deep vein thrombosis was present in about 30% of cases, that it was more frequent in patients treated in the ICU, and that people with COVID-19 and thrombosis had higher mortality than people without thrombosis (6). Hyperinflammatory response, hypoxia, endothelial damage, as well as comorbidities, immobility, age and history of previous thrombosis are key risk factors for hypercoagulability and subsequent complications (5).

D-dimer is mentioned as the most important indicator in the context of COVID-19 coagulopathy. Elevated D-dimer values were recorded in COVID-19 patients with a more severe form of the disease. Also, it was shown that higher D-dimer values correlated with disease severity and mortality. Certain studies (7-9) confirmed that D-dimer values greater than 1 mg/ml, 0.5 mg/ml, or 0.5 mg/l upon admission were associated with an 18-fold, 7.3-fold, or 4-fold increase in mortality risk, respectively. It has also been proven that elevated D-dimer values are associated with a more severe clinical presentation (10). The results of our recently published prospective study showed that elevated levels of D-dimer, as well as coagulation factor VIII (FVIII), in a certain category of patients (elderly, with a more severe presentation of COVID infection) can be maintained even after 6 months of COVID infection. This indicates that the degree of hypercoagulability is maintained in a certain number of patients even after the infection has passed (11). In addition to high D-dimer values, indicators of COVID coagulopathy can be low platelet values, prolonged prothrombin and partial thromboplastin time, as well as fibrinogen, FVIII, von Willebrand factor (vWF), and reduced antithrombin (AT) values as well as ADAMTS13 (7, 11-13).

This research aims to evaluate changes in hemostatic parameters and to determine their prognostic significance in patients with a severe form of COVID-19 disease.

Material and methods

This study was designed as a retrospective cohort study, which included 146 patients treated from June 2020 to September 2020 in the Intensive Care Unit (ICU) of the Clinical Hospital Center "Bežanijska Kosa" in Belgrade, diagnosed with COVID-19 pneumonia. The inclusion criteria were as follows: the age over 18, proven current SARS-CoV2 infection, and admission to the Intensive Care Unit. Respondents younger than 18 years of age and respondents whose stay in the intensive care unit was not due to a severe form of COVID-19, as well as incomplete data on respondents, were excluded from the research.

Upon admission to the ICU, the usual anamnestic data (hetero-anamnestic data related to the patient's comorbidities and accompanying therapy) and demographic data were collected and entered into the electronic database. All patients, according to the protocol for treatment at the ICU, had blood samples taken for the determination of routine laboratory analyses, such as complete blood count, coagulation profile, biochemical analyses, and markers of inflammation. All these values were recorded in the hospital's electronic database, along with all important patient clinical data.

A computed tomography (CT) score was determined for each patient. The score was graded according to the degree of involvement of the lung tissue by consolidation seen on the CT scan of the chest. Each lung lobe was scored from 1 to 5 depending on the prevalence of consolidation (1. < 5% involvement; 2. 5-25%; 3. 26-49%; 4. 50-75%; 5. > 75%). The values of the total CT score were obtained by adding up the individual score for each lobe, and the score values could range from 0 to 25. The severity of the clinical presentation, as determined by the CT score, was categorized into mild (score <8), moderate (9-15), and severe (>15) (14).

The treatment of patients and the definition of a severe clinical picture were carried out according to the national protocol of the Republic of Serbia for the treatment of COVID-19 (15).

In the statistical analysis, descriptive and analytical statistics methods were used, depending on the type of data distribution. If the distribution was normal, the T-test was used for analysis. For distributions deviating from normality, the Mann-Whitney U test was employed

for non-parametric analysis. The non-parametric tests were the Chi-square ($\chi 2$) test or Fisher's exact probability test. The ROC curve was used to determine the optimal D-dimer cut-off value and its sensitivity (Sn) and specificity (Sp). The Kaplan-Meier method and the log-rank test were used to assess survival. $p < 0.05$ was considered statistically significant. The computer program "R" was used to process statistical data.

Results

The research included 146 respondents, 103 (70.5%) male and 43 (29.5%) female. The average age was 65 years, range from 29 to 89 years. Of the total number of respondents, more than a half died during the treatment, 82 (56.2%), while 64 (43.8%) were discharged after the treatment. Of the 82 patients with a fatal outcome, 58 (70.7%) were male, and 24 (29.3%) were female. The incidence of fatal outcomes matches the representation in relation to sex.

Comorbidities were present in 109 (74.7%) subjects. Arterial hypertension was recorded in 99 (67.8%) subjects, diabetes mellitus in 50 (34.2%), coronary disease in 25 patients (17.1%), while other comorbidities were less common (obesity and cardiomyopathy in 8.2%, COPD in 4.8%, and asthma in 2.7% of cases).

In relation to the severity of the clinical picture, the majority of respondents had a CT score of 20, i.e., 14.2%. There were 10.4% of respondents with the maximum score. Upon admission to the ICU, most patients were intubated, with 52.7% receiving invasive mechanical ventilation and 34.2% receiving non-invasive ventilation. The lowest percentage of people was on high-flow oxygen therapy, 3.4%, while 9.6% of patients were on low-flow oxygen therapy through an oxygen mask **(Table 1)**.

The values of the subjects' coagulation parameters were as follows: D-dimer 768 (1066) ng/ml, INR 1.1 (0.3), APTT 24.7 (8.3) and fibrinogen 3.8 (1.5) g/ l. The measured values of D-dimer, INR, APTT and fibrinogen did not differ significantly between men and women (D-dimer p=0.51; INR p=0.409; aPTT p=0.826; fibrinogen p=0.212).

Patients with hypertension had higher D-dimer values, 888 (2715) ng/ml, compared to 554 (705.5) ng/ml in normotensive p=0.002. Differences in D-dimer values were not observed between subjects with and without corresponding comorbidities **(Table 2)**.

INR values recorded upon admission to the ICU were higher in patients with cardiomyopathy 1.3 (1.5) than those without 1.1 (0.2)s p= 0.028. INR values did not differ between patients with and without other comorbidities **(Table 3)**.

Table 1. Clinical characteristics of subjects suffering from COVID-19 admitted to the Intensive Care Unit

Clinical features	
Age	*med (IQR)* 65 (21) years
Sex	*n* % 103/43 70.5/29.5
Comorbidity	*n (%)*
Arterial hypertension	99 (67.8)
Coronary disease	25 (17.1)
Cardiomyopathy	12 (8.2)
Diabetes mellitus	50 (34.2)
Obesity	12 (8.2)
Asthma	4 (2.7)
COPD	7 (4.8)
The severity of the clinical presentation according to the CT score	*n (%)*
Mild	
Moderate	7 (4.8)
Severe	25 (17.1) 114 (78.1)
Type of respiratory support	*n (%)*
Low Flow Oxygen	14 (9.6)
High Flow Oxygen	5 (3.4)
Non-invasive mechanical ventilation	50 (34.2)
Invasive mechanical ventilation	77 (52.7)
Number of days of hospitalization	*med (IQR)* 18 (16)
Treatment outcome	*n (%)*
Discharge	64 Male 45 (70.3) Female 19 (29.7)
Fatal outcome	82 Male 58(70.7); Female 24(29.3)

Table 2. D-dimer values of patients with COVID-19 according to comorbidities (values are shown as median (IQR))

Comorbidity	Presence	D-dimer values (ng/ml)	p value
Arterial hypertension	YES	888 (2715)	0.002*
	NO	554 (705.5)	
Diabetes mellitus	YES	680.5 (746)	0.212
	NO	823.5 (3217.7)	
Obesity	YES	1051 (1000)	0.428
	NO	760 (1286.3)	
COPD	YES	741 (1908.7)	0.865
	NO	775 (1213.8)	
Asthma	YES	873.5 (1005.2)	0.980
	NO	760 (1263.8)	
Coronary disease	YES	740 (2990)	0.719
	NO	768 (1123)	
Cardiomyopathy	YES	752 (1066)	0.967
	NO	768 (1229)	

Note: *-statistically significant difference
COPD - Chronic obstructive pulmonary disease

Table 3. INR values of COVID-19 patients according to comorbidities (values are shown as median (IQR))

Comorbidity	Presence	INR values	p value
Arterial hypertension	YES	1.1 (0.3)	0.965
	NO	1.1 (0.3)	
Diabetes mellitus	YES	1.1 (0.2)	0.051
	NO	1.1 (0.3)	
Obesity	YES	1 (0.3)	0.774
	NO	1.1 (0.3)	
COPD	YES	1.2 (0.4)	0.420
	NO	1.1 (0.3)	
Asthma	YES	1 (0.26)	0.135
	NO	1.1 (0.3)	
Coronary disease	YES	1.2 (0.3)	0.562
	NO	1.1 (0.2)	
Cardiomyopathy	YES	1.3 (1.5)	0.028*
	NO	1.1 (0.2)	

Note: *-statistically significant difference
COPD - Chronic obstructive pulmonary disease

Table 4. aPTT values of COVID-19 patients according to comorbidities (values are shown as median (IQR))

Comorbidity	Presence	aPTT (s) values	p value
Arterial hypertension	YES	25.2 (10.3)	0.436
	NO	24.2 (6.5)	
Diabetes mellitus	YES	23.6 (10.5)	0.546
	NO	25.3 (7.4)	
Obesity	YES	21.5 (6.4)	0.257
	NO	25.2 (8.5)	
COPD	YES	30.6 (11.6)	0.916
	NO	24.6 (7.9)	
Asthma	YES	21.7 (13.1)	0.267
	NO	24.6 (8.4)	
Coronary disease	YES	27.6 (10)	0.083
	NO	24.6 (7.5)	
Cardiomyopathy	YES	29.5 (12.8)	0.070
	NO	24.4 (7.3)	

COPD - Chronic obstructive pulmonary disease

Table 5. Values of fibrinogen strains with COVID-19 according to comorbidities (values are shown as median (IQR))

Comorbidity	Presence	Fibrinogen values (g/L)	p value
Arterial hypertension	YES	3.8 (1.65)	0.505
	NO	3.8 (1.15)	
Diabetes melitus	YES	4.2 (2.2)	0.169
	NO	3.7 (1.2)	
Obesity	YES	3.9 (1.3)	0.722
	NO	3.8 (1.5)	
COPD	YES	3.7 (1.8)	0.759
	NO	3.8 (1.5)	
Asthma	YES	5.4 (2.2)	0.047*
	NO	3.8 (1.5)	
Coronary disease	YES	4.1 (1.1)	0.081
	NO	3.8 (1.5)	
Cardiomyopathy	YES	3.4 (1.3)	0.270
	NO	3.8 (1.5)	

Note: *-statistically significant difference
COPD - Chronic obstructive pulmonary disease

The measured values of the partial thromboplastin time did not differ in patients with certain comorbidities and those without, at the admission to the ICU. Detailed values are shown in **Table 4**.

People with asthma had fibrinogen values of 5.4 (2.2) g/l on admission significantly higher than those without asthma 3.8 (1.5) p=0.047. Fibrinogen values in other subjects with and without various comorbidities were not observed. Detailed values are shown in **Table 5**.

Regarding the disease outcome, significantly higher values of D-dimer upon admission to the ICU were recorded in subjects who died during treatment 888 (1226.5) ng/ml compared to patients who were discharged from treatment 666 (1207.3) ng/ml (p = 0.03).

No differences were determined in relation to other parameters of hemostasis **(Table 6)**.

The ROC curve is used to determine the cut-off value of the D-dimer to predict the fatal outcome. The optimal value was 760 mg/ml. Of the total deceased, 51 subjects had D-dimer values greater than or equal to 760 ng/ml, and the remaining deceased (31) had values less than 760 ng/ml. The sensitivity of 60.8% and the specificity of 59.4% of D-dimer was calculated **(Figure 1)**. The Kaplan-Meier survival curve and the log-rank test revealed that D-dimer values of 760 ng/ml or higher are a statistically significant predictor of mortality during hospitalization (p = 0.04) **(Figure 2)**.

Table 6. Values of coagulation parameters of COVID patients measured at admission to the ICU in relation to the outcome of treatment

Variable	Death outcome	Median (IQR)	p value
D-dimer (ng/l)	YES	888 (1226.5)	0.033*
	NO	666 (1207.3)	
INR	YES	1.1 (0.3)	0.437
	NO	1.1 (0.3)	
aPTT (s)	YES	25.9 (10.3)	0.518
	NO	23.9 (6.2)	
Fibrinogen (g/l)	YES	3.8(1.7)	0.157
	NO	3.8 (1.2)	

Note: *-statistically significant difference

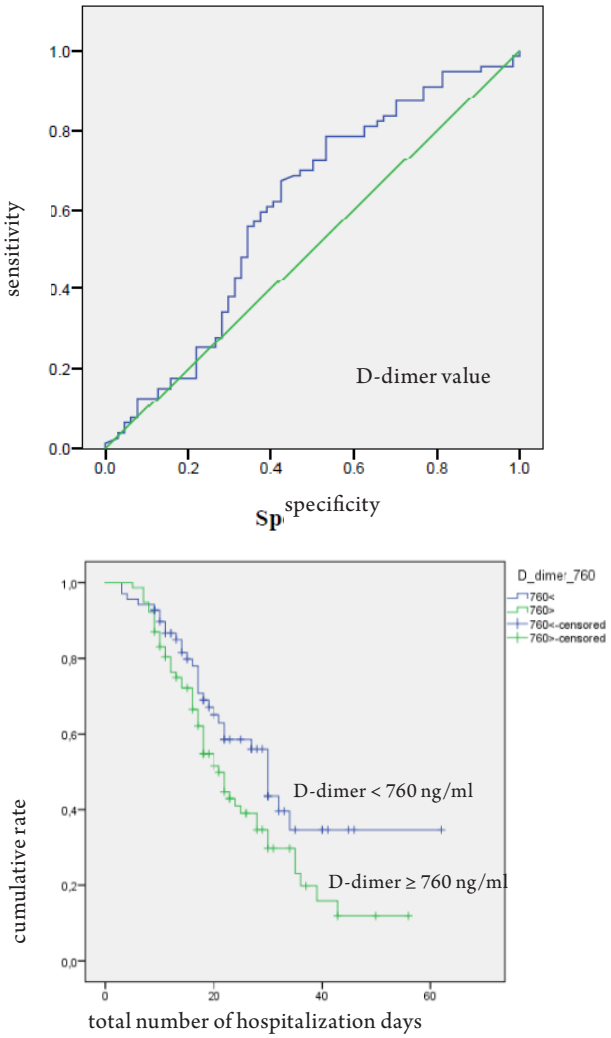


Figure 1. ROC curves of D-dimer values for predicting outcomes of treatment

Thrombosis was detected in 11 patients (7.5%). Among these, 8 had arterial thrombosis (including myocardial infarction in 5 patients and thrombosis of the brachial, radial, and ulnar arteries in 3 patients), while 3 had venous thrombosis, specifically pulmonary thromboembolism. Of the total number of subjects with thrombosis, eight died, while three were discharged after treatment. D-dimer values did not differ significantly between subjects with and without thrombosis (p = 0.46).

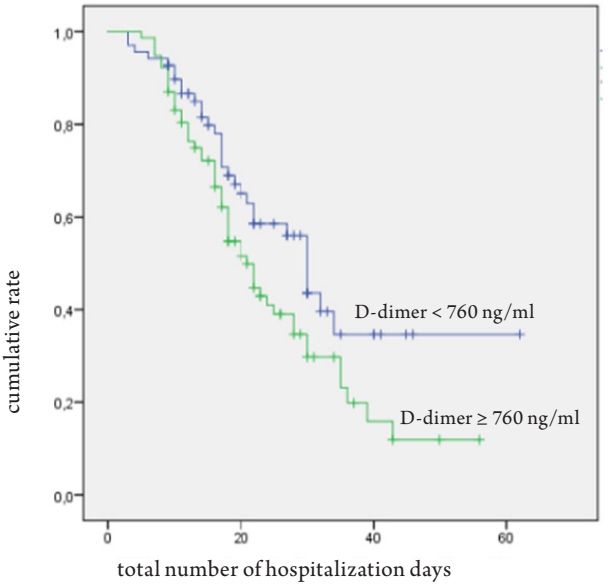


Figure 2. Kaplan-Meier survival curves for D-dimer measured at ICU admision

Discussion

The research revealed distinct patient profiles in terms of age, gender, comorbidities, hemostatic parameters, and elevated D-dimer values among SARS-CoV-2 positive individuals with pneumonia who were treated in Intensive Care Units. The average age of patients who required treatment in the ICU was 65 years. Men had a greater need for treatment in the ICU as well as a higher mortality, while the total mortality was 56.2%. The most common documented chronic disease among the respondents was hypertension (67.8%), followed by diabetes mellitus (34.2%) and coronary disease (17.1%). People with hypertension had higher D-dimer values compared to people without the given comorbidity. Also, people with cardiomyopathy had significantly higher INR values compared to those without it, and higher fibrinogen values were recorded in people with asthma. Significant differences for the remaining parameters of hemostasis were not detected in relation to the present comorbidities. Higher D-dimer values upon admission to the ICU were recorded in persons who died during treatment, while values equal to or greater than 760 ng/ml were associated with a higher risk of in-hospital mortality.

More than 78% of those treated in the ICU had a CT score greater than 15, which speaks in favour of the severe clinical picture of the patients. Patients with a CT score greater than 15 have a high risk of death, followed by a longer stay in the ICU (16). As the CT score increases, the risk of death also rises (17).

Endeshaw et al., in a study conducted on 100 patients, proved that patients with hypertension had significantly higher D-dimer values of 1.1 mg/l, compared to controls of 0.37 mg/l. Additionally, the more severe arterial hy-

pertension was, the higher D-dimer was (18). In addition, the values of D-dimer and fibrinogen are positively related to the duration of hypertension, which suggests that the higher the values the longer the person was hypertensive (19). Hypertension is one of the most common comorbidities in COVID-19 patients (20).

D-dimer represents a significant marker of hemostatic activity and indicates the formation of a fibrin clot, i.e. its breakdown under the action of the fibrinolytic system. Significantly higher D-dimer values were recorded in persons suffering from COVID-19 (21). At the beginning of the pandemic, it was thought that high D-dimer values resulted from an increased frequency of arterial and venous thrombosis, the development of disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS), etc. However, the underlying factor is the host's robust inflammatory response to the SARS-CoV-2 virus. The virus induces the synthesis and release of mediators such as IL-6, TNF, G-CSF, etc., leading to mononuclear cell activation. Thus, activated cells express tissue factors that lead to thrombin activation and fibrin conversion. Also, the virus directly causes damage to endothelial cells and the release of cell contents, including plasminogen activator and vWF. Under the influence of plasminogen activators, plasminogen is converted into plasmin, initiating fibrinolysis. The ultimate goal of this process is the breakdown of fibrin into fibrin degradation products. If the fibrin clot is stabilized by coagulation Factor XIII (FXIII), its subsequent breakdown results in the formation of D-dimer (22).

The results of our study showed that D-dimer values of more than 760 mg/ml (measured on admission to the ICU) can represent a great predictor of mortality. Also, numerous studies have reported similar data. A meta-analysis that included 100 studies over 42,000 patients concluded that high D-dimer levels upon admission are associated with the risk of developing a more severe form of the disease, as well as death (23). Popovska Jovičić et al. in their research, where they included 288 patients diagnosed with SARS-CoV2 infection, proved that D-dimer values of 0.82 ug/mL measured upon admission can indicate a potential risk of death during the hospitalization (24). A multicentre study in Spain reported that admission D-dimer values higher than 945 μg/L FEU have a predictive value regarding in-hospital mortality (25). Within the first ten days of hospital treatment, there is a trend of increasing D-dimer, after which the value decreases (26).

It should be noted that the results of our prospective study in which patients were monitored after hospitalization for COVID 19 infection showed that certain categories of patients (the elderly and those with a more severe clinical presentation of COVID infection) can maintain elevated D-dimer and FV III levels, even 6 months after hospitalization. This finding indicates that the degree of hypercoagulability is maintained in a certain number of patients even after the infection has passed, therefore, it represents a risk for thrombosis in the post-Covid period (11).

When discussing the risk of thrombosis associated with acute COVID-19 infection, thrombotic events were verified in 11 (7.5%) subjects, of which 8 were arterial and 3 pulmonary embolisms.

The D-dimer values of persons with verified thrombosis were 1260 (31810) ng/ml and did not significantly differ from the D-dimer values of persons without verified thrombosis, which can be explained by the higher frequency of arterial thrombosis. In other studies, the incidence of thrombosis in ICU patients was significantly hihger (27-29). In contrast to our research, in other studies venous compared to arterial thromboses were detected more frequently (30, 31), and D-dimer levels above 3,000 μg/L measured in patients with COVID-19 can be a predictor of pulmonary thromboembolism (32).

Regarding the limitations of the study, it should be emphasized it was a retrospective study that included a relatively small number of respondents. These are the first data on hemostatic changes in critically ill COVID-19 patients in our country who were treated in the ICU. As such, they represent a significant contribution to understanding this issue and highlight the need for prospective studies with larger sample sizes.

CONCLUSION

COVID coagulopathy is a complication that increases the mortality of people infected with the SARS-CoV2 virus. The main feature is the state of hypercoagulability, which is confirmed by elevated D-dimer values. Our research showed that people suffering from COVID-19 had high D-dimer levels upon admission to the ICU and this value was significantly higher in people who died. D-dimer values greater than or equal to 760 ng/ml upon admission to the ICU may have prognostic significance for survival during the hospitalization.

Author Contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by Marija Zdravković and Marija Milenković. The first draft of the manuscript was written by Marija Milenković. Mirjana Kovač and Marija Zdravković edited the manuscript. All authors read and approved the final manuscript.

References

1. Sarker R, Roknuzzaman ASM, Nazmunnahar, Shahriar M, Hossain MJ, Islam MR. The WHO has declared the end of pandemic phase of COVID-19: Way to come back in the normal life. Health Sci Rep. 2023 Sep 5;6(9):e1544. doi: 10.1002/hsr2.1544. PMID: 37674622; PMCID: PMC10478644.
2. Narayanan SA, Jamison DA Jr, Guarnieri JW, Zaksas V, Topper M, Koutnik AP,et al. A comprehensive SARS-CoV-2 and COVID-19 review, Part 2: host extracellular to systemic effects of SARS-CoV-2 infection. Eur J Hum Genet. 2024 Jan;32(1):10-20. doi: 10.1038/s41431-023-01462-1 PMID: 37938797; PMCID: PMC10772081.
3. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020 Feb 22;395(10224):565-574. doi: 10.1016/S0140-6736(20)30251-8. PMID: 32007145; PMCID: PMC7159086.
4. Paludan SR, Mogensen TH. Innate immunological pathways in COVID-19 pathogenesis. Sci Immunol. 2022 Jan 7;7(67):eabm5505. doi: 10.1126/sciimmunol.abm5505. PMID: 34995097.
5. Milenković M, Kovač M. Poremećaji hemostaze. In: Kovačević P, Barić G, Dragić S, Jandrić M, et al. Osnovi intenzivne medicine. Medicinski fakultet Univerziteta u Banja Luci , Udruženje intenzivista za nehirurške grane, Banja Luka 2022, str. 513-571.
6. Kollias A, Kyriakoulis KG, Lagou S, Kontopantelis E, Stergiou GS, Syrigos K. Venous thromboembolism in COVID-19: A systematic review and meta-analysis. Vasc Med. 2021 Aug;26(4):415-425. doi: 10.1177/1358863X21995566. PMID: 33818197; PMCID: PMC8024143.
7. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020 Mar 28;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3. PMID: 32171076; PMCID: PMC7270627.
8. Korkusuz R, Karandere F, Senoglu S, Kocoglu H, Yasar KK. The prognostic role of D-dimer in hospitalized COVID-19 patients. Bratisl Lek Listy. 2021;122(11):811-815. doi: 10.4149/BLL_2021_129. PMID: 34672673.
9. Shah S, Shah K, Patel SB, Patel FS, Osman M, Velagapudi P, et al. Elevated D-Dimer Levels Are Associated With Increased Risk of Mortality in Coronavirus Disease 2019: A Systematic Review and Meta-Analysis. Cardiol Rev. 2020 Nov/Dec;28(6):295-302. doi: 10.1097/CRD.0000000000000330. PMID: 33017364; PMCID: PMC7437424.
10. Lippi G, Favaloro EJ. D-dimer is Associated with Severity of Coronavirus Disease 2019: A Pooled Analysis. Thromb Haemost. 2020 May;120(5):876-878. doi: 10.1055/s-0040-1709650. PMID: 32246450; PMCID: PMC7295300.
11. Kovac M, Balint MT, Milenkovic M, Basaric D, Tomic B, Balint B, Ignjatovic V. Assessment of Factor VIII Activity and D-Dimer Levels in the Post-COVID Period. Hamostaseologie. 2024 Mar 1. doi: 10.1055/a-2238-4744. PMID: 38428838.
12. Han H, Yang L, Liu R, Liu F, Wu KL, Li J, Liu XH, Zhu CL. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clin Chem Lab Med. 2020 Jun 25;58(7):1116-1120. doi: 10.1515/cclm-2020-0188. PMID: 32172226.
13. Fei Y, Tang N, Liu H, Cao W. Coagulation Dysfunction. Arch Pathol Lab Med. 2020 Oct 1;144(10):1223-1229. doi: 10.5858/arpa.2020-0324-SA. PMID: 32551814.
14. Bhandari S, Rankawat G, Bagarhatta M, Singh A, Singh A, Gupta V, Sharma S, Sharma R. Clinico-Radiological Evaluation and Correlation of CT Chest Images with Progress of Disease in COVID-19 Patients. J Assoc Physicians India. 2020 Jul;68(7):34-42. PMID: 32602679.
15. Pelemiš M, Stevanović G, Turkulov V, Vučinić V, Matijašević J, Milošević B, i saradnici. Nacionalni protokol Republike Srbije za lečenje COVID-19 infekcije, Verzija 8. 2020.
16. Jayachandran AK, Nelson V, Shajahan ME. Chest CT severity score as a predictor of mortality and short-term prognosis in COVID-19. J Family Med Prim Care. 2022 Aug;11(8):4363-4367. doi: 10.4103/jfmpc.jfmpc_209_22. PMID: 36353028; PMCID: PMC9638539.
17. Abbasi B, Akhavan R, Ghamari Khameneh A, Zandi B, Farrokh D, Pezeshki Rad M, et al. Evaluation of the relationship between inpatient COVID-19 mortality and chest CT severity score. Am J Emerg Med. 2021 Jul;45:458-463. doi: 10.1016/j.ajem.2020.09.056. PMID: 33039235; PMCID: PMC7521211.
18. Endeshaw C, Solomon G, Menakath M, Zeleke G, Tadesse A, Tadesse L, al. Assessment of plasma D-dimer level and its correlation with disease severity among hypertensive patients. Journal of Science and Inclusive Development. 2020 2(2):21-40. doi: 10.20372/jsid/2020-31.
19. Rafaqat S, Khalid A, Riaz S, Rafaqat S. Irregularities of Coagulation in Hypertension. Curr Hypertens Rep. 2023 Oct;25(10):271-286. doi: 10.1007/s11906-023-01258-0. Epub 2023 Aug 10. PMID: 37561240.
20. Tadic M, Cuspidi C, Grassi G, Mancia G. COVID-19 and arterial hypertension: Hypothesis or evidence? J Clin Hypertens (Greenwich). 2020 Jul;22(7):1120-1126. doi: 10.1111/jch.13925. PMID: 32627330; PMCID: PMC7362072.
21. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med. 2020 Jun 25;58(7):1131-1134. doi: 10.1515/cclm-2020-0198. PMID: 32119647.
22. Vincent JL, Levi M, Hunt BJ. Prevention and management of thrombosis in hospitalised patients with COVID-19 pneumonia. Lancet Respir Med. 2022 Feb;10(2):214-220. doi: 10.1016/S2213-2600(21)00455-0. Epub 2021 Nov 25. PMID: 34838161; PMCID: PMC8616572.
23. Varikasuvu SR, Varshney S, Dutt N, Munikumar M, Asfahan S, Kulkarni PP, Gupta P. D-dimer, disease severity, and deaths (3D-study) in patients with COVID-19: a systematic review and meta-analysis of 100 studies. Sci Rep. 2021 Nov 8;11(1):21888. doi: 10.1038/s41598-021-01462-5. PMID: 34750495; PMCID: PMC8576016.
24. Popovska Jovičić B, Raković I, Gavrilović J, Sekulić Marković S, Petrović S, Marković V, et al. Vitamin D, Albumin, and D-Dimer as Significant Prognostic Markers in Early Hospitalization in Patients with COVID-19. J Clin Med. 2023 Apr 12;12(8):2825. doi: 10.3390/jcm12082825. PMID: 37109161; PMCID: PMC10145116.
25. García de Guadiana-Romualdo L, Morell-García D, Favaloro EJ, Vílchez JA, Bauça JM, Alcaide Martín MJ, et al. Harmonized D-dimer levels upon admission for prognosis of COVID-19 severity: Results from a Spanish multicenter registry (BIOCOVID-Spain study). J Thromb Thrombolysis. 2022 Jan;53(1):103-112. doi: 10.1007/s11239-021-02527-y. PMID: 34272635; PMCID: PMC8284690.
26. Creel-Bulos C, Liu M, Auld SC, Gaddh M, Kempton CL, Sharifpour M, et al. Trends and diagnostic value of D-dimer levels in patients hospitalized with coronavirus disease 2019. Medicine (Baltimore). 2020 Nov 13;99(46):e23186. doi: 10.1097/MD.0000000000023186. PMID: 33181697; PMCID: PMC7668476.
27. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Müller MCA, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020 Aug;18(8):1995-2002. doi: 10.1111/jth.14888. PMID: 32369666; PMCID: PMC7497052.
28. Cui S, Chen S, Li X, Liu S, Wang F. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. J Thromb Haemost. 2020 Jun;18(6):1421-1424. doi: 10.1111/jth.14830. PMID: 32271988; PMCID: PMC7262324.
29. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020 Jul;191:145-147. doi: 10.1016/j.thromres.2020.04.013. PMID: 32291094; PMCID: PMC7146714.
30. Poissy J, Goutay J, Caplan M, Parmentier E, Duburcq T, Lassalle F, Jeanpierre E, Rauch A, Labreuche J, Susen S; Lille ICU Haemostasis COVID-19 Group. Pulmonary Embolism in Patients With COVID-19: Awareness of an Increased Prevalence. Circulation. 2020 Jul 14;142(2):184-186. doi: 10.1161/CIRCULATIONAHA.120.047430. PMID: 32330083.

31. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X; CRICS TRIGGERSEP Group (Clinical Research in Intensive Care and Sepsis Trial Group for Global Evaluation and Research in Sepsis). High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med. 2020 Jun;46(6):1089-1098. doi: 10.1007/s00134-020-06062-x. PMID: 32367170; PMCID: PMC7197634.

32. Galland J, Thoreau B, Delrue M, Neuwirth M, Stepanian A, Chauvin A; Lariboisière COVID Group; et al. White blood count, D-dimers, and ferritin levels as predictive factors of pulmonary embolism suspected upon admission in noncritically ill COVID-19 patients: The French multicenter CLOTVID retrospective study. Eur J Haematol. 2021 Aug;107(2):190-201. doi: 10.1111/ejh.13638. PMID: 34288162; PMCID: PMC8239942.

PROMENE U HEMOSTATSKOM SISTEMU KOD KRITIČNO OBOLELIH OD KOVIDA 19

Marija Milenković[1,2],Mirjana Kovač[3], Ivan Rović[1], Đuro Šijan[1], Bogdan Crnokrak[2,4], Marija Zdravković[2,4]

Sažetak

Uvod/cilj istraživanja: Koagulopatija usled kovida 19 predstavlja poremećaj sistema hemostaze koji se javlja kod kritično obolelih bolesnika zaraženih SARS-CoV2 virusom i koja povećeva rizik od mortaliteta. Cilj istraživanja je procena promena hemostaznih parametara i određivanje njihovog prognostičkog značaja kod bolesnika sa teškom formom bolesti kovid 19.

Metode: Studija je dizajnirana kao retrospektivna kohortna studija, koja je obuhvatila 146 pacijenta lečenih od juna do septembra 2020. godine u Jedinici intenzivnog lečenja (JIL) Kliničko bolničkog centra „Bežanijska kosa" u Beogradu pod dijagnozom pneumonije izazvane kovidom 19. Uključujući kriterijumi su bili starost iznad 18 godina, dokazana aktuelna SARS-CoV2 infekcija i prijem u JIL.

Rezultati: Tokom lečenja su preminula 82 bolesnika (56.2%), dok je 64 (43.8%) otpušteno nakon sprovedenog lečenja. Značajno više vrednosti D-dimera na prijemu u JIL zabeležene su kod ispitanika koji su preminuli u toku lečenja 888 (1226,5) ng/ml u odnosu na osobe koje su otpuštene sa lečenja 666 (1207,3) ng/ml (p = 0,03). Razlike nisu dokazane za INR, aPTT i fibrinogen. Vrednosti D-dimera na prijemu u JIL više ili jednake 760ng/ml statistički su značajan prediktor smrtnog ishoda u toku hospitalizacije (p = 0,04).

Zaključak: Koagulopatija usled kovida 19 predstavlja komplikaciju koja povećava mortalitet osoba zaraženih SARS-CoV2 virusom. Osnovno obeležje jeste stanje hiperkoagulabilnosti, koje se detektuje povišenim vrednostima D-dimera. Vrednosti D-dimera više ili jednake 760 ng/ml na prijemu u JIL mogu imati prognostički značaj za preživljavanje u toku hospitalizacije.

Ključne reči: kovid 19, koagulopatija, D-dimer, jedinica intenzivnog lečenja

Primljen: 01.07.2024. I **Revizija:** 18.07.2024. I **Prihvaćen:** 25.07.2024.

Medicinska istaživanja 2024; 57(3):91-98

Medicinska istaživanja 2024; 57(3):99-108 | DOI 10.5937/medi57-51182

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Evaluation of hsa-mir-675-5p expression and its diagnostic and prognostic relevance in oral cancer

Goran Stojković[1,2], Marija Savić Veselinović[3], Nikola Todorović[1], Bojana Bukurov[1,2], Miljan Folić[1,2], Tjaša Ivošević[1,4], Katarina Zeljić[3]

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

[2] Hospital for Otorhinolaryngology and Maxillofacial Surgery, University Clinical Center Serbia, Belgrade, Serbia

[3]University of Belgrade, Faculty of Biology, Belgrade, Serbia

[4]Center for Anaesthesiology and Resuscitation, University Clinical Center of Serbia, Belgrade, Serbia

Recived: 23 May 2024

Revised:18 July 2024

Accepted: 25 July 2024

Check for updates

Funding information:
This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia [grant number: 451-03-65/2024-03/200178].

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Katarina Zeljić
University of Belgrade, Faculty of Biology,
3 Studentski trg, 11000 Belgrade, Serbia
E-mail: katarina.zeljic@bio.bg.ac.rs

Summary

Introduction: Oral cancer is the most common subtype of cancer in the head and neck region, with an increasing incidence worldwide. Unfortunately, no specific biomarkers are used in everyday clinical practise. Small non-coding RNA molecules, microRNA (miRNA), are considered sensitive biomarkers for early diagnosis as well as prognosis in patients with oral cancer. Especially, microRNA derived from the H19 locus are poorly investigated for their potential role in oral cancer.

Aim: The aim of this study was to evaluate expression of hsa-miR-675-5p in tumor and non-tumor tissues of oral cancer patients and to associate it with pathohistological features.

Material and Methods: The study group consisted of 35 patients with oral cancer. Tumor and surrounding non-tumor tissues were taken from each patient**.** Relative expression was measured using the quantitative reverse transcription - real time PCR method**.**

Results: The relative expression of hsa-miR-675-5p was lower in oral cancer tumor than in non-tumor tissue suggesting its tumor suppressive role. hsa-miR-675-5p has diagnostic potential for sensitive distinction of tumor and non-tumor tissues in oral cancer patients. There was no difference in overall survival rates between patients with low and high hsa-miR-675-5p expression, confirming that hsa-miR-675-5p cannot be used as a prognostic biomarker in patients with oral cancer.

Conclusion: hsa-miR-675-5p can be considered as a potential diagnostic but not a prognostic molecular biomarker in oral cancer.

Keywords: oral cancer, hsa-miR-675-5p, biomarker

Cite this article as: Stojković G, Savić Veselinović M, Todorović N, Bukurov B, Folić M, Ivošević T, Zeljić K. Evaluation of hsa-mir-675-5p expression and its diagnostic and prognostic relevance in oral cancer; Medicinska istaživanja 2024; 57(3):99-108 DOI 10.5937/medi57-51182

INTRODUCTION

Epidemiological data worldwide indicate a troubling increase in the incidence of oral cancer (1). While surgical removal of tumor remains a mainstay treatment, recurrence rates among the patients with negative resection margins can be as high as one-third (2). To reduce the risk of recurrence post-surgery, current histopathological procedures should be improved by adding a molecular approach to analyze tissue changes.

MicroRNAs (miRNAs) represent a class of small, non-coding RNA molecules, typically 18-24 nucleotides in length, involved in posttranscriptional regulation by either mRNA degradation or repression of translation of target gene (3, 4). With over 2 000 miRNAs identified in humans to date, their regulatory role extends over vital biological processes such as: cell proliferation, differentiation, development, apoptosis and immune response. The focus of research into miRNAs in the biomedical field is based on the fact that miRNAs have promising biomarker potential in various pathologies, including cancer (5, 6). Notably, their stability in tissue and liquid biopsies uncerscores their utility as molecular biomarkers (6). In our previous study, we delineated three-miRNAs signature (miR-31-3p, miR-139-5p and miR-30a-5p) for diagnostic use in oral cancer and also identified miRNAs (miR-135b-5p, miR-18a-5p and miR-30a-5p) indicative of poor survival (7). It is important to continue the search for molecular biomarkers to improve future diagnostic, prognostic and therapeutic practice in oral cancer patients.

The IGF2-H19 locus (11p15.5), paternally imprinted and harboring both coding and noncoding genes, is frequently deregulated in cancer (8), yet its involvement in oral cancer remains elusive. Of interest is hsa-miR-675, expressed from the *H19* gene within the IGF2-H19 locus, with documented increased expression of hsa-miR-675-3p in esophageal cancer (9, 10) and underexpression of hsa-miR-675-5p in adrenocortical carcinoma (11). Given its dysregulation across cancer types, its potential implication in oral cancer can be assumed also. To our knowledge, status of hsa-miR-675 in oral cancer remains unexplored, leaving its significance unresolved. Furthermore, the biomarker potential of hsa-miR-675 remains ambiguous.

Our objective was to characterise H19 derived hsa-miR-675 through a bioinformatic approach and assess relative levels of hsa-miR-675 in publicly available databases and clinical samples of oral tumor and surrounding non-tumor tissues. We aim to associate these findings with pathohistological features and survival outcomes in oral cancer patients.

MATERIAL AND METHODS

Exploration of hsa-miR-675 using a bioinformatic approach

hsa-miR-675 was functionally analysed by DIANA-miRPath v4.0 web-based software (http://www.microrna.gr/miRPathv4) (12). Both forms of hsa-miR-675, -5p and -3p, were searched. TargetScan v8.0 was used for gene target selection. To determine significantly enriched molecular pathways with target genes predicted to be regulated by the miRNA of our interest, the Kyoto Encyclopedia of Genes and Genomes (KEGG) was used under the criteria of pathway union, p value threshold of 0.05, and false discovery rate (FDR) correction. If p value was less than 0.05, pathway was considered significantly enriched. Heatmap of significantly enriched pathways was constructed by DIANA-miRPath v4.0.

Network of hsa-mir-675 (-5p and -3p forms) - gene interactions were constructed and visualised by miRNet v2.0 software (https://www.mirnet.ca) (13). miRTarBase v8.0 and TarBase v8.0 were used as a resource of validated interactions between hsa-miR-675 and target genes. On all target genes was performed enrichment analysis using hypergeometric test and KEGG database. If adjusted p value was less than 0.05, pathway was considered significantly enriched.

The University of California Santa Cruz (UCSC) Xena platform (http://xena.ucsc.edu/) was used for visual analysis of hsa-miR-675 expression in head and neck squamous cancer (HNSC) compared to normal tissue using the Genomic Data Commons - The Cancer Genome Atlas (GDC TCGA) database (14). The GDC TCGA HNSC database contains phenotype data for 612 samples. After filtering out samples and restricting the search to oral cancer cases of white ethnicity with data on hsa-miR-675 expression, 254 samples remained **(Figure 1A)**. Of these, 235 were primary tumors and 19 were solid normal tissue. There were 18 matched samples of primary tumors and normal tissue. The hsa-miR-675 stem loop expression data and relevant demographic (gender, age, alcohol consumption, smoking history) and clinical data (histological grade, TNM status, clinical stage, overall survival) were downloaded from the UCSC Xena platform for further statistical analysis. Kaplan-Meier curve of overall survival were generated in UCSC Xena and compared by log-rank test. For survival analysis hsa-miR-675 expression was classified into low and high based on median value.

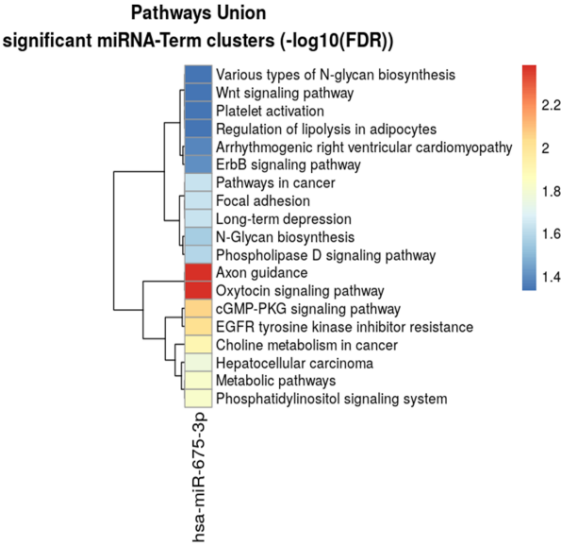
Patients and biological samples

Tissue samples from oral cancer patients were collected in period between 2018 and 2020. This same series of patients (N=35) was used for analysis in our previously published study, with demographic and pathohistological

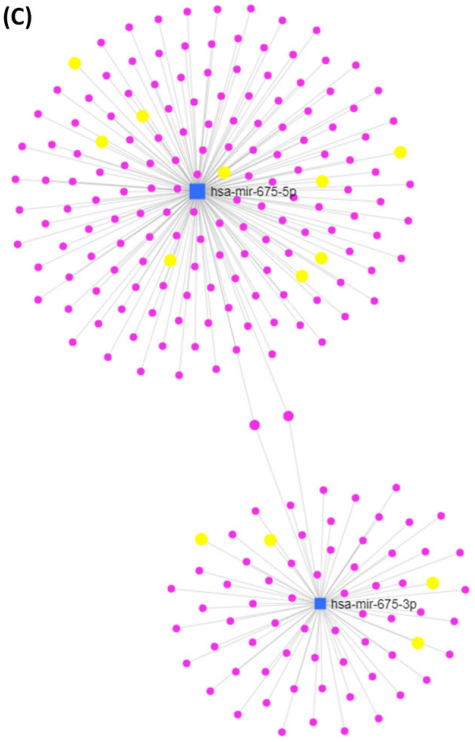
(A)

KEGG pathway	Target genes (N)	p-value
Axon guidance	61	**1.64578E-05**
Oxytocin signaling pathway	54	**2.45035E-05**
cGMP-PKG signaling pathway	56	**7.7691E-05**
EGFR tyrosine kinase inhibitor resistance	31	**0.000113062**
Choline metabolism in cancer	37	**0.000180689**
Metabolic pathways	375	**0.000276854**
Phosphatidylinositol signaling system	35	**0.00031311**
Hepatocellular carcinoma	54	**0.000401065**
Focal adhesion	62	**0.000628199**
Long-term depression	24	**0.000744748**
Pathways in cancer	140	**0.000741836**
Phospholipase D signaling pathway	48	**0.000909317**
N-Glycan biosynthesis	20	**0.001053068**
ErbB signaling pathway	29	**0.001611169**
Arrhythmogenic right ventricular cardiomyopathy	27	**0.001840491**
Wnt signaling pathway	50	**0.002372702**
Platelet activation	41	**0.002431426**
Regulation of lipolysis in adipocytes	22	**0.002188501**
Various types of N-glycan biosynthesis	16	**0.002567327**

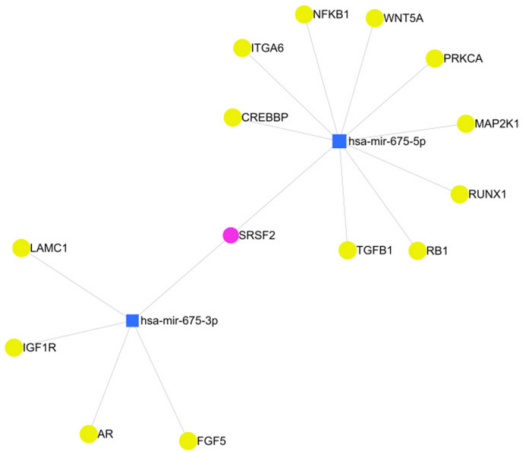
(B)



(C)



(D)



(E)

KEGG	Target genes (N)	p value	Adjusted p
Melanogenesis	9	**0.0000196**	**0.00196**
Prostate cancer	7	**0.000333**	**0.0167**
Pathways in cancer	13	**0.00074**	**0.0247**
TGF-beta signaling pathway	6	**0.00169**	**0.0422**
Prion diseases	3	**0.00381**	**0.0762**
Chronic myeloid leukemia	5	**0.005**	**0.0833**

Figure 1. Significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for hsa-miR-675-3p presented as list **(A)** and heatmap **(B)**. Intensity of colours represents p value (-log10(FDR)). Target genes (N) – number of genes in the pathway. hsa-miR-675-5p and hsa-miR-675-3p gene interaction networks **(C)**. Yellow circles represent genes involved in significantly enriched pathways in cancer **(D)**.
Blue squares represent miRNA, circles represent genes regulated by miRNA form. Genes names are given along to yellow circles.
List of significant KEGG enriched in genes from gene-miRNA interaction network **(E)**. KEGG with significant p and adjusted p values are presented.
p<0.05 are in bold.

characteristics of the study group detailed therein (7). Ethical approval for the collection and utilization of biological samples for research purposes was granted from the Ethics committee of the Faculty of Medicine, University of Belgrade (approval number: 1550/VII-6).

Patients diagnosed with oral cancer provided informed consent for their patricipation in the study. During surgical tumor excision, specimens from both tumor and surrounding non-tumor tissue (approximatelly 2 cm from macroscopically identified tumor margins) was taken for research, immersed in RNA later solution (Invitrogen, USA) and stored at -80°C. Tissue verification and tumor staging were in accordance with the guidelines outlined in the Union for International Cancer Control Staging Manual, 8th Edition.

Quantification of hsa-miR-675-5p relative expression

The *mir*Vana™ kit (Invitrogen, USA) was used to isolate total RNA from collected biological samples. RNA at a concentration of 20ng/µl was used for cDNA synthesis with the TaqMan™ MicroRNA Reverse Transcription Kit (Invitrogen, USA) according to the manufacturer's recommendations. TaqMan™ MicroRNA Assay was used for hsa-miR-675-5p (ID 002005) quantification by real time PCR. Normalization was done by RNU6B (ID 001093) as an endogenous control. Cycle Treshold (Ct) values were used to calculate the relative expression of the hsa-miR-675-5p target using the comparative ΔCt method (ΔCt=Ct of target – Ct of endogenous control). Ct values were measured in triplicate for each sample, and the average value was used for further analysis. Relative expression, presented as $2^{-\Delta Ct}$, is used for statistical tests.

Statistical analysis

The collected data were analysed and graphically displayed using GraphPad Prism software, version 9. Chi-square ($\chi 2$) test was used for association of categorical variables. The normality of the data was tested using the Shapiro-Wilk test. If the continuous data were normally distributed, a parametric t-test was performed, for non-normally distributed paired data, the Wilcoxon rank test was performed. Receiver Operating Curve (ROC) was used to evaluate diagnostic potential of analysed miRNA. Association of gene expression with patients overall survival was estimated by Kaplan-Meier survival curve and log-rank test. Cox regression analysis was performed to estimate hazard risk depending on hsa-miR-675-5p expression. All p values were two-tailed and if p value was less than 0.05, results were considered significant.

RESULTS

Bioinformatic exploration of hsa-miR-675

Nineteen KEGG pathways showed significant enrichment in genes predicted to be regulated by hsa-miR-675, with metabolic pathways and pathways in cancer being the most enriched **(Figure 1A, 1B)**. Using miRNet v2.0, we constructed a network of interactions between hsa-miR-675-5p and hsa-miR-675-3p, and target genes. Network analysis revealed that hsa-miR-675-5p regulates a larger number of genes (163 genes) compared to hsa-miR-675-3p (70 genes) **(Figure 1C)**. Further analysis using the hypergeometric test and the KEGG database confirmed significant enrichment in cancer with the largerst number of genes affected (genes *RUNX1*, *MAP2K1*, *PRKCA*, *RB1*, *WNT5A*, *CREBPP*, *ITGA6*, *NFKB1*, *TGFB1*) **(Figure 1D, 1E)** affirming the involvment of hsa-miR-675 in carcinogenesis. This justifies the selection of hsa-miR-675 for further analysis in the open transcriptomic database for oral cancer as well as in clinical samples.

Exploring hsa-miR-675 in the open GDC TCGA HNSC database via UCSC Xena **(Figure 2A)** revealed no significant difference in expression between primary tumor samples and solid normal tissue among oral cancer patients (p=0.504, t-test Welch's correction), **Figure 2B**. Similary, no significant difference was found in matched tumor and non-tumor tissue from 18 oral cancer patients (p=0.277, t-test Welch's correction), **Figure 2B**.

ROC analysis on whole group including 254 samples, indicated that hsa-miR-675 was not effective as a diagnostic biomarker for distinguishing oral tumor from non-tumor tissue (AUC=0.538, 95% CI=0.421-0.654, p=0.579), **Figure 1C**. The same trend persisted when analyzing a subset of matched samples (AUC=0.620, 95% CI= 0.427-0.813, p=0.217). Furthermore, Kaplan-Meier curve analysis showed no significant difference in overall survival between patients with low and high expression of hsa-miR-675 (p=0.169, log-rank test), **Figure 2D**, indicating that hsa-miR-675 cannot be considered as a prognostic biomarker.

Table 1 presents the association of hsa-miR-675 expression with demographic and pathohistological features, revealing significant associations with the location of primary tumor (p=0.011, $\chi 2$ test), histological grade (p=0.013, $\chi 2$ test), tumor stage (p=0.013, $\chi 2$ test) and tumor size (p=0.019, $\chi 2$ test), categorized into low and high expression groups based on the median expression level.

Relative expression, diagnostic and prognostic potential of hsa-miR-675-5p

In our study group, the relative expression of hsa-miR-675-5p was significantly higher in non-tumor than in tumor samples from oral cancer patients tissue (p=0.006, Wilcoxon signed rank test), **Figure 3**. The expression

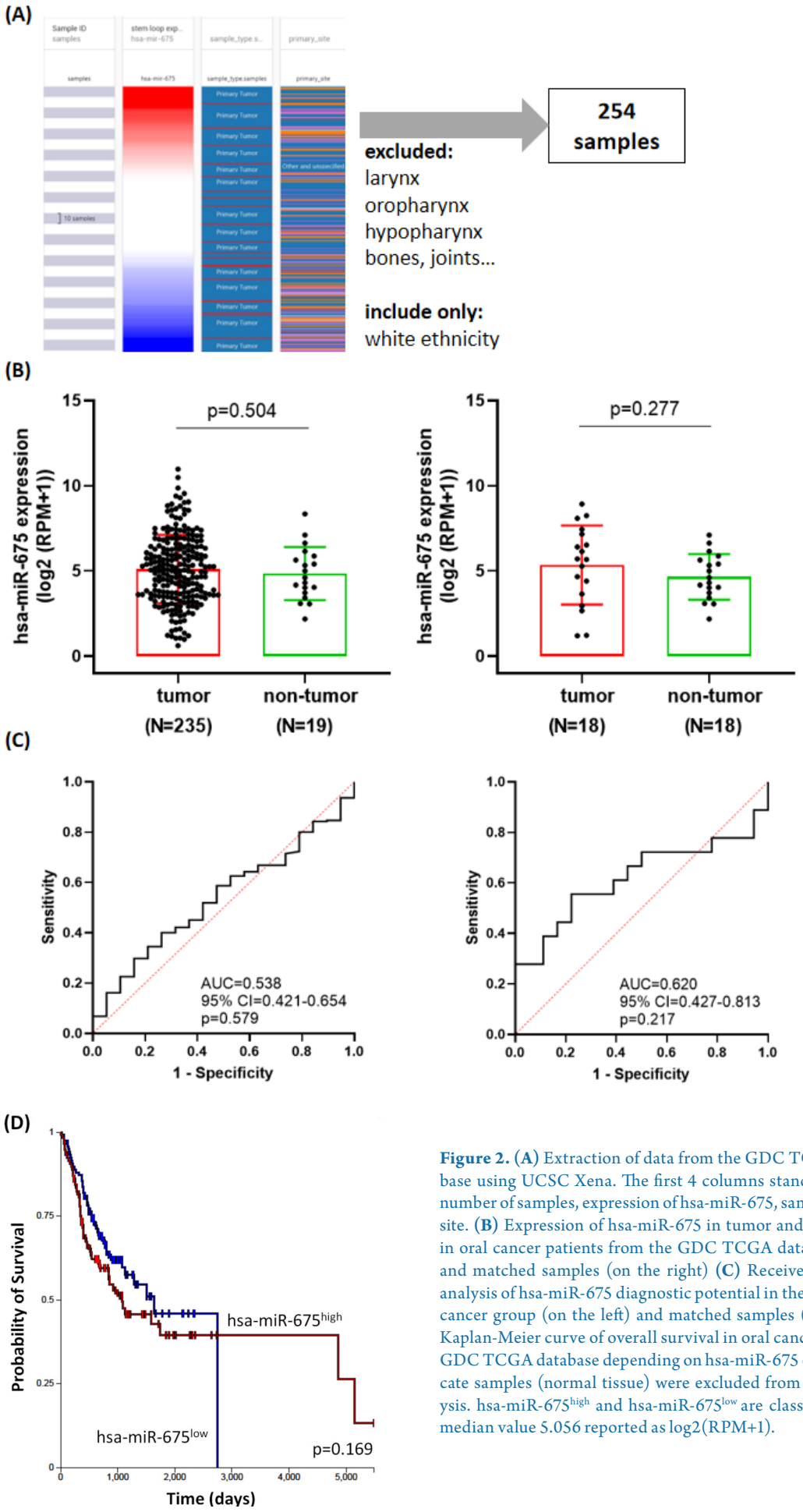


Figure 2. **(A)** Extraction of data from the GDC TCGA HNSC database using UCSC Xena. The first 4 columns stand for, respectively, number of samples, expression of hsa-miR-675, sample type, primary site. **(B)** Expression of hsa-miR-675 in tumor and non-tumor tissue in oral cancer patients from the GDC TCGA database (on the left) and matched samples (on the right) **(C)** Receiver operating curve analysis of hsa-miR-675 diagnostic potential in the GDC TCGA oral cancer group (on the left) and matched samples (on the right) **(D)** Kaplan-Meier curve of overall survival in oral cancer group from the GDC TCGA database depending on hsa-miR-675 expression. Duplicate samples (normal tissue) were excluded from the survival analysis. hsa-miR-675^{high} and hsa-miR-675^{low} are classified according to median value 5.056 reported as log2(RPM+1).

Table 1. Association of hsa-miR-675 expression in oral cancer with demographic and pathohistological features of oral cancer patients from GDC TCGA database downloaded from the UCSC Xena

GDC TCGA demographic and pathohistological features		Relative expression of hsa-miR-675 in tumor tissue				
		hsa-miR-675^{low} N=118		hsa-miR-675^{high} N=117		
		N	%	N	%	p
Sex	male	76	64.4	79	67.5	0.614
	female	42	35.6	38	32.5	
Age (years, median)	≤61	63	53.4	65	55.6	0.739
	>61	55	46.6	52	44.4	
Smoking habits	nonsmoker	64	54.2	59	50.4	0.559
	smoker + ex smoker	54	45.8	58	49.6	
Alcohol consumption	no intake	37	31.4	38	32.5	0.854
	moderate + high	81	68.6	79	67.5	
Location of primary tumor	base of tongue	15	12.7	9	7.7	**0.011**
	floor of the mouth	22	18.6	19	16.2	
	gum	8	6.8	1	0.9	
	lip	2	1.7	1	0.9	
	palate	3	2.5	2	1.7	
	other and unspecified parts of tongue	44	37.3	71	60.7	
	other and unspecified part of mouth	24	20.3	14	12	
Histological gradus	well differentiated	25	21.9	12	10.4	**0.013**
	moderately differentiated	59	51.8	76	66.1	
	poorly differentiated	26	22.8	27	23.5	
	anaplastic	4	3.5	0	0	
	grade can not be evaluated / NA	4	-	2	-	
T staging	T1, T2	24	20.3	41	35	**0.013**
	T3, T4	90	76.3	73	62.4	
	NA	4	3.4	3	2.6	
Tumor size	≤2cm	16	14.5	22	19.1	**0.019**
	2-4cm	24	21.8	41	35.7	
	>4cm	70	63.6	52	45.2	
N staging	N0	56	50.9	57	49.5	0.842
	N1	54	49.1	58	50.5	
	NA	8	-	2	-	

hsa-miR-675^{high} and hsa-miR-675^{low} are classified according to median value 0.056 reported as log2(RPM+1);
NA – not available; GDC TCGA – Genomic Data Commons The Cancer Genome Atlas; N – number of cases; N0 – without regional lymph node involvement; N1- regional lymph node involvement; p<0.05 is presented in bold

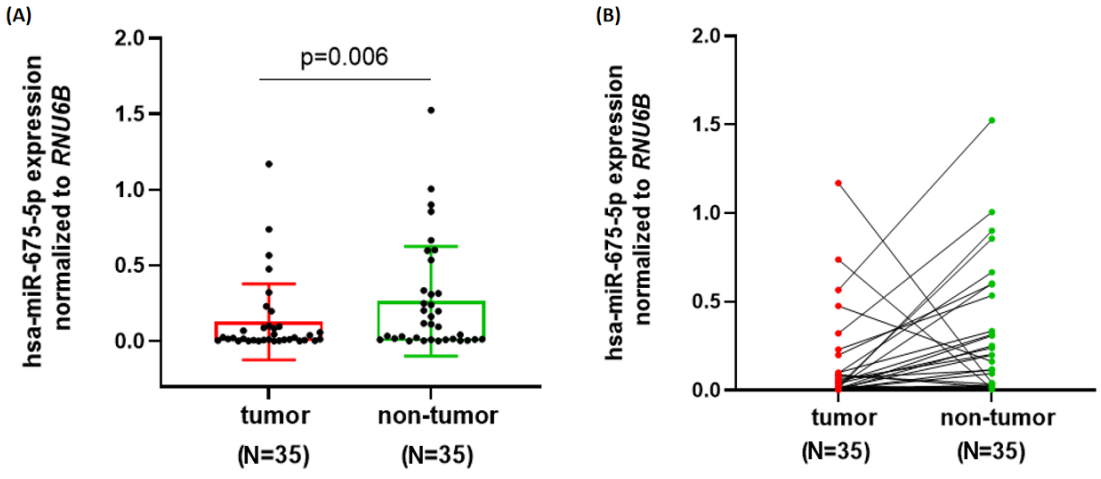


Figure 3. hsa-miR-675-5p relative expression (presented as mean ± SD of $2^{-\Delta Ct}$ value) of oral cancer patients in **(A)** tumor and non-tumor tissue **(B)** paired tumor and non-tumor tissue.
SD – standard deviation

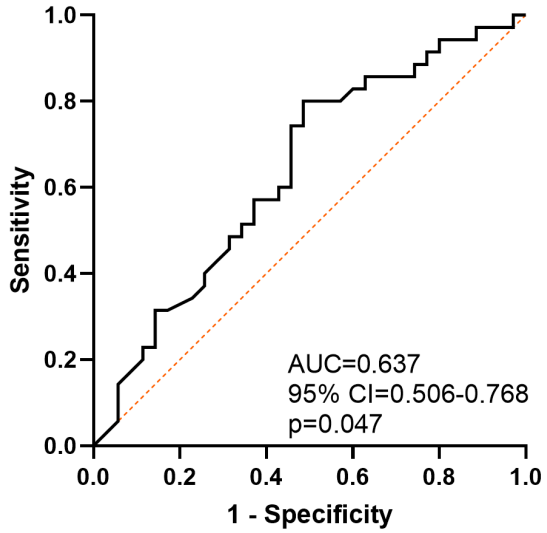


Figure 4. Receiver operating curve analysis of hsa-miR-675-5p diagnostic potential in oral cancer.
AUC – Area under curve
95% CI – 95% Confidence interval

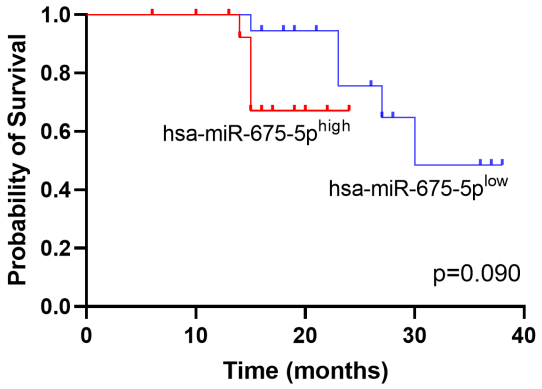


Figure 5. Kaplan-Meier curve of overall survival of oral cancer patients depending on hsa-miR-675-5p expression.
hsa-miR-675-5p^{high} and hsa-miR-675-5p^{low} are classified according to median value 0.021 reported as $2^{-\Delta Ct}$.

level in non-tumor samples was 2-fold higher than in tumor samples. In both groups, relative expression was variable with a mean $2^{-\Delta Ct}$ value of 0.127 ± 0.250 in tumor and 0.264 ± 0.362 in non-tumor tissue.

ROC curve analysis indicated acceptable discriminatory power of hsa-miR-675-5p for distinguishing oral tumor from non-tumor (AUC=0.637, 95% CI=0.506-0.768, p=0.047), **Figure 4**, with a proposed cut-off value of 0.106, estimated using the maximum Youden index (sensitivity + specificity -1) with a specificity and sensitivity of 80% and 51.4%, respectively.

The median value of hsa-miR-675-5p expression in tumor tissue ($2^{-\Delta Ct}$ value of 0.021) was used to classify it into low (hsa-miR-675-5p^{low}) or high (hsa-miR-675-5p^{high}) expression groups. There was no difference in survival between oral cancer patients with hsa-miR-675-5p^{low} and hsa-miR-675-5p^{high} expression (p=0.090, log-rank test), **Figure 5**. Survival rates were not significantly different when patients were stratified by tumor stage and size.

Relative expression of hsa-miR-675-5p and demographic and pathohistological features

Table 2 shows that no significant associations were found between the expression of either low or high hsa-miR-675-5p in tumor tissue and the demographic and pathohistological features of oral cancer patients. Cox regression analysis further indicated that hsa-miR-675-5p was not associated with oral cancer outcome (hazard risk=3.596, 95% CI=0.706-18.315, p=0.123).

DISCUSSION

The search for suitable molecular biomarkers in oral cancer research continues to offer vast potential. With the plethora of genes, non-coding transcripts and their interactions, the design of comprehensive gene panel for testing apperas promising. Nonetheless, focusing on the analysis of a single gene candidate is a valuable starting point for developing a gene panel for diagnostic and prognostic assesments of oral cancer patients. In this study, we investigated hsa-miR-675 as it arises from IGF2/H19 locus, often deregulated in different cancers, but unstudied in oral cancer.

The availability of public transcriptomic datasets across various cancer types underscores the importance of leveraging these data. However, conducting expression analysis in own clinical samples remains crucial and justified. In our investigation of hsa-miR-675, we started our exploration with bionformatics approach, initially checking KEGG pathways enriched in genes targeted by hsa-miR-675 and validated gene-miRNA interaction network. Notably, the disruption of signalling pathways, particulary metabolic pathwas and pathways in cancer, aligns with the hallmark characteristics of cancer cells (15). Our findings strongly suggest the involvement of hsa-miR-675 in cancerogenesis, thus validating our choice for further analysis.

Contrary to our expectations, expression analysis of hsa-miR-675 in the GDC TCGA, samples failed to reveal significant differences between cancer samples and solid normal tissues, thereby refuting hsa-miR-675 as a diagnostic and prognostic biomarker in oral cancer. This discrepancy maystem from the composition of the TCGA oral cancer cohort, which predominantely comprised cancer samples, potentially diluting any differences with normal tissues. Furthermore, the limited availability of matched samples highlights the need for robust analyses in more homogenous sample sets. Matched samples were only available for 18 oral cancer samples, representing a relatively modest study group. In a group twice as large as that studied by the GDC TCGA, we found significant

Table 2. hsa-miR-675-5p relative expression association with demographic and pathohistological features of oral cancer patients

Demographic and pathohistological features		Relative expression of hsa-miR-675-5p in tumor tissue				
		hsa-miR-675-5p^{low} N=18		hsa-miR-675-5p^{high} N=17		
		N	%	N	%	p value
Sex	male	14	77.8	12	70.6	0.627
	female	4	22.2	5	29.4	
Age (years, median)	<59	11	61.1	7	41.2	0.238
	>59	7	38.9	10	58.8	
Smoking habits	nonsmoker	4	22.2	6	35.3	0.392
	smoker + ex smoker	14	77.8	11	64.7	
Alcohol consumption	no intake	5	27.8	5	29.4	0.915
	moderate + high	13	72.2	12	70.6	
Oral hygiene	good	11	61.1	8	47.1	0.625
	poor	7	38.9	9	52.9	
Location of primary tumor	tongue	12	66.7	13	76.5	0.096
	hard palate	4	22.2	0	0	
	floor of the mouth	2	11.1	4	23.5	
Histological grade	well differentiated	5	27.8	5	29.4	0.944
	moderately differentiated	9	50	9	52.9	
	poorly differentiated	4	22.2	3	17.6	
Tumor size	≤2cm	1	5.6	1	5.9	0.672
	2-4cm	9	50	6	35.3	
	>4cm	8	44.4	10	58.8	
T staging	T1, T2	8	44.4	6	35.3	0.581
	T3, T4	10	55.6	11	64.7	
N staging	N0	9	50	9	52.9	0.862
	N1	9	50	8	47.1	
M staging	M0	18	100	17	100	-
	M1	0	0	0	0	
Recurrences	no	13	72.2	13	76.5	0.774
	yes	5	27.8	4	23.5	

hsa-miR-675-5p^{high} and hsa-miR-67-5p^{low} are classified according to median value 0.021 reported as $2^{-\Delta Ct}$.
N – number of cases; N0 – without regional lymph node involvement; N1- regional lymph node involvement; M0 – without metastases; M1 – present metastases.

hsa-miR-675-5p differences between tumor and non-tumor tissue. Our results of down-regulated hsa-miR-675-5p in oral cancer tissue are in line with findings reported for adrenocortical carcinoma (11), while opposite to findings in esophageal carcinoma (10) and laryngeal carcinoma and cell lines of head and neck cancer (16). In nasopharyngeal carcinoma and tongue cancer cell line, H19 and hsa-miR-675-5p were upregulated (17, 18). Based on these data, it can be assumed that hsa-miR-675 has dual role, both oncogen and tumor suppressor depending on the tumor type. The fact that hsa-miR-675-5p is less expressed in oral cancer tissue suggest that hsa-miR-675-5p could be considered as a potential therapeutic target in suspicious malignant lesions in oral cavity. These assumptions need to be clarified in future studies.

The data presented herein offer potential for early identification of oral cancer patients, even before clinical signs of the disease appear. Validating these findings entails assesing hsa-miR-675-5p levels across various clinical samples, including premalignant oral changes. Additionally, exploring the presence of hsa-miR-675-5p in body fluids, such as serum, plasma, saliva could confirm its candidacy as a non-invasive biomarker. Incorporating hsa-miR-675-5p into existing three-miRNAs signature panel for testing in oral cancer patients warrants further investigation in larger cohorts (7). All recommended investigations should be performed in a larger study group.

The prognostic potential of hsa-miR-675-5p was not confirmed, as there were no differences in overall survival between the groups with low and high hsa-miR-675-5p expression. The median of relative expression was used for classification since it is most commonly used in survival analysis. However, it is justified to set different cut-off values for the classification. In contrast to our results, in laryngeal carcinoma, high expression of hsa-miR-675 was associated with poor prognosis, disease-free survival and recurrence, indicating the predictive potential of hsa-miR-675 (16).

The exact mechanism underlying the involvment of hsa-miR-675-5p in oral cancer development is still un-

known. It has been shown that has-miR-675-5p promotes invasion and metastasis through downregulation of target gene SFN (17). Functional studies, including hsa-miR-675-5p mimic and knock-down, performed firstly in *in vitro* setting, should provide more information on role of this miRNA as well as it's host gene *H19* in cancerogenesis. A previous study reported increase in H19 is associated with an increase in hsa-miR-675 in tongue cancer patients (19). Lower expression of *H19* was found in tumor tissue than in non-tumor tissue of tongue cancer, which is consistent with our results since the majority of patients in our study group had oral cancer localised to the tongue. However, anatomic location of the tumor in oral cavity was not associated with hsa-miR-675-5p expression in our study, which justify considering different anatomic location alltogether in our study.

To fully elucidate the role of hsa-miR-675-5p in oral cancerogenesis, it would be useful to investigate the co-expression between hsa-miR-675-5p and its host *H19* as well as other genes in the IGF2/H19 locus. Since IGF2/H19 is an imprinted locus, it is recommended to determine methylation level in relation to the expression of hsa-miR-675-5p.

Co-expression of this miRNA and their target genes should be analyzed, too. So far, oral cancer literature data indicate that almost all structure genes gained through our bioinformatic approach, involved in significantly enriched pathways in cancer, are up regulated. Higher expression was obtained for mRNA of genes *RUNX1* (20), *MAP2K1* (21), *PRKCA* (22) *ITGA6* (23), *NFKB1* (24), as well as for protein products of genes *PRKCA* (25), *RB1* (26) and *WNT5A* (27). These results are in concordance with our results, if hsa-miR-675-5p is down regulated, it is expected that its target genes are up regulated.

CONCLUSION

The results of our study suggest a potential tumor-suppressive role of hsa-miR-675-5p and a highlight its utillity as a diagnostic biomarker in oral cancer. Further investigations should focus on the measurement of hsa-miR-675-5p in liquid biopsies, including saliva, serum and plasma across a larger cohort of oral cancer patients.

Author contributions:

Investigation: GS, MSV, KZ; Clinical samples collection: GS, NT, BB, MF, TI; Clinical data curation: GS, NT; BB, MF, TI; Research data curation: KZ; Formal analysis: KZ, MSV; Methodology: KZ, MSV; Validation: GS, KZ, MSV; Visualisation: KZ; Funding: GS, KZ; Supervision: KZ; Writing – original draft: KZ; Writing – review and editing: GS, MSV, NT, BB, MF, TI, KZ; Final approval of the article: GS, MSV, NT, BB, MF, TI, KZ.

Ethical approval:

Ethical approval for the collection and use of biological samples for research purposes was obtained from the Ethics committee of the Faculty of Medicine, University of Belgrade (approval number: 1550/VII-6).

REFERENCES

1. Sun R, Dou W, Liu W, Li J, Han X, Li S, et al. Global, regional, and national burden of oral cancer and its attributable risk factors from 1990 to 2019. Cancer Med 2023; 12: 13811-13820. doi: 10.1002/cam4.6025
2. Král D, Tvrdý P, Šašková L, Zapletalová J, Michálek J and Pink R. Evaluation of resection margins in oral squamous cell carcinoma. Acta Chir Plast 2023; 64: 110-115. doi: 10.48095/ccachp2022110
3. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297. doi: 10.1016/s0092-8674(04)00045-5
4. Bartel DP. Metazoan MicroRNAs. Cell 2018; 173: 20-51. doi: 10.1016/j.cell.2018.03.006
5. Chakrabortty A, Patton DJ and Smith BF. miRNAs: Potential as Biomarkers and Therapeutic Targets for Cancer. Genes (Basel) 2023; 14: 1375. doi: 10.3390/genes14071375
6. Condrat CE, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, et al. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. Cells 2020; 9: 276. doi: 10.3390/cells9020276
7. Stojkovic G, Jovanovic I, Dimitrijevic M, Jovanovic J, Tomanovic N, Stankovic A, et al. Meta-signature guided investigation of miRNA candidates as potential biomarkers of oral cancer. Oral Dis 2023; 29: 1550-1564. doi: 10.1111/odi.14185
8. Matouk IJ, Halle D, Gilon M and Hochberg A. The non-coding RNAs of the H19-IGF2 imprinted loci: a focus on biological roles and therapeutic potential in Lung Cancer. J Transl Med 2015; 13: 113. doi: 10.1186/s12967-015-0467-3
9. Xiao Q, Chen T, Wu Y, Wu W, Xu Y, Gong Z, et al. MicroRNA-675-3p promotes esophageal squamous cell cancer cell migration and invasion. Mol Med Rep 2018; 18: 3631-3640. doi: 10.3892/mmr.2018.9372
10. Zhou YW, Zhang H, Duan CJ, Gao Y, Cheng YD, He D, et al. miR-675-5p enhances tumorigenesis and metastasis of esophageal squamous cell carcinoma by targeting REPS2. Oncotarget 2016; 7: 30730-30747. doi: 10.18632/oncotarget.8950
11. Scicluna P, Caramuta S, Kjellin H, Xu C, Fröbom R, Akhtar M, et al. Altered expression of the IGF2-H19 locus and mitochondrial respiratory complexes in adrenocortical carcinoma. Int J Oncol 2022; 61. doi: 10.3892/ijo.2022.5430
12. Tastsoglou S, Skoufos G, Miliotis M, Karagkouni D, Koutsoukos I, Karavangeli A, et al. DIANA-miRPath v4.0: expanding target-based miRNA functional analysis in cell-type and tissue contexts. Nucleic Acids Res 2023; 51: W154-w159. doi: 10.1093/nar/gkad431
13. Chang L, Zhou G, Soufan O and Xia J. miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. Nucleic Acids Res 2020; 48: W244-w251. doi: 10.1093/nar/gkaa467
14. Goldman MJ and Craft B. Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol 2020; 38: 675-678. doi: 10.1038/s41587-020-0546-8
15. Martínez-Reyes I and Chandel NS. Cancer metabolism: looking forward. Nat Rev Cancer 2021; 21: 669-680. doi: 10.1038/s41568-021-00378-6
16. Guan GF, Zhang DJ, Wen LJ, Xin D, Liu Y, Yu DJ, et al. Overexpression of lncRNA H19/miR-675 promotes tumorigenesis in head and

neck squamous cell carcinoma. Int J Med Sci 2016; 13: 914-922. doi: 10.7150/ijms.16571

17. Zhang T, Lei F, Jiang T, Xie L, Huang P, Li P, et al. H19/miR-675-5p Targeting SFN Enhances the Invasion and Metastasis of Nasalpharyngeal Cancer Cells. Curr Mol Pharmacol 2019; 12: 324-333. doi: 10 .2174/1874467212666190719120446
18. Chang L and Wang D. Ginsenoside Rd inhibits migration and invasion of tongue cancer cells through H19/miR-675-5p/CDH1 axis. J Appl Oral Sci 2022; 30: e20220144. doi: 10.1590/1678-7757-2022-0144
19. Piao Z, Zou R, Lin Y, Li Z, Bai Z, Zhou L, et al. Overexpression of lncRNA H19 leads to reduced proliferation in TSCC cells through miR-675-5p/GPR55. Transl Cancer Res 2020; 9: 891-900. doi: 10.21037/tcr.2019.12.45
20. Feng X, Zheng Z, Wang Y, Song G, Wang L, Zhang Z, et al. Elevated RUNX1 is a prognostic biomarker for human head and neck squamous cell carcinoma. Exp Biol Med (Maywood) 2021; 246: 538-546. doi: 10.1177/1535370220969663
21. Mohammadi K RM, Mohammadpour H, Jamshidian F. Evaluation of Changes in miR-7113-3p, miR-6721-5p and MAP2K1 gene expressions in tumor and normal tissues of patients with oral cancer. Basic & Clinical Cancer Research 2023; 14: 102-112. doi:
22. Saproo S, Sarkar SS, Gautam V, Konyak CW, Dass G, Karmakar A, et al. Salivary protein kinase C alpha and novel microRNAs as diagnostic and therapeutic resistance markers for oral squamous cell carcinoma in Indian cohorts. Front Mol Biosci 2022; 9: 1106963. doi: 10.3389/fmolb.2022.1106963
23. Zhang C, Cai Q and Ke J. Poor Prognosis of Oral Squamous Cell Carcinoma Correlates With ITGA6. Int Dent J 2023; 73: 178-185. doi: 10.1016/j.identj.2022.05.010
24. Tang XH, Urvalek AM, Osei-Sarfo K, Zhang T, Scognamiglio T and Gudas LJ. Gene expression profiling signatures for the diagnosis and prevention of oral cavity carcinogenesis-genome-wide analysis using RNA-seq technology. Oncotarget 2015; 6: 24424-24435. doi: 10.18632/oncotarget.4420
25. Parzefall T, Schnoell J, Monschein L and Foki E. PRKCA Overexpression Is Frequent in Young Oral Tongue Squamous Cell Carcinoma Patients and Is Associated with Poor Prognosis. Cancers (Basel) 2021; 13. doi: 10.3390/cancers13092082
26. Thomas S, Balan A and Balaram P. The expression of retinoblastoma tumor suppressor protein in oral cancers and precancers: A clinicopathological study. Dent Res J (Isfahan) 2015; 12: 307-314. doi: 10.4103/1735-3327.161427
27. Prgomet Z, Andersson T and Lindberg P. Higher expression of WNT5A protein in oral squamous cell carcinoma compared with dysplasia and oral mucosa with a normal appearance. Eur J Oral Sci 2017; 125: 237-246. doi: 10.1111/eos.12352

EVALUACIJA EKSPRESIJE, DIJAGNOSTIČKOG I PROGNOSTIČKOG ZNAČAJA HSA-MIR-675-5P KOD ORALNOG KARCINOMA

Goran Stojković[1,2], Marija Savić Veselinović[3], Nikola Todorović[1], Bojana Bukurov[1,2], Miljan Folić[1,2], Tjaša Ivoševič[1,4], Katarina Zeljić[3]

Sažetak

Uvod: Oralni karcinom je najčešći podtip karcinoma u predelu glave i vrata, sa rastućom incidencom širom sveta. U svakodnevnoj kliničkoj praksi se ne koriste specifični biomarkeri za dijagnozu i prognozu ovog entiteta. Mali nekodirajući RNK molekuli, mikroRNK (miRNK), smatraju se dobrim kandidatima za biomarkere za uspostavljanje rane i senzitivne dijagnoze, kao i prognozu kod pacijenata sa oralnim karcinomom. mikroRNK koja se transkribuje sa H19 lokusa do sada nije ispitivana kod oralnog karcinoma.

Cilj: Cilj rada je bilo merenje relativne ekspresije hsa-miR-675-5p u tumorskom i netumorskom tkivu pacijenata sa oralnim karcinomom, kao i ispitivanje asocijacije ove miRNK sa patohistološkim karakteristikama pacijenata.

Materijal i metode: Studijsku grupu činilo je 35 pacijenata sa oralnim karcinomom. Od pacijenta uključenih u studiju prilikom ekscizije tumora uzet je uzorak tkiva tumora i okolnog netumorskog tkiva. Relativna ekspresija je merena kvantitativnom reverznom transkripcijom - PCR metodom u realnom vremenu.

Rezultati: Relativna ekspresija hsa-miR-675-5p bila je značajno niža u tumorskom nego u netumorskom tkivu, što ukazuje na njegovu supresivnu ulogu u tumoru. hsa-miR-675-5p ima dijagnostički potencijal za razlikovanje tumorskog od netumorskog tkiva kod pacijenata sa oralnim karcinomom. Nije bilo razlike u preživljavanju između pacijenata sa niskim i visokim nivoima ekspresije hsa-miR-675-5p, što ukazuje da se hsa-miR-675-5p ne može koristiti kao prognostički biomarker kod oralnog karcinoma.

Zaključak: hsa-miR-675-5p se može smatrati potencijalnim dijagnostičkim, ali ne i prognostičkim molekularnim biomarkerom u slučaju oralnog karcinoma.

Ključne reči: oralni karcinom, hsa-miR-675-5p, biomarker

Primljen: 23.05.2024. I **Revizija:** 18.07.2024. I **Prihvaćen:** 25.07.2024.

Medicinska istaživanja 2024; 57(3):99-108

Medicinska istaživanja 2024; 57(3):109-113 | DOI 10.5937/medi57-50559

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Treatment of painful flexible flatfoot in children after failed conservative treatment using a minimally invasive surgery technique

Bojan Bukva[1,2], Marko Majstorović[1,2], Siniša Dučić[1,2], Branislav Krivokapić[1,3], Vladimir Radlović[1,2], Goran Đuričić[1,4], Ninoslav Begović[1,5], Jasna Stojković[1,6], Tatjana Knežević[1,6], Dejan Nikolić[1,6]

[1]Faculty of Medicine, University of Belgrade, Serbia
[2]Department of Pediatric Surgery, University Children's Hospital, Belgrade, Serbia
[3]Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia
[4]Department of Radiology, University Children's Hospital, Belgrade, Serbia
[5]Institute for Mother and Child "Dr Vukan Čupić", Belgrade, Serbia
[6]Department of Physical Medicine and Rehabilitation, University Children's Hospital, Belgrade, Serbia

Recived: 20 April 2024
Revised:04 August 2024
Accepted: 07 August 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Bojan Bukva
University Children's Hospital,
10 Tiršova Street, 11000 Belgrade, Serbia
Email: bojanbukva@yahoo.com

Summary

Introduction/Aim: Flexible flatfoot (FFF) is one of the most common skeletal deformities in the pediatric population, especially in preadolescence. This retrospective observational study evaluates results of a calcaneal subtalar extra-articular arthrodesis (subtalar arthroereisis) with one cannulated screw for the treatment of painful flexible flatfoot in children, after previously failed conservative treatment.

Material and Methods: Preadolescent and adolescent pediatric patients were observed at the Department of Pediatric Orthopedic Surgery. The study included 28 feet of 15 children (5 girls and 10 boys) during a 6-year period, from 2016 to 2021 (average 36 months). The average age was 11.46 years (range 8-15 years). We performed the same operative technique under fluoroscopy for all patients. Radiographic parameters were taken before, after operative treatment, and after hardware removal. The following radiological parameters were measured: talo-calcanear (TC), talo-navicular (TN), talo-first metatarsal bone (T1MT) and calcanear pitch (CP) angles, in the anteroposterior and/or lateral views. Functional outcomes (extension and eversion of the foot) before surgery and after hardware removal were compared.

Results: After operative treatment, the midfoot radiographic parameters were not statistically significantly altered; however, the subtalar joint and forefoot were statistically significantly improved: both radiologically and clinically. The range of extension and eversion of the foot were remarkably reduced without disturbing the morphological and functional aspects of the talocrural and subtalar joints.

Conclusion: Extra-articular arthrodesis, using a cannulated screw is a minimally invasive technique, represents an optimal method for the operative treatment of symptomatic flexible flatfoot in children. This technique is simple, efficient and does not disrupt the anatomy of the foot.

Keywords: flexible flatfoot, extra-articular arthrodesis, minimally invasive surgery, children

Cite this article as: Bukva B, Majstorović M, Dučić S, Krivokapić B, Radlović V, Đuričić G, Begović N, Stojković J, Knežević T, Nikolić D. Treatment of painful flexible flatfoot in children after failed conservative treatment using a minimally invasive surgery technique; Medicinska istaživanja 2024; 57(3):109-113 DOI 10.5937/medi57-50559

INTRODUCTION

Flexible flatfoot (FFF) is the most common skeletal deformity in the pediatric population (1). It is represented as abnormally low, or absent, m e d i a l arch of the foot, in association with excessive eversion of the heel during weight-bearing and an abducted forefoot, producing a midfoot sag. During standing position on the toe tips, the longitudinal arch of the foot will reform because of the windlass mechanism of the plantar fascia.

Usually, FFF does not cause any symptoms. Rarely, FFF can cause calf or foot pain, gait disturbances or disability, at which point it becomes alarming (2). Factors that need to be taken into account in the treatment of FFF are: age, joint hypermobility, general hyperlaxity, degree of deformity and patient concerns (3,4). Initial treatment of FFF is physiotherapy and orthotics, but if those conservative methods fail, operative treatments should be considered (5). The goals of an operative treatment are: pain relief, foot alignment, stability of the foot during stance, and correction of an abnormal gait (5).

Extra-articular arthrodesis using a cannulated screw (subtalar arthroereisis) represents a minimal invasive surgical method of treatment. It involves the placement of an implant, or bone graft, within the sinus tarsi to restrict excessive motion at the subtalar joint. Subtalar arthroereisis corrects the hindfoot valgus deformity and restores plantar-flexion without overly disrupting the anatomy of the foot (2,6).

The aim of this study was to evaluate radiological and clinical parameters of a minimal invasive surgical technique for the treatment of flexible flatfoot in children, using an extra-articular arthrodesis with a cannulated screw, after failed conservative treatment.

MATERIAL AND METHODS

Study sample

We retrospectively analyzed the results of 28 feet (15 patients), aged from 8 to 15 years (average 11.46±3.33 years), during a six-year period (from January 2016 to December 2021). This included 5 girls and 10 boys with lower leg or foot symptoms (pain and discomfort) after failed physiotherapy. The average follow-up was 26 months (range 18-78 months).

Inclusion criteria for this study was flexible flatfoot with pain or discomfort in the lower leg, calf or foot, in pediatric patients with open growth plates, with no previous surgeries of the foot, ankle or lower leg. According to Johnson and Strom classification system, all our patients were stage II, which include moderate swelling and tenderness along posterior tibial tendon, marked weakness in "heel-rise test", flexible positive deformity in talonavicular joint and positive "too many toes sign" (7).

Patients with systemic disease, neurological disorders and patients with chronic diseases or bone dysplasia were excluded. Patients on chemo- or radiotherapy and patients on corticosteroids were also excluded.

Ethical approval was obtained from the Human Ethics Research Committee.

Surgical procedure

An oblique dorso-lateral 2-cm incision was made over the sinus tarsi of the foot, following the natural skin lines. The underlying extensor brevis muscle was dissected from its surrounding tissues and retracted distally. The sinus tarsi was cleaned of all fat and soft tissue. The varus-valgus position of the heel was assessed on clinical examination intraoperatively. The calcaneus was rotated under the talus and the foot was held with the ankle in equinus and in inversion. Under fluoroscopic guidance, the threaded guidewire was driven from the talar neck projection line into the calcaneus (in the antero-posterior [AP] and lateral view). A short tunnel was drilled over the guidewire, followed by insertion of an AO (Arbeitsgemeinschaft für Osteosynthesefragen) cannulated screw (3.5-4.5mm diameter and 30-35 mm in length, depending on the size of the bone). During surgery, it is important that the screw does not enter the plantar cortex of the calcaneus. Postoperatively, a cast was not applied, with weight bearing occurring two days after surgery. The X-rays in the standing position (in AP and lateral view) were done during the first postoperative week. None of our patients had a triceps surae contracture, so elongation of the Achilles tendon was not performed.

Patients were evaluated according to the clinical and radiological findings. Following parameters were observed: demographic data (age, gender, side of surgery), clinical data (passive dorsiflexion and passive eversion of the foot), and radiological data [talo-calcanear (TC), talo-navicular (TN), talo-first metatarsal bone (T1MT) and calcaneal pitch (CP)] in the antero-posterior (AP) and lateral (PRO) views]. Clinical and radiological data were correlated before surgery and after hardware removal (18-36 months after the surgery). Goniometer was used to measure clinical data and values were expressed in angle degrees.

Statistical analysis

Data was analyzed using the Kolmogorov Smirnov test (for examination of a variable's deviation from the normal distribution) and one-way repeated measures using ANOVA. Data was presented as mean and standard deviation. When required, the Bonferroni correction was used to assess particular significant differences. Partial eta squared (partial η^2) was used for effect size assessment. Type one error was set at α=5%. Statistical significance was set at a p-level of $p<0.05$. For statistical analysis of the data we used the data analysis software system Statistica for Windows (version 13.0., Dell Inc., Tulsa, OK, USA).

Table 1. Analysis of radiological parameters in the treatment of flexible flatfoot

Parameter	Before surgery*	After surgery*	F-value**	p-value**	η^2value***
TC (AP)	28.11±8.11	21.14±7.26	93.309	p<0.001	0.776
TC (Lat)	29.64±8.61	23.64±7.44	26.321	p<0.001	0.494
T1MT (AP)	19.79±5.79	13.11±5.10	69.943	p<0.001	0.721
T1MT (Lat)	16.75±7.76	12.46±6.05	12.099	p=0.001	0.309
TN (AP)	16.36±5.79	15.07±6.35	2.178	p=0.152	0.075
TN (Lat)	11.25±4.93	11.43±6.40	0.012	p=0.914	0.000
CP	12.00±3.97	16.46±2.95	37.956	p<0.001	0.584

* Expressed in angle degrees (mean values±SD); ** One-way repeated measures ANOVA; *** Partial eta squared (partial η^2) for effect size assessment; TC (AP): talo-calcaneal angle in antero-posterior view; TC(Lat): talo-calcaneal angle in lateral view; T1MT (AP): talo-first metatarsal angle in antero-posterior view; T1MT (Lat): talo-first metatarsal angle in lateral view; TN (AP): talo-navicular angle in antero-posterior view; TN (Lat): talo-navicular angle in lateral view; CP: "calcaneal pitch" angle

RESULTS

We studied 28 feet (in 15 patients) with an average follow-up of 26 months (range 18-78 months). From all participants 13 out of the 15 patients had surgery on both feet. Using the Kolmogorov-Smirnov test, it was calculated that all observed variables consistently had a normal distribution. The data set had no significant outliers. We found no statistical significance in either radiological or clinical parameters between both left and right feet.

In analysis of radiological parameters, after the surgery there were statistically significant differences in the talo-calcaneal (TC), talo-firstmetatarsal (T1MT) and calcaneal pitch (CP) angles, while there was no statistical significance with respect to the talo-navicular (TN) angle **(Table 1)**.

The values of radiological parameters related to hindfoot; the subtalar (talocalcaneal) joint (TC) and "calcaneal pitch" (CP), and to forefoot (T1MT) were significantly improved after the surgery, whereas there was mild improvement in midfoot values, expressed in talo-navicular (TN) angle values.

When comparing pre- and post-surgery assessment, most improvement was seen in the clinical parameters (range of extension and eversion) **(Table 2)**. Passive extension and eversion of the foot were statistically improved before and after the surgery **(Table 2)**.

DISCUSSION

Many surgical procedures for the correction of symptomatic flexible flat feet (FFF) have been proposed. The ideal operative procedure should result in a painless foot and lower leg, with a physiological longitudinal arch, full range of motion and function of the talocrural and subtalar joints (2). Operative treatment of FFF is rarely indicated and should not be performed under the age of eight years (2). In all cases, contracture of the triceps surae muscles should be corrected.

Operative procedures can be classified as:

- soft tissue procedures (plications, tendon transfers, tendon lengthening)
- bone and joint procedures (osteotomies, bone excisions, arthrodesis)
- use of implants (bone or synthetic) into the calcaneus into the sinus tarsi (8-14)

Chronic pain and gait disturbances are possible and rare complications of subtalar arthrodesis (15). In 1972, Recardo Alvarez published the original technique for subtalar extra-articular arthorodesis (subtalar arthroereisis) – since then, many variant techniques have been reported (16). Subtalar arthoereisis has an immediate mechanical effect, in elevation of the talus in sinus tarsi, without any disruption of foot anatomy. It also has a proprioceptive effect, controlling the compressive and directional muscular forces (17). The subtalar joint has a critical role in proprioception of the foot in contact with the ground (17). Some authors propose that sinus tarsi pain originates mostly from mechanoceptors and nociceptors (17,18). However, some publications showed that patients with sinus tarsi pain and instability in the subtalar joint have a prolonged peroneal reaction time (PRT), causing irregular peroneal muscle activity, ultimately leading to pain and instability in the sinus tarsi (19-21). In this retrospective study with cannulated screw insertion into sinus tarsi in patients with painful flatfeet, the radiological

Table 2. Analysis of clinical parameters in the treatment of flexible flatfoot.

Parameter	Before surgery*	After surgery*	F-value**	p value**	η^2value***
Extension	24.75±7.25	11.68±7.76	293.668	p<0.001	0.916
Eversion	18.93±5.33	6.18±5.08	303.263	p<0.001	0.918

* Expressed in angle degrees (mean values±SD); ** One-way repeated measures ANOVA; *** Partial eta squared (partial η^2) for effect size assessment

and clinical improvement was obtain, particularly in the forefoot and hindfoot. And the most important, all participants were painless after the surgery.

In this study, the major complaint for all participants was forefoot supination and intoeing gait after surgery, which we did not consider as complication. Also, we did not consider the effusion in the subtalar joint and in the sinus tarsi as the complication. All of those signs were temporary, and resolved 3 months post-surgery. The major complication in this study (occurring in one participant) was the perforation of the distal part of the calcaneus by the cannulated screw, requiring subsequent screw removal.

Some authors report that fracture of the fourth metatarsal bone can occur in corrective surgical techniques, since the fifth ray of the foot is more mobile and contracture of the peroneal muscles due to the antalgic position of the foot occurs (16). There were no such complications in patients in this study.

There are limitations to this study: a small number of patients, retrospective, no qualitative patient satisfaction survey and there was no control group of children with symptomatic FFF treated using a different surgical technique for comparison.

CONCLUSION

Most children with flexible flatfeet are asymptomatic and do not require treatment. Symptomatic children, however, may benefit from conservative (non-operative) treatment. Non-operative treatment (physical therapy, shoe insoles and orthotics) is usually successful. If this fails, operative treatment is an option. Extra-articular arthrodesis, using a cannulated screw (subtalar arthroereisis), remains an excellent method for surgical flexible flatfeet correction. It does not disrupt the physiological function, or the anatomy of the foot, and has immediate beneficial post-surgery effects. The technique is simple, easily and quickly performed with the screw providing mechanical support and a beneficial proprioceptive effect in the sinus tarsi. The mechanical load over the medial side of the foot (talo-naviculo-cuneiform joint, plantar aponeurosis and medial ligaments) is minimized and an ideal physiological load balance of the foot can be achieved. Indications for surgery must be precise to avoid any possibility of overtreatment.

REFERENCES

1. Vukasinovic Z. Specijalna ortopedija. Beograd: IOHB Banjica; 2004.
2. Mosca VS. Flexible flatfoot in children and adolescents. J Child Orthop. 2010; 4: 107-121. doi: 10.1007/s11832-010-0239-9, PMID: 21455468.
3. Mosca VS. Flexible flatfoot and skewfoot. J Bone Joint Surg. 1995; 77A: 1937-45.
4. Bourdet C, Seringe R, Adamsbaum C, Glorion C, Wicart P. Flatfoot in children and adolescents. Analysis of imaging findings and therapeutic implications. Orthop Traumatol Surg Res. 2013; 99: 80-87. doi: 10.1016/j.otsr.2012.10.008, PMID: 23260367.
5. Giannini S, Girolami M, Ceccarelli F. The surgical treatment of infantile flat foot. A new expanding endo-orthotic implant. Ital J Orthop Traumatol. 1985; 11: 315-22. PMID: 4086278.
6. Hoke M. An operation for the correction of extremely relaxed flat feet. J Bone Joint Surg. 1931; 13: 773-83.
7. Johnson KA, Strom DE. Tibialis posterior tendon dysfunction. Clin Orthop Relat Res. 1989; 239: 196–206. PMID: 2912622.
8. Lee MS, Vanore JV, Thomas JL, Catanzariti AR, Kogler G, Kravitz SR, et al. Diagnosis and treatment of adult flatfoot. J Foot Ankle Surg. 2005; 44: 78-113. doi: 10.1053/j.jfas.2004.12.001, PMID: 15768358.
9. Addante JB, Chin MW, Loomis JC. Subtalar joint arthroereisis with SILASTIC silicone sphere: a retrospective study. J Foot Surg. 1992; 31: 47–51. PMID: 1315351
10. Adelman VR, Szczepanski JA, Adelman RP. Radiographic evaluation of endoscopic gastrocnemius recession, subtalar joint arthroereisis, and flexor tendon transfer for surgical correction of stage II posterior tibial tendon dysfunction: a pilot study. J Foot Ankle Surg. 2008; 47: 400-408. doi: 10.1053/j.jfas.2008.06.005, PMID: 18725119.
11. Brancheau SP, Walker KM, Northcutt DR. An analysis of outcomes after use of the Maxwell-Brancheau Arthroereisis implant. J Foot Ankle Surg. 2012; 51: 3-8. doi: 10.1053/j.jfas.2011.10.019, PMID: 22196453.
12. Koning PM, Heesterbeek PJ, de Visser E. Subtalar arthroereisis for pediatric flexible pes planovalgus: fifteen years experience with the cone-shaped implant. J Am Podiatr Med Assoc. 2009; 99: 447-453. doi: 10.7547/0990447, PMID: 19767554.
13. Kwon JY, Myerson MS. Management of the flexible flat foot in the child: a focus on the use of osteotomies for correction. Foot Ankle Clin. 2010; 15: 309-322. doi: 10.1016/j.fcl.2010.02.001, PMID: 20534358.
14. Metcalfe SA, Bowling FL, Reeves ND. Subtalar joint arthroereisis in the management of pediatric flexible flatfoot: a critical review of the literature. Foot Ankle Int. 2011; 32: 1127-1139. doi: 10.3113/FAI.2011.1127, PMID: 22381197.
15. Hazany S, Ly N, Hazany D, Bader S, Ostuka N. Outcomes of subtalar arthroereisis for the planovalgus foot. J Surg Orthop Adv. 2012; 21: 147-150. doi: 10.3113/jsoa.2012.0147, PMID: 23199943.
16. De Pellegrin M, Moharamzadeh D, Strobl WM, Biedermann R, Tschauner C, Wirth T. Subtalar extra-articular screw arthroereisis (SESA) for the treatment of flexible flatfoot in children. J Child Orthop. 2014; 8: 479-487. doi: 10.1007/s11832-014-0619-7, PMID: 25413354
17. Frank CB. Ligament structure, physiology and function. J Musculoskelet Neuronal Interact. 2004; 4: 199-201. PMID: 15615126.
18. Stagni R, Leardini A, O'Connor JJ, Giannini S. Role of passive structures in the mobility and stability of the human subtalar joint: a literature review. Foot Ankle Int. 2003; 24: 402-409. doi: 10.1177/107110070302400505, PMID: 12801196.
19. Rein S, Hagert E, Hanisch U, Lwowski S, Fieguth A, Zwipp H. Immunohistochemical analysis of sensory nerve endings in ankle ligaments: a cadaver study. Cells Tissues Organs. 2013; 197: 64-76. doi: 10.1159/000339877, PMID: 22964904.
20. Rein S, Hanisch U, Zwipp H, Fieguth A, Lwowski S, Hagert E. Comparative analysis of inter- and intraligamentous distribution of sensory nerve endings in ankle ligaments: a cadaver study. Foot Ankle Int. 2013; 34: 1017-24. doi: 10.1177/1071100713480862, PMID: 23456084.
21. Khin-Myo-Hla, Ishii T, Sakane M, Hayashi K. Effect of anesthesia of the sinus tarsi on peroneal reaction time in patients with functional instability of the ankle. Foot Ankle Int. 1999; 20: 554-559. doi: 10.1177/107110079902000903, PMID: 10509681.

LEČENJE BOLNOG RAVNOG STOPALA KOD DECE KORIŠĆENJEM MINIMALNO INVAZIVNE HIRURŠKE PROCEDURE NAKON NEUSPELOG KONZERVATIVNOG LEČENJA

Bojan Bukva, Marko Majstorović, Siniša Dučić, Branislav Krivokapić, Vladimir Radlović, Goran Đuričić, Ninoslav Begović, Jasna Stojković, Tatjana Knežević, Dejan Nikolić

Sažetak

Uvod: Fleksibilno ravno stopalo je jedan od najčešćih deformiteta u pedijatrijskoj populaciji, naročito u periodu preadolescencije. Ova retrospektivna opservaciona studija se odnosi na evaluaciju rezultata lečenja ekstra-artrikularnom subtalarnom artrodezom pomoću jednog kanuliranog zavrtnja (subtalarna artroereiza), u lečenju bolnih ravnih stopala, a nakon neuspelog konzervativnog lečenja,

Materijal i metode: Ispitanici su bili preadolesceti i adolescent lečeni na Odeljenju dečje ortopedije i traumatologije Univerzitetske dečje klinike u Beogradu. Studija je obuhvatala ukupno 28 stopala , odnosno 15 dece (5 devojčica i 10 dečaka), tokom perioda od 6 godina (prosečno 36 meseci). Prosečni uzrast pacijenata je bio 11.46 godina (opseg 8-15 godina). Svi pacijenti su lečeni istom hirurškom procedurom pod kontrolom RTG pojačivača slike. Radiografski parametri su mereni pre i posle hirurške intervencije, kao i posle vađenja osteosintetskog materijala. Posmatrali smo sledeće radiografske parametre: talo-kalkanearni (TC), talo-navikularni (TN), talo-prvi metatarzalni ugao (T1MT) i uzdignutost petne kosti ("calcanear pitch" -CP) u antero-posteriornoj i profilnoj projekciji. Takođe, poredili smo funkcionalne (kliničke) rezultate, u vidu stepena opružanja (ekstenzije) i izvrtanja (everzije) stopala pre hirurške intervencije, posle intervencije i nakon vađenja osteosintetskog materijala.

Rezultati: Nakon hirurškog lečenja navedeni radiografski parametri u nivou srednjeg dela stopala nisu statistički značajnije promenjeni u odnosu na preoperativne, ali u nivou prednjeg segmenta stopala i subtalarnog zgloba navedeni klinički i radiografski parametri su značajno poboljšani. Obim pokreta opružanja (ekstenzije) i izvrtanja (everzije) su statistički značajno redukovani, bez morfoloških i funkcionalnih poremećaja talokruralnog i subtalarnog zgloba.

Zaključak: Ekstra-artikularna subtalarna artrodeza pomoću jednog kanuliranog zavrtnja, kao metoda minimalno invazivne hirurške procedure, predstavlja optimalni metod hirurškog lečenja simptomatskih fleksibilnih ravnih stopala kod dece. Ova tehnika je jednostavna za izvođenje, efikasna i ne remeti fiziološku anatomiju stopala.

Vrsta studije: retrospektivna studija, nivo dokaza IV

Ključne reči: fleksibilno ravno stopalo, subtalarna artroereiza; minimalno invazivna hirurgije, deca, pedijatrija

Primljen: 20.04.2024. I **Revizija:** 04.08.2024. I **Prihvaćen:** 07.08.2024.

Medicinska istaživanja 2024; 57(3):109-113

Medicinska istaživanja 2024; 57(3):109-113 | DOI 10.5937/medi57-52226

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Early treatment response of breast cancer brain metastases to gamma knife stereotactic radiosurgery

Jovanović Marija[1, 4], Davidović Kristina[1, 4], Petković Ana[2], Šeha Biljana[2], Jeremić Danilo[3,4], Milićević Mihailo[2,4], Bogdanović Ivan[2, 4], Rosanda Ilić[2, 4]

[1] University Clinical Center of Serbia, Center of Radiology, Belgrade, Serbia
[2] University Clinical Center of Serbia, Clinic for Neurosurgery, Belgrade, Serbia
[3] Institute of Orthopedics Banjica, Belgrade, Serbia
[4] University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Recived: 16 July 2024
Revised: 06 August 2024
Accepted: 19 August 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Jovanovic Marija
University Clinical Center of Serbia, Center of Radiology,
2 Pasterova Street, Belgrade, Serbia
Email: macvanskimarija@yahoo.com

Summary

Introduction: Brain metastases represent the most common intracranial malignancy in the adult population, while breast cancer represents the leading cause of brain metastases among women. Brain metastases have increased in recent years due to improved therapeutic control of systemic disease and better diagnostic tools. Stereotactic radiosurgery (SRS) is used in patients with brain tumors to achieve local disease control, preserve the quality of life, and extend patient survival. This study aimed to evaluate the effects of SRS in patients with brain metastases from breast cancer through analysis of magnetic resonance imaging (MRI) parameters of the brain.

Methods: Brain MRI was conducted in 30 adult female patients before and 3-6 months after SRS treatment. Radiological analysis was used to estimate lesion volumes before and after SRS.

Results: Patients were categorized into four groups based on therapeutic response: 1 - complete response (CR) with loss of the lesion, 2 - partial response (PR) with more than 50% reduction in lesion volume, 3 - disease progression (PD) with more than 25% increase in lesion volume, 4 - stable disease (SD) if the patient did not display PR or PD. Among all patients, it was found that CR was present in 0 (0%), PR in 15 (50%), PD in 1 (3%), and SD in 14 (47%) patients. Following the SRS treatment, a statistically significant reduction in tumor volume was observed ($p<0.001$).

Conclusion: Radiological volumetric analysis of brain metastases after SRS showed a statistically significant reduction in lesion volume, demonstrating effective local disease control.

Keywords: brain metastases, breast cancer, stereotactic radiosurgery, magnetic resonance imaging

Cite this article as: Jovanović M, Davidović K, Petković A, Seha B, Jeremić D, Milićević M, Bogdanović I, Ilić R. Early treatment response of breast cancer brain metastases to gamma knife stereotactic radiosurgery; Medicinska istaživanja 2024; 57(3):115-121 DOI 10.5937/medi57-52226

INTRODUCTION

Brain metastases represent the most common intracranial malignancy in the adult population, while breast cancer represents the leading cause of brain metastases among women (1, 2). Brain metastases from breast cancer occur in 20-40% of cases during the disease, with a higher risk in patients older than 41 years, those with human epidermal growth factor receptor 2 (HER2) positive or triple-negative breast cancer subtypes, and those with existing metastases at 2-3 extracranial sites (1-3). Approximately 50% of brain metastases from breast cancer are solitary, while multicentric lesions occur in the remaining cases (1). The increase in brain metastases over the last decade can be attributed to advancements in therapeutic modalities, longer patient survival, improved lesion detection, and more frequent use of magnetic resonance imaging (MRI) of the brain (1, 4, 5).

Stereotactic radiosurgery (SRS) is currently the method of choice for the non-invasive treatment of brain metastases, providing local disease control with minimal toxicity to the surrounding brain structures (4). It involves delivering a high dose of focal Gamma radiation to a precisely defined volume, typically in a single fraction, in patients with 1-10 brain metastases totaling less than 15 ml in volume. Assessing the effect of SRS on brain metastatic lesions is crucial for planning further local and systemic therapies for patients, with MRI of the brain being a pivotal method both for diagnosis and for therapeutic planning and post-therapy monitoring of patients.

In recent years, various research groups have proposed different criteria to achieve more objective measures for evaluating the post-therapy response of brain tumors (6-18). Many authors, including the leading RANO (The Response Assessment in Neuro-Oncology) working group, advocate for linear 1-dimensional (1D) or 2-dimensional (2D) models of analysis by measuring 1 or 2 largest diameters of the lesion (6-12) while volumetric 3-dimensional (3D) analysis has been performed in fewer individual studies (13-20). Given that brain metastasis is treated with SRS based on its volume, volumetric analysis is the most objective method for evaluating tumor response to applied therapy (21, 22).

Although the number of studies confirming the significance of volumetric measurements of lesions is increasing, most focus on analyzing metastases regardless of their primary origin (13-20). To date, only two studies have specifically addressed the volumetric analysis of metastatic lesions from breast cancer (23, 24). Therefore, this study aimed to assess the early therapeutic effect of SRS in local disease control of brain metastases originating from breast cancer through MRI volumetric analysis, thereby contributing to the scientific literature in this field.

MATERIALS AND METHODS

A retrospective study was conducted at the Department of Magnetic Resonance Imaging at the Center for Radiology of the University Clinical Center of Serbia (UCCS) involving 30 adult female patients with solitary brain metastases originating from breast cancer. The study was approved by the Ethics Committee of UCCS (number 1264/12).

From 2023 to 2024, all patients underwent stereotactic radiosurgery (SRS) treatment at the Department of Stereotactic Neurosurgery—Gamma Knife Center for Neuro-oncology, Clinic of Neurosurgery, UCCS. The average dose of focused SRS radiation delivered was 20 Gy.

MRI of the brain was performed for all patients before and 3-6 months after SRS using a 3 Tesla MRI with a 32-channel head coil. The MRI protocol for intracranial examination included: axial and sagittal T2-weighted imaging (T2WI) [time of echo/time of recovery (TE/TR) = 5000/98 ms, slice thickness/gap = 5/1 mm, field of view (FOV) = 23 cm], axial T1-weighted imaging (T1WI) [TE/TR = 220/4.8 ms, slice thickness/gap = 5.1 mm, FOV = 23 cm], coronal T2-weighted FLAIR (fluid-attenuated inversion recovery) sequence [TE/TR = 9000/97 ms, time of inversion (TI) = 2500 ms, slice thickness/gap = 5/1 mm, FOV = 23 cm], and diffusion-weighted imaging (DWI) [TR/TE = 3800/68 ms, slice thickness/gap = 5/1 mm, FOV = 23 cm] with b-values of 0 and 1000 s/mm^2, along with calculation of apparent diffusion coefficient (ADC) maps.

Following intravenous administration of gadolinium-based contrast agent (0.1 mmol/kg body weight; gadobutrol; Gadovist, Bayer, UK), a three-dimensional T1-weighted MPRAGE (magnetization-prepared rapid acquisition gradient-echo) sequence [TE/TR = 2400/3.6 ms, TI = 1000 ms, slice thickness/gap = 5/1 mm, FOV = 24 cm] was acquired.

Brain MRI findings were analyzed using commercial software (Syngo Via, Siemens, Germany). Lesion dimensions were measured on post-contrast T1-weighted images, calculating the anteroposterior (d1), laterolateral (d2), and craniocaudal (d3) diameters in millimeters. Lesion volume was calculated using the formula:

$$V = \frac{3}{4} \pi \times d1/2 \times d2/2 \times d3/2.$$

Figure 1 shows a representative MRI image of the brain in a patient with brain metastasis before and after SRS.

Statistical analysis

Descriptive and analytical statistical methods were used in this study. Central tendency measures (mean) and dispersion measures (standard deviation, SD) were used for continuous numerical variables. Categorical variables

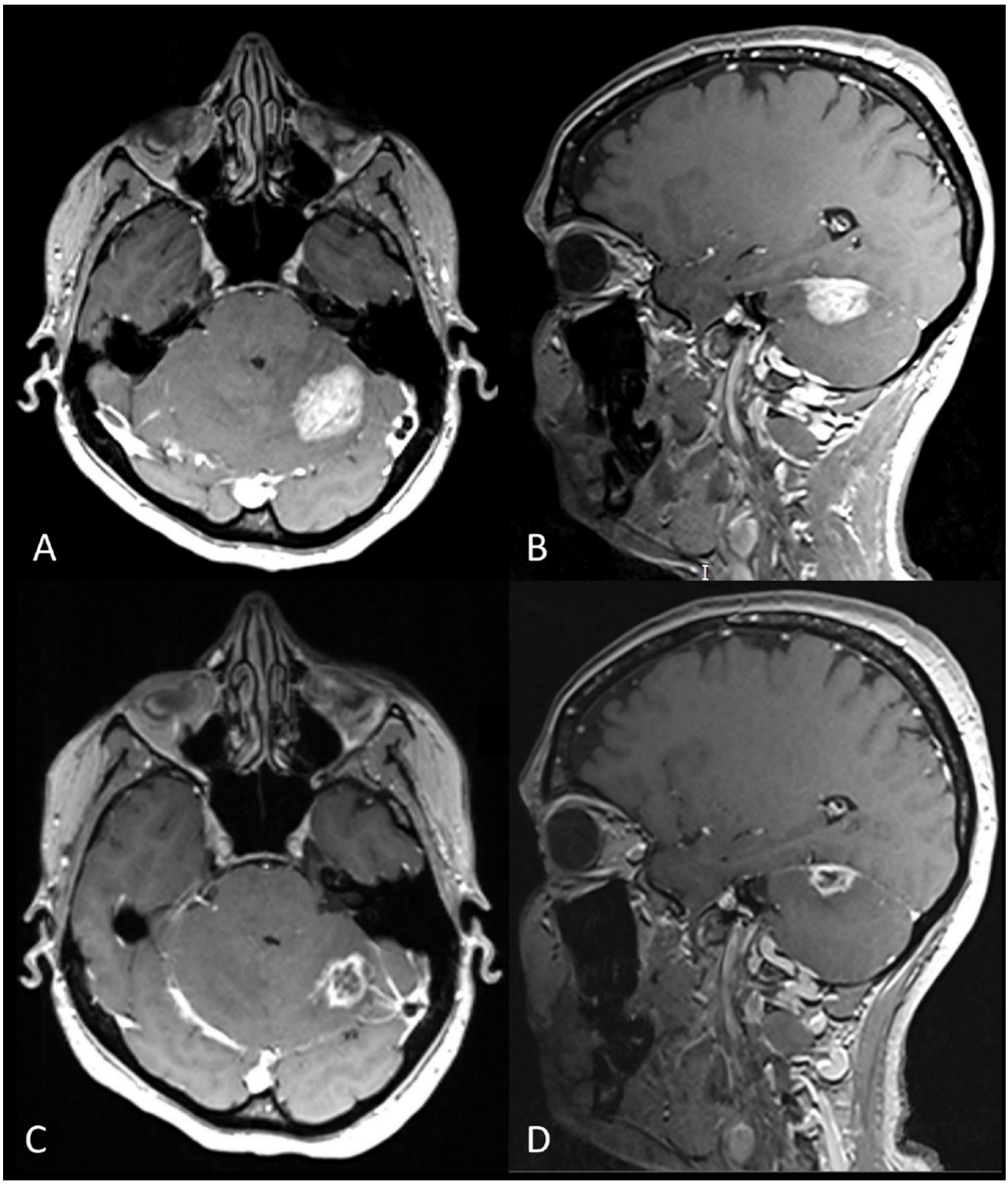


Figure 1. MRI of brain metastasis before and 3 months after SRS in a 57-year-old patient with breast carcinoma. (A) Axial postcontrast T1WI before SRS, (B) sagittal postcontrast T1WI before SRS, (C) axial postcontrast T1WI after SRS, (D) sagittal postcontrast T1WI after SRS. Expansive lesion in the left cerebellar hemisphere with inhomogeneous postcontrast enhancement and edema, with partial response (PR) after SRS.

were presented as absolute numbers with percentages. Results were presented in tables and graphs. All data were analyzed using SPSS 20.0 (IBM Corp., Armonk, NY) and R 3.4.2 (R Core Team, Vienna, Austria) statistical software packages. A value of less than 0.05 for type-1 statistical error was considered statistical significance (alpha) for hypothesis testing.

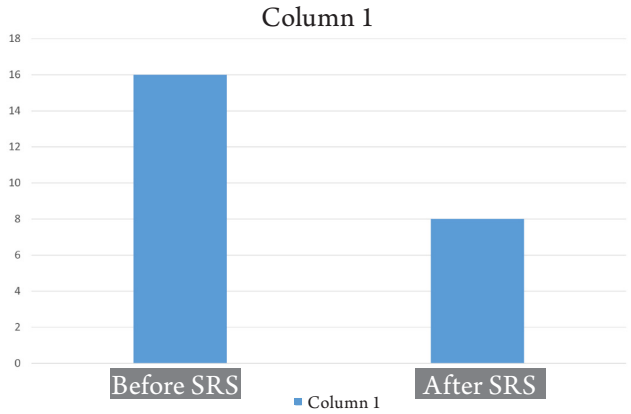
RESULTS

The study included a total of 30 female patients with solitary brain metastases originating from breast cancer. The average age of the participants was 60 (ranging from 46 to 64 years).

Table 1. Arithmetic mean value and standard deviation (SD) of lesion diameter and volume before and after SRS size (statistically significant change - p<0.001).

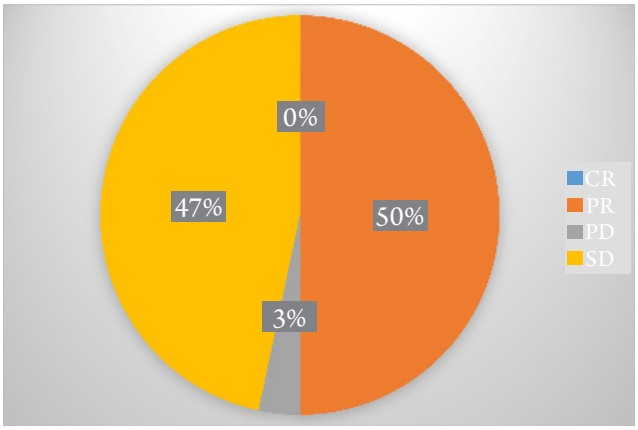
	AP1 (mm)	AP2 (mm)	LL1 (mm)	LL2 (mm)	CC1 (mm)	CC2 (mm)	V1 (cm3)	V2 (cm3)	%
Mean	13.53	10.87	12.77	9.53	12.43	9.30	1.57	0.77	46.87
SD	8.48	8.61	8.55	7.59	8.55	5.76	2.81	1.58	23.81

Following the SRS treatment, a statistically significant change in tumor volume was observed, indicating a significant reduction in lesion size (p<0.001). The mean with standard deviation of lesion diameters and volumes before and after SRS is presented in **Table 1**, and the change in brain metastasis volume values before and after SRS expressed in cm^3 is shown in **Graph 1**.



Graph 1. Volume changes of brain metastasis before and after SRS in cm3 size (statistically significant change – p<0.001).

By calculating the percentage change in lesion volume values before and after SRS, patients were categorized into 4 groups based on therapeutic response: 1 - Complete Response (CR), 2 - Partial Response (PR) with more than 50% reduction in lesion volume, 3 - Progressive Disease (PD) with more than 25% increase in lesion volume, 4 - Stable Disease (SD) if the patient's condition did not meet PR or PD criteria. Among the total number of patients, it was found that CR was present in 0 patients (0%), PR in 15 patients (50%), PD in 1 patient (3%), and SD in 14 patients (47%).



Graph 2. Therapeutic response of brain metastasis after SRS in percentages.
CR – complete response, PR – partial response, PD – progressive disease, SD – stable disease

The distribution of therapeutic responses in patients with solitary brain metastases from breast cancer after SRS, expressed in percentages, is illustrated in **Graph 2**.

DISCUSSION

In this study, we analyzed changes in brain metastasis volumes before and 3-6 months after SRS therapy in patients with primary breast cancer. By evaluating brain MRI parameters, the study enabled the assessment of lesion size changes and therapeutic responses to SRS. Radiological and statistical analyses confirmed a significant reduction in metastatic lesion volume shortly after SRS, validating its effectiveness in local disease control of brain metastases.

SRS is a preferred therapeutic modality for treating brain metastases, used either as monotherapy or adjunctive therapy alongside whole-brain radiotherapy (WBRT) or surgical resection (1, 4, 25, 26). WBRT, known for its significant neurotoxic effects, can lead to long-term neurocognitive decline and cerebellar dysfunction in treated patients. Furthermore, due to these potential side effects, its clinical use has been limited to palliative care settings (1).

In contrast, SRS delivers a high dose of focal Gamma radiation to a precisely defined volume, achieving local disease control with minimal toxicity to surrounding healthy brain structures. It is typically recommended for patients with a good clinical status and up to 4 brain metastases or up to 10 lesions with a total volume not exceeding 15 ml (1, 4, 25, 26). SRS is most commonly delivered as a single radiation fraction ranging from 15 to 24 Gy. In contrast, 2-5 fractions are recommended for larger lesions (>3 cm in diameter), lesions near critical structures like the brainstem, optic nerves, or optic chiasm, or in patients who have previously received cranial radiation therapy (1, 4, 25). The primary goals of SRS are long-term local tumor control, improved quality of life, and extended patient survival. As a result, the use of SRS for treating brain metastases has significantly increased.

In local disease control, SRS and surgical treatments are competitive therapeutic approaches. Neurosurgical tumor resection favors scenarios such as unknown pri-

mary tumors, unreliable neuroradiological lesion diagnosis, large cystic or necrotic lesions, the need for high-dose corticosteroid therapy, significant compressive effects of the lesion on surrounding structures, or the necessity for lesion molecular profiling for therapeutic purposes. Despite studies demonstrating the benefits of surgical resection, many patients are not optimal candidates due to their poor overall medical condition, other medical comorbidities, inaccessible tumor locations, or multiple intracranial metastases, with some patients declining surgical treatment due to high risks.

Assessing the effect of SRS therapy on brain metastases is crucial for planning both local and systemic therapies for patients, with MRI of the brain being the most precise method for evaluating post-therapeutic findings. Over the recent years, several working groups have proposed various criteria to achieve more objective, quantitative radiological assessments of tumor response post-therapy. These models typically include analyzing brain tumor responses on post-contrast T1W MRI sequences. The World Health Organisation (WHO), McDonald's, The Response Evaluation Criteria In Solid Tumors (RECIST), and The Response Assessment in Neuro-Oncology (RANO) working groups have developed linear models for analyzing brain tumor response post-therapy. In contrast, volumetric analysis has been conducted in fewer individual studies (6-11, 13-18). By measuring one (unidimensional, 1D) or two (bidimensional, 2D) maximum tumor diameters on post-contrast T1W MRI sequences, 1D RECIST and 2D RANO analyses define CR as complete absence of the lesion post-therapy, PR as at least a 30% reduction in the largest diameters of the lesion, PD as at least a 20% increase in the largest diameters of the lesion, and SD as a state not classified as PR or PD (8-10, 27). According to the WHO and McDonald working groups, 2D tumor analysis defines CR as a complete absence of the lesion post-therapy, PR as at least a 50% reduction in the largest diameters of the lesion, PD as at least a 25% increase in the largest diameters of the lesion, and SD as a state that is not classified as PR or PD (7, 9, 27). While the aforementioned authors performed 1D or 2D measurements of brain metastasis size, the authors of this study conducted 3D measurements with lesion volume calculation, followed by therapeutic response classification according to the WHO and McDonald working groups.

Follwell et al. were among the first to analyze the volume of 178 brain metastases of various primary origins in 70 patients treated with SRS (18). They compared the therapeutic response of brain metastases based on 1D RECIST criteria and 3D lesion volume, defining PD as a condition with a 71.5% increase in lesion volume, PR as a 58.5% decrease in lesion volume, and SD for those lesions that were neither PD nor PR. Over a two-year follow-up, they found that assessing brain metastasis therapeutic response to SRS based on changes in lesion volume was more accurate than using 1D RECIST criteria. Popat et al. emphasize the importance of calculating brain metastasis volumes as a prerequisite for assessing the feasibility of SRS, as reflected in the mandatory analysis for evaluating the therapeutic effect of SRS applied in this study (21).

The significance of volumetric measurements of metastatic lesions has increased in recent years, although most studies have focused on analyzing metastases regardless of their primary origin (13-20). Only two studies have been published and examining the volume of brain metastases originating from breast cancer (23, 24).

Maucevic et al. studied the volume of solitary and multiple brain metastases originating from breast cancer after SRS in 151 patients (23). However, the authors of this study did not clearly define tumor response based on changes in lesion volume after SRS was applied. In contrast to the Maucevic study, the authors of this study analyzed brain metastases originating from breast cancer with a more complex classification of therapeutic responses.

Consistent with the results of this study, Kowalchuk et al. demonstrated that SRS of solitary and multiple brain metastases significantly reduces lesion volume after therapy in patients with triple-negative breast cancer (24). The authors explained that the low overall survival during the seven-month follow-up of these patients could be explained by systemic disease progression rather than a local progression of intracranial metastasis after SRS treatment. Given the advancements in systemic therapy development for this triple-negative breast cancer subtype, greater effectiveness of SRS in treating brain metastases in these patients is expected in the future (1, 24). In contrast to the aforementioned study, the authors of this study analyzed only solitary brain metastases regardless of the histopathological subtype of breast cancer.

This current study has several limitations. The sample size was small, and the study included patients with metastases originating from primary breast tumors regardless of their histopathological subtype. The study was limited to analyzing conventional MRI parameters, while advanced MRI techniques such as diffusion, susceptibility, MR spectroscopy, and perfusion were not applied. Lastly, clinical evaluation of patient performance status, progression-free survival, quality of life, and overall survival was not analyzed.

Further research on the changes in brain metastasis volume before and after SRS in a more significant number of patients, grouped by histopathological subtypes of primary tumors, with analysis of multiple parameters of conventional and advanced MRI techniques and longer patient follow-up, would facilitate the development and clinical application of radiological methods in post-therapeutic monitoring of patients with brain metastases.

CONCLUSION

This study has demonstrated that SRS therapy in patients with brain metastases originating from breast cancer leads to a statistically significant reduction in lesion volume in the early post-therapy period, confirming the validity of SRS application in local control of metastatic disease. The findings of this study should be further validated by larger studies examining the significance of volumetric measurements of lesions.

Ethical Statement: The study was conducted by the Declaration of Helsinki and was approved by the Ethics Committee of the University Clinical Center of Serbia.

Conflict of interest: None to declare.

REFERENCES:

1. Raghavendra AS, Ibrahim NK. Breast Cancer Brain Metastasis: A Comprehensive Review. JCO Oncol Pract 2024; 00:1-12. doi: 10.1200/OP.23.00794
2. Derks SHAE, van der Veldt AAM, Smits M. Brain metastases: the role of clinical imaging. Br J Radiol. 2022; 95(1130): 20210944. doi: 10.1259/bjr.20210944
3. Wang T, Chen J, Yang J, Fu M, Hua W, Jia W, et al. CSCO expert consensus on the diagnosis and treatment of breast cancer brain metastasis. Transl Breast Cancer Res 2022; 30:3:22. doi: 10.21037/tbcr-22-30
4. Le Rhun E, Guckenberger M, Smits M, Dummer R, Bachelot T, Sahm F, et al. EANO–ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up of patients with brain metastasis from solid tumours. Ann Oncol 2021; 32(11): 1332-1347. doi: 10.1016/j.annonc.2021.07.016.
5. Douri K, Iorio-Morin C, Mercure-Cyr R, Figueiredo G, Jean Touchette C, Masson-Côté L, et al. Response Assessment in Brain Metastases Managed by Stereotactic Radiosurgery: A Reappraisal of the RANO-BM Criteria. Curr Oncol 2023; 30(11): 9382-9391. doi: 10.3390/curroncol30110679
6. Benzakoun J, Robert C, Legrand L, Pallud J, Meder JF, Oppenheim C, et al. Anatomical and functional MR imaging to define tumoral boundaries and characterize lesions in neuro-oncology. Cancer Radiothér 2020; 24 (5): 453-62. doi: 10.1016/j.canrad.2020.03.005
7. Macdonald DR, Cascino TL, Schold SC, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 1990; 8: 1277–80. doi: 10.1200/JCO.1990.8.7.1277
8. Galanis E, Buckner JC, Maurer MJ, Sykora R, Castillo R, Ballman KV, et al. Validation of neuroradiologic response assessment in gliomas: Measurement by RECIST, two-dimensional, computer-assisted tumor area, and computer-assisted tumor volume methods. Neuro Oncol 2006; 8(2): 156-65. doi: 10.1215/15228517-2005-005.
9. Park JO, Lee SI, Song SY, Kim K, Kim WS, Jung CW, et al. Measuring response in solid tumors: Comparison of RECIST and WHO response criteria. Jpn J Clin Oncol 2003; 33: 533–7. doi: 10.1093/jjco/hyg093.
10. Lin NU, Lee EQ, Aoyama H, Barani IJ, Barboriak DP, Baumert BG, et al. Response assessment criteria for brain metastases: proposal from the RANO group. Lancet Oncology 2015; 16: 270 – 8. doi: 10.1016/S1470-2045(15)70057-4.
11. Wen PY, Chang SM, Van den Bent MJ, Vogelbaum MA, Macdonald DR, Lee EQ. Response Assessment in Neuro-Oncology Clinical Trials. J Clin Oncol 2017; 35(21): 2439 – 49. doi: 10.1200/JCO.2017.72.7511.
12. Spalek MJ, Mandat T. Salvage Treatment for Progressive Brain Metastases in Breast Cancer. Cancers 2022; 14(4): 1096. https://doi.org/10.3390/cancers14041096
13. Kim WH, Kim DG, Han JH, et al. Early significant tumor volume reduction after radiosurgery in brain metastases from renal cell carcinoma results in long-term survival. Int J Radiat Oncol Bio Phys 2012; 82(5): 1749-55. doi: 10.1016/j.ijrobp.2011.03.044.
14. Van der Weide H, de Kunder S, Houben R, Bosmans G, Lambin P, Baumert BG. Influence of metastatic volume on local control and survival assessed by 3D volumetric measurements after radiosurgery in patients with brain metastases and planned observation. Strahlenther Onkol 2011; 187: 523 – 4.
15. Yang DY, Sheehan J, Liu YS, Changlai SP, Pan HC, Chen CJ, et al. Analysis of factors associated with volumetric data errors in gamma knife radiosurgery. Stereotact Funct Neurosurg 2009; 87: 1 – 7. doi: 10.1159/000177622.
16. Pan HC, Cheng FC, Sun MH, Chen CC, Sheehan J. Prediction of volumetric data errors in patients treated with gamma knife radiosurgery. Stereotact Funct Neurosurg 2007; 85: 184 – 91. doi: 10.1159/000101297
17. Sharpton SR, Oermann EK, Moore DT, Schreiber E, Hoffman R, Morris DE, et al. The Volumetric Response of Brain Metastases After Stereotactic Radiosurgery and Its Post-treatment Implications. Neurosurgery 2014; 74: 9 – 16. doi: 10.1227/NEU.0000000000000190.
18. Follwell MJ, Khu KJ, Cheng L, Xu W, Mikulis DJ, Millar BA, et al. Volume specific response criteria for brain metastases following salvage stereotactic radiosurgery and associated predictors of response. Acta Oncologica 2012; 51: 629–35. doi: 10.3109/0284186X.2012.681066.
19. Kanayama N, Ikawa T, Ohira S, Hirata T, Morimoto M, Ogawa K, et al. Volumetric reduction of brain metastases after stereotactic radiotherapy: Prognostic factors and effect on local control. Cancer Med 2022; 11(24): 4806–15. doi: 10.1002/cam4.4809
20. Oft D, Schmidt MA, Weissmann T, Roesch J, Mengling V, Masitho S, et al. Volumetric regression in brain metastases after stereotactic radiotherapy: time course, predictors, and significance. Front Oncol. 2020;10: 590980. https://doi.org/10.3389/fonc.2020.590980
21. Popat S, Welsh L. Brain metastases in solid tumours: new guidelines for a new era. Annals of Oncology 2021; 32 (11): 1322-4. doi: 10.1016/j.annonc.2021.08.1992.
22. Ocaña-Tienda B, Pérez-Beteta J, Romero-Rosales JA, Asenjo B, Ortiz de Mendivil A, Pérez Romasanta LA, et al. Volumetric analysis: Rethinking brain metastases response assessment. Neuro-Oncology Advances 2024; 6 (1): 1-9. https://doi.org/10.1093/noajnl/vdad161
23. Muacevic A, Kreth FW, Tonn JC, Wowra B. Stereotactic radiosurgery for multiple brain metastases from breast carcinoma. Cancer 2004; 100: 1705–11. https://doi.org/10.1002/cncr.20167
24. Kowalchuk RO, Niranjan A, Hess J, Antonios JP, Zhang MY, Braunstein, S, et al. Stereotactic radiosurgery and local control of brain metastases from triple-negative breast cancer. J Neurosurg 2023; 138: 1608–14. doi: 10.3171/2022.10.JNS221900.
25. Palmer JD, Trifiletti DM, Gondi V, Chan M, Minniti G, Rusthoven CG, et al. Multidisciplinary patient-centered management of brain metastases and future directions. Neuro-Oncol Adv 2020; 2(1): 1–17. doi: 10.1093/noajnl/vdaa034
26. Tonse R, Tom MC, Mehta MP, Ahluwalia MS, Kotecha R. Integration of Systemic Therapy and Stereotactic Radiosurgery for Brain Metastases. Cancers 2021; 13: 3682-97. https://doi.org/10.3390/cancers13153682
27. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000; 92: 205–16. doi: 10.1093/jnci/92.3.205.

RANI TERAPIJSKI ODGOVOR NA GAMA NOŽ STEREOTAKSIČNU RADIOHIRUGIJU U SLUČAJU METASTAZA NA MOZGU KOD PRIMARNOG KARCINOMA DOJKE

Jovanović Marija[1, 4], Davidović Kristina[1, 4], Petković Ana[2], Šeha Biljana[2], Jeremić Danilo[3,4], Milićević Mihailo[2,4], Bogdanović Ivan[2, 4], Rosanda Ilić[2, 4]

Sažetak

Uvod: Metastaze mozga su najučestaliji intrakranijalni malignitet adultne populacije, a u ženskoj populaciji najčešće potiču od primarnog karcinoma dojke. Incidencija metastaza mozga raste poslednjih godina usled bolje terapijske kontrole sistemske bolesti i bolje dijagnostike stanja pacijenta. Primena stereotaksične radiohirurgije (stereotactic radiosurgery, SRS) kod pacijenata sa tumorima mozga ima za cilj da obezbedi kako lokalnu kontrolu bolesti, tako i očuvanje kvaliteta života i duže preživljavanje pacijenata. Cilj ovog rada je da proceni efekat SRS kod pacijentkinja sa metastazama mozga kod primarnog karcinoma dojke analizom parametara magnetno-rezonantnog prikaza (magnetic resonance imaging, MRI) mozga.

Metode: Kod 30 adultnih pacijentkinja načinjen je MRI endokranijuma pre i 3-6 meseci nakon SRS tumora. Radiološkom analizom izračunati su volumeni lezija pre i nakon SRS.

Rezultati: Pacijentkinje su svrstane u četiri grupe prema terapijskom odgovoru: 1 – kompletni terapijski odgovor (CR) kod potpunog gubitka lezije, 2 – parcijalni terapijski odgovor (PR) u slučaju smanjenja volumena lezije za više od 50%, 3 – progresija bolesti (PD) kod povećanja volumena lezije za više od 25%, 4 – stabilna bolest (SD) ukoliko stanje pacijenta nije označeno kao PR ili PD. Od ukupnog broja pacijentkinja, CR je bi prisutan kod 0 (0%), PR kod 15 (50%), PD kod 1 (3%), a SD kod 14 (47%) pacijentkinja. Nakon primenjene SRS utvrđena je statistički značajna razlika u promeni volumena tumora ($p<0.001$).

Zaključak: Radiološkom volumetrijskom analizom metastaza mozga nakon SRS ustanovljena je statistički značajna redukcija u volumenu lezija i time lokalna kontrola bolesti.

Ključne reči: metastaze mozga, karcinom dojke, stereotaksična radiohirurgija, magnetnorezonantni prikaz

Primljen: 16.07.2024. I **Revizija:** 06.08.2024. I **Prihvaćen:** 19.08.2024.

Medicinska istaživanja 2024; 57(3):115-121

Medicinska istaživanja 2024; 57(3):123-127 | DOI: 10.5937/medi57-49569

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REVIEW ARTICLE

Cardiovascular diseases associated with obstructive sleep apnea syndrome

Marija Zdravković[1,2], Ratko Lasica[2,3], Sofija Nikolić[1], Milica Brajković[1,2]

[1] University Hospital Medical Center Bežanijska kosa, Belgrade, Serbia
[2] Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[3] Department of Cardiology, University Clinical Center of Serbia, Belgrade, Serbia

Recived: 29 February 2024
Revised: 23 April 2024
Accepted: 23 April 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Milica Brajković
University Hospital Medical Center Bežanijska kosa
Dr Žorža Matea, 11080 Belgrade, Serbia
Email: brajkovic.milica77@gmail.com

Summary

Obstructive Sleep Apnea (OSA) is a syndrome characterized by repeated episodes of breathing cessation during sleep, which can be partial (hypopneas) or complete (apneas). Intermittent hypoxia is the fundamental pathophysiological mechanism in the development of all associated diseases with obstructive sleep apnea. OSA is linked to various forms of cardiovascular diseases, and their association is correlated with poorer health outcomes. It is present in as much as 40% to 60% of patients with pre-existing cardiovascular diseases, making the causal relationship between cardiovascular diseases and obstructive sleep apnea the focus of this article.

Keywords: obstructive sleep apnea, cardiovascular diseases, intermittent hypoxemia

Cite this article as: Zdravković M, Lasica R, Nikolić S, Brajković M. Cardiovascular diseases associated with obstructive sleep apnea syndrome; Medicinska istaživanja 2024; 57(3):123-127 DOI: 10.5937/medi57-49569

INTRODUCTION

Obstructive sleep apnea (OSA) is a syndrome characterized by recurrent episodes of partial or complete collapse of the upper airways during sleep. At the moment of collapse, the airflow through the airways is reduced or completely absent despite continuous efforts to inhale (1).

The most likely reasons for the collapse of the airways include the anatomy of the upper airways, the ability of the dilator muscles of the upper airways to respond to respiratory challenges during sleep, the tendency to wake up from increased respiratory drive during sleep (arousal threshold), and the stability of the breathing control system. The consequence of reduced airflow through the airways is inadequate alveolar ventilation, leading to poor gas exchange and increased activity of the sympathetic nervous system (1,2).

Snoring and interruptions in breathing during sleep lead to excessive daytime sleepiness and a lack of concentration which are characteristic features of patients with obstructive sleep apnea.

Obstructive sleep apnea is a serious condition associated with various metabolic disorders linked to increased mortality. Furthermore, there is evidence indicating that it represents an independent risk factor for a range of adverse cardiovascular outcomes (3). Cardiovascular diseases (CVD) are widely prevalent in the general population and constitute a leading cause of mortality. Therefore, significant attention is given to risk factors influencing their development and subsequent prevention (4). It is estimated to affect 34% of men and 17% of women in the general population, and 40% to 60% of patients with CVD (5).

Mechanisms linking OSA and CVD are not yet fully elucidated. A spectrum of different factors is involved, such as increased sympathetic activity, changes in intrathoracic pressure, and oxidative stress. One piece of evidence supporting a causal relationship between OSA and CVD is the alterations in blood pressure values and the improvement in left ventricular systolic function after continuous positive airway pressure (CPAP) therapy (6).

PATHOPHYSIOLOGY

As previously mentioned, intermittent hypoxia during the night is the fundamental pathophysiological mechanism in the development of associated diseases in patients with this syndrome. One of the main pathophysiological mechanisms of cardiovascular diseases in OSA is sympathetic activation in response to intermittent ischemia, driven by increased activity of chemoreceptors at the carotid bodies. This subsequently influences the development of an imbalance in myocardial oxygen demand and supply (7).

Additionally, increased oxidative stress is one of the most commonly described mechanisms responsible for the development of cardiovascular consequences. In normal conditions, there is an equilibrium between free radicals and antioxidants, and disrupting this balance leads to a disturbance known as oxidative stress.

Oxidative stress exerts its harmful effects in multiple ways, either by activating NADPH oxidase and synthesizing superoxide, which impacts the reduction of nitric oxide (NO), or by increasing the oxidation of biological compounds such as lipids, proteins, and DNA, or by reducing the activity of antioxidant enzymes (8).

NO is the primary vasodilator synthesized in the endothelium and it possesses abundant vasoprotective properties, including the inhibition of platelet aggregation and the expression of adhesion molecules. Due to its clear and well-known effects, the consequences of NO deficiency primarily involve elevated blood pressure values.

In patients with OSA, elevated levels of 8-isoprostane, compounds similar to prostaglandins, have been observed. The formation of isoprostanes involves free radicals as catalyzing agents in the reaction. Isoprostanes are compounds that enhance vasoconstrictor tone, thereby increasing the likelihood of developing arterial hypertension in patients with OSA (7). Systemic inflammation and its effects are crucial aspects in patients with OSA. In addition to everything mentioned earlier, intermittent hypoxia also induces the activation of inflammatory cells and the release of inflammatory mediators.

Vascular endothelium can be damaged by various stressors, including free oxygen radicals, blood pressure force, circulating cholesterol, or fatty acids. Endothelial injury stimulates the expression of leukocyte adhesion molecules and endothelial adhesion molecules, initiating the well-known process of atherosclerotic plaque formation, which is a prerequisite for the development of atherosclerosis (8).

A significant number of patients with OSA have metabolic syndrome, especially those who are obese. Elevated levels of triglycerides along with high LDL cholesterol values carry a high cardiovascular risk, primarily due to the atherosclerotic process. Studies have also shown that endogenous cholesterol synthesis is increased in obese individuals with OSA (9). Insufficient activity of the lipoprotein lipase enzyme is considered responsible for elevated LDL cholesterol levels. The activity of this enzyme is under the control of insulin, cortisol, and adrenaline. Therefore, it is crucial to emphasize the role of OSA in insulin resistance (10,11).

CARDIOVASCULAR DISEASES AND OBSTRUCTIVE SLEEP APNEA

From everything previously stated, we can conclude that unregulated blood pressure values are a hallmark in patients with OSA and are present in about 50% of patients.

What is characteristic are hypertensive episodes that occur during the night with the absence of the morning physiological blood pressure drop, otherwise known as non-dipper hypertension. They are often poorly controlled with standard antihypertensive therapy unless the syndrome itself is adequately treated. Up to 30% of hypertensive individuals may suffer from unrecognized OSA. The prevalence of hypertensive crises in patients with coexisting OSA and hypertension is as high as 15.7% (12,13).

In addition to the various etiologies previously described, one of the more serious consequences in patients with OSA is the development of manifest heart failure (3). As previously mentioned in the text, a characteristic feature of OSA is the interruptions in breathing during sleep, which occur despite the patient's inspiratory effort. At the moment of effort, there is a drop in intrathoracic pressure, leading to hemodynamic consequences. The decrease in intrathoracic pressure increases the pressure in the left ventricle, raising afterload and causing distension of the right ventricle. This, in turn, shifts the interventricular septum to the left, affecting the filling of the left ventricle and ultimately contributing to a reduction in stroke volume (3,14).

The combination of increased afterload on the left ventricle and a faster heart rate due to heightened sympathetic activity leads to a mismatch between myocardial oxygen supply and demand. This acute condition predisposes the patient to cardiac ischemia and arrhythmias, and chronically it can result in left ventricular hypertrophy and the development of heart failure (14).

Increased sympathetic tone occurs during each episode of upper airway obstruction and remains present even during waking moments in patients with OSA. Sympathetic discharges can provoke abnormal electrical activity in the atria. In addition to acute mechanisms occurring during each obstructive event, OSA can lead to cardiac remodeling, which, in turn, increases the susceptibility to cardiac arrhythmias and, of course, the possibility of sudden cardiac death as the most severe complication (15).

In severe forms of OSA, the frequency of arrhythmias can be as high as 50%. The most common are recurrent atrial fibrillation, nonsustained ventricular tachycardia (VT), sinus arrest, second-degree AV block, and premature ventricular contractions (PVCs). It is important to note that bradyarrhythmia is a common type of arrhythmia in patients with OSA and it is a consequence of increased vagal tone. A European multicenter study showed a high prevalence of obstructive sleep apnea syndrome, approximately 60%, among patients with implanted pacemakers, regardless of pacing indications (16).

It is crucial to emphasize that the occurrence of coronary heart disease and sudden cardiac death is more common in patients with OSA compared to the general population. It is characteristic that patients with OSA who die suddenly most often pass away while sleeping between 10 PM and 6 AM, in contrast to other patients who typically experience sudden death in the early morning hours (15,17).

DIAGNOSIS OF OBSTRUCTIVE SLEEP APNEA SYNDROME

To prevent and timely treat the described consequences before the onset of fatal complications, it is essential to promptly diagnose obstructive sleep apnea. The gold standard for diagnosing this syndrome is polysomnography, which precisely quantifies the extent of respiratory and sleep disorders.

To diagnose obstructive sleep apnea, a patient must experience more than 5 respiratory interruptions per hour, accompanied by desaturation and lasting for at least 10 seconds. Depending on the number of respiratory interruptions during sleep, whether they are partial (hypopneas) or complete (apneas), OSA is classified as mild (AHI 5-15/h), moderate (AHI 15-30/h), or severe (AHI over 30/h). To facilitate the diagnostic process and assess the severity of obstructive sleep disease, several clinical questionnaires have been developed, such as the STOP-BANG questionnaire, Epworth Sleepiness Scale, and Berlin questionnaire (18).

TREATMENT

The treatment of obstructive sleep apnea is complex and includes positional therapy, behavioral therapy, intraoral prosthetic systems, surgical treatment, as well as positive pressure therapy using CPAP and BIPAP devices. The choice of therapeutic modality depends on several factors, primarily the severity of symptoms assessed through clinical questionnaires, the patient's comorbidities, and, of course, the degree of diagnosed OSA. Continuous positive airway pressure (CPAP) and bilevel positive airway pressure (BIPAP) therapy is undoubtedly considered the gold standard for treating OSA and represents the first-line therapy for patients with moderate to severe OSA (19).

Prominent daytime sleepiness and nighttime symptoms tend to diminish after a short period of consistent CPAP use. After 3-6 months of continuous treatment, patients often experience improvements in memory and attention. Some studies provide evidence that CPAP treatment has a positive impact on cardiovascular outcomes. CPAP therapy significantly lowers diastolic and systolic blood pressure, thereby reducing the incidence of fatal cardiovascular events. In addition, insulin resistance and altered lipid profiles in the serum are closely linked to OSA. Although the effect of CPAP on metabolic changes is widely researched, the results are still inconclusive. Inflammatory responses, which significantly contribute to the atherosclerotic process, increasing cardiovascular

and cerebrovascular morbidity, are proven to be significantly reduced with CPAP treatment (20).

Taken together, these data indicate that CPAP is extremely effective in controlling the symptoms and consequences of OSA. Very few unwanted effects have been recorded, and they are mainly associated with discomfort from wearing the mask. It is crucial to note that the effectiveness of CPAP strictly depends on its consistent use, and recurrence of symptoms occurs within 1-3 days of discontinuing the treatment.

In addition to treating the underlying cause, in this case, obstructive sleep apnea, it is necessary to treat the consequences with the goal of reducing cardiovascular risk. Statin therapy and its benefits in preventing cardiovascular diseases are unquestionable. However, evidence regarding the positive effects of lipid-lowering therapy in patients with OSA is controversial. Various multicenter studies have been conducted, but significant improvements in endothelial function after 12 weeks of atorvastatin use in patients with severe OSA have not been observed. It is evident that statin therapy has improved blood pressure values, potentially impacting overall cardiovascular risk (9).

In clinical practice, PCSK9 inhibitors, along with statins, have been shown to reduce cardiovascular risk in patients with stable atherosclerotic cardiovascular disease or recent acute coronary syndrome. As a relatively new and insufficiently studied biomarker, the regulation of PCSK9 in OSA is still poorly understood (21).

In recent years, the effects of mesenchymal stem cells have been studied due to their potent regenerative, pro-angiogenic, and immunomodulatory properties. As known, repetitive hypoxia induces an inflammatory response, which is a precondition for the development of atherosclerosis. Studies on animal models have been conducted, and the results show that mesenchymal stem cells effectively alleviate vascular injuries, inflammation, and fibrosis caused by OSA. It is crucial to emphasize that their effectiveness needs to be demonstrated in clinical studies (22).

CONCLUSION

Obstructive sleep apnea is a syndrome associated with a significant number of comorbidities and is a crucial factor in their development. It is important to emphasize that this syndrome is not synonymous with snoring but it is a significant independent factor for the development of cardiovascular diseases, which can have a fatal outcome if not treated in a timely manner.

The authors contributed equally to this work.

REFERENCES

1. Wilfried De Backer, Obstructive sleep apnoea– hypopnoea syndrome in: Paolo Palange, Gernot Rohde editors in Respiratory medicine 3rd edition : The European Respiratory society, 2019. P 522-527.
2. Eckert DJ, Malhotra A. Pathophysiology of adult obstructive sleep apnea. Proc Am Thorac Soc. 2008 Feb 15;5(2):144-53. doi: 10.1513/pats.200707-114MG. PMID: 18250206; PMCID: PMC2628457.
3. Danica LP, Krotin M, Zdravkovic M, Soldatovic I, Zdravkovic D, Brajkovic M, Gardijan V et al. Early left ventricular systolic and diastolic dysfunction in patients with newly diagnosed obstructive sleep apnoea and normal left ventricular ejection fraction. ScientificWorldJournal. 2014 Feb 27; 2014:898746. doi: 10.1155/2014/898746. PMID: 24723836; PMCID: PMC3958663.
4. Shahar E, Whitney CW, Redline S, Lee ET, Newman AB, Nieto FJ et al. Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. Am J Respir Crit Care Med. 2001 Jan;163(1):19-25. doi: 10.1164/ajrccm.163.1.2001008. PMID: 11208620.
5. Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM. Increased prevalence of sleep-disordered breathing in adults. Am J Epidemiol. 2013 May 1;177(9):1006-14. doi: 10.1093/aje/kws342. Epub 2013 Apr 14. PMID: 23589584; PMCID: PMC3639722.
6. Jean-Louis G, Zizi F, Clark LT, Brown CD, McFarlane SI. Obstructive sleep apnea and cardiovascular disease: role of the metabolic syndrome and its components. J Clin Sleep Med. 2008 Jun 15;4(3):261-72. PMID: 18595441; PMCID: PMC2546461.
7. Eisele HJ, Markart P, Schulz R. Obstructive Sleep Apnea, Oxidative Stress, and Cardiovascular Disease: Evidence from Human Studies. Oxid Med Cell Longev. 2015; 2015:608438. doi: 10.1155/2015/608438. Epub 2015 Jun 8. PMID: 26167241; PMCID: PMC4475750.
8. Unnikrishnan D, Jun J, Polotsky V. Inflammation in sleep apnea: an update. Rev Endocr Metab Disord. 2015 Mar;16(1):25-34. doi: 10.1007/s11154-014-9304-x. PMID: 25502450; PMCID: PMC4346503.
9. Zeljković A, Milojević A, Vladimirov S, Zdravković M, Memon L, Brajković M et al. Alterations of cholesterol synthesis and absorption in obstructive sleep apnea: Influence of obesity and disease severity. Nutr Metab Cardiovasc Dis. 2022 Dec;32(12):2848-2857. doi: 10.1016/j.numecd.2022.09.006. Epub 2022 Sep 17. PMID: 36323608.
10. Popadic V, Brajkovic M, Klasnja S, Milic N, Rajovic N, Lisulov DP et al. Correlation of Dyslipidemia and Inflammation With Obstructive Sleep Apnea Severity. Front Pharmacol. 2022 May 25; 13:897279. doi: 10.3389/fphar.2022.897279. PMID: 35694268; PMCID: PMC9179947.
11. Zdravkovic M, Popadic V, Klasnja S, Milic N, Rajovic N, Divac A et al. Obstructive Sleep Apnea and Cardiovascular Risk: The Role of Dyslipidemia, Inflammation, and Obesity. Front Pharmacol. 2022 Jun 15; 13:898072. doi: 10.3389/fphar.2022.898072. PMID: 35784707; PMCID: PMC9240428.
12. Crinion SJ, Ryan S, Kleinerova J, Kent BD, Gallagher J, Ledwidge M et al. Nondipping Nocturnal Blood Pressure Predicts Sleep Apnea in Patients With Hypertension. J Clin Sleep Med. 2019 Jul 15;15(7):957-963. doi: 10.5664/jcsm.7870. PMID: 31383232; PMCID: PMC6622521.
13. Khamsai S, Chootrakool A, Limpawattana P, Chindaprasirt J, Sukeepaisarnjaroen W, Chotmongkol V et al. Hypertensive crisis in patients with obstructive sleep apnea-induced hypertension. BMC Cardiovasc Disord. 2021 Jun 23;21(1):310. doi: 10.1186/s12872-021-02119-x. PMID: 34162333; PMCID: PMC8220687.
14. Khattak HK, Hayat F, Pamboukian SV, Hahn HS, Schwartz BP, Stein PK. Obstructive Sleep Apnea in Heart Failure: Review of Prevalence, Treatment with Continuous Positive Airway Pressure, and Prognosis. Tex Heart Inst J. 2018 Jun 1;45(3):151-161. doi: 10.14503/THIJ-15-5678. PMID: 30072851; PMCID: PMC6059510.
15. Blackwell JN, Walker M, Stafford P, Estrada S, Adabag S, Kwon Y. Sleep Apnea and Sudden Cardiac Death. Circ Rep. 2019;1(12):568-574. doi: 10.1253/circrep.cr-19-0085. Epub 2019 Dec 10. PMID: 32201748; PMCID: PMC7083593.

16. Geovanini GR, Lorenzi-Filho G. Cardiac rhythm disorders in obstructive sleep apnea. J Thorac Dis. 2018 Dec;10(Suppl 34):S4221-S4230. doi: 10.21037/jtd.2018.12.63. PMID: 30687538; PMCID: PMC6321897.

17. Gottlieb DJ, Yenokyan G, Newman AB, O'Connor GT, Punjabi NM, Quan SF et al. Prospective study of obstructive sleep apnea and incident coronary heart disease and heart failure: the sleep heart health study. Circulation. 2010 Jul 27;122(4):352-60. doi: 10.1161/CIRCULATIONAHA.109.901801. Epub 2010 Jul 12. PMID: 20625114; PMCID: PMC3117288.

18. Goyal M, Johnson J. Obstructive Sleep Apnea Diagnosis and Management. Mo Med. 2017 Mar-Apr;114(2):120-124. PMID: 30228558; PMCID: PMC6140019.

19. Spicuzza L, Caruso D, Di Maria G. Obstructive sleep apnoea syndrome and its management. Ther Adv Chronic Dis. 2015 Sep;6(5):273-85. doi: 10.1177/2040622315590318. PMID: 26336596; PMCID: PMC4549693.

20. Ruzicka M, Knoll G, Leenen FHH, Leech J, Aaron SD, Hiremath S. Effects of CPAP on Blood Pressure and Sympathetic Activity in Patients With Diabetes Mellitus, Chronic Kidney Disease, and Resistant Hypertension. CJC Open. 2020 Mar 27;2(4):258-264. doi: 10.1016/j.cjco.2020.03.010. PMID: 32695977; PMCID: PMC7365815.

21. Milojević A, Zdravković M, Brajković M, Memon L, Gardijan V, Vekić J et al. Effects of Apnea, Obesity, and Statin Therapy on Proprotein Convertase Subtilisin/Kexin 9 Levels in Patients with Obstructive Sleep Apnea. Med Princ Pract. 2022;31(3):293-300. doi: 10.1159/000524087. Epub 2022 Mar 15. PMID: 35292607; PMCID: PMC9274940.

22. Zdravkovic M, Harrell CR, Jakovljevic V, Djonov V, Volarevic V. Molecular Mechanisms Responsible for Mesenchymal Stem Cell-Based Modulation of Obstructive Sleep Apnea. Int J Mol Sci. 2023 Feb 13;24(4):3708. doi: 10.3390/ijms24043708. PMID: 36835120; PMCID: PMC9958695.

KARDIOVASKULARNE BOLESTI UDRUŽENE SA OPSTRUKTIVNOM BOLESTI SPAVANJA

Marija Zdravković[1,2], Ratko Lasica[2,3], Sofija Nikolić[1], Milica Brajković[1,2]

Sažetak

Opstruktivna apneja u snu (OSA) je sindrom koji karakterišu ponavljajuće epizode prekida disanja tokom spavanja pri čemu prekidi mogu biti delimični (hipopneje) ili potpuni (apneje). Intermitentna hipoksija je osnovni patofiziološki mehanizam u razvoju svih udruženih bolesti sa opstruktivnom apnejom u snu. OSA je povezana sa različitim oblicima kardiovaskularnih bolesti, a njihova udruženost je povezana sa lošijim zdravstvenim ishodom. Prisutna je kod čak 40% do 60% pacijenata sa već postojećim kardiovaskularnim oboljenjima zbog čega je tema ovog rada upravo uzročno posledična veza kardiovaskularnih bolesti i opstruktivne apneje u snu.

Ključne reči: opstruktivna apneja u snu, kardivaskularne bolesti, intermitenta hipoksemija

Primljen: 29.02.2024. I **Revizija:** 23.04.2024. I **Prihvaćen:** 28.04.2024.

Medicinska istaživanja 2024; 57(3):123-127

Medicinska istaživanja 2024; 57(3):129-136 | DOI: 10.5937/medi57-49490

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REVIEW ARTICLE

Acinetobacter baumannii pneumonia associated with mechanical ventilation due to COVID-19: epidemiology, clinical characteristics and therapy

Tatjana Gazibara[1], Branko Beronja[2], Aleksandra Karan[3], Biljana Lukić[3]

[1] University of Belgrade, Faculty of Medicine, Institute of Epidemiology, Belgrade, Serbia
[2] University of Belgrade, Faculty of Medicine, Belgrade, Serbia
[3] General Hospital "Dr Radivoj Simonović", Sombor, Serbia

Recived: 26 February 2024
Revised: 22 May 2024
Accepted: 22 May 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Tatjana Gazibara
Institute of Epidemiology,
26a Višegradska Street, 11000 Belgrade, Serbia
Email: tatjanagazibara@yahoo.com; tatjana.gazibara@med.bg.ac.rs

Summary

Less than 3% of people who get infected with COVID-19 need hospital treatment. However, up to one-third of the hospitalized patients with COVID-19 require invasive mechanical ventilation. Ventilator-associated pneumonia (VAP), caused by the multidrug-resistant *Acinetobacter baumannii* (*A. baumannii*), is an emerging infection in the intensive care units and can have fatal consequences for those patients who already have critical COVID-19. Countries of the Balkan peninsula have an exceptionally high prevalence of invasive carbapenem-resistant *Acinetobacter spp* in the hospital setting. Diagnosing this type of pneumonia is a challenging process. Furthermore, treatment complexities arise because of multidrug resistance. Novel therapeutic agents, such as sulbactam/durlobactam and zosurabalpin could be the new therapeutic opportunity for *A. baumannii*-induced VAP. Antimicrobial resistance of *A. baumannii* is not entirely understood, although several mechanisms have been identified. To adequately manage VAP it is important to isolate causative agents, have awareness of the resistance pattern, carefully dispense antibiotics, and identify risk factors. In this review, we discuss epidemiological characteristics, pathophysiological mechanisms, clinical presentation and diagnosis, as well as the current and novel treatments of *A. baumannii*-induced VAP.

Keywords: COVID-19, ventilator-associated pneumonia, *Acinetobacter baumannii*, multidrug resistance

Cite this article as: Gazibara T, Beronja B, Karan A, Lukić B. Acinetobacter baumannii pneumonia associated with mechanical ventilation due to COVID-19: epidemiology, clinical characteristics and therapy; Medicinska istaživanja 2024; 57(3):129-136 DOI: 10.5937/medi57-49490

INTRODUCTION

Beside tremendous societal challenges of the coronavirus disease (COVID-19) pandemic (1-3), the underlying changes in the antimicrobial ecology since the onset of the pandemic seem to be a major threat, particularly in the hospital setting. COVID-19 has a heterogeneous clinical presentation, ranging from asymptomatic and mild forms to severe and critical clinical forms, which require treatment in the intensive care unit (ICU) (4). In relation with all patients with symptomatic COVID-19, severe illness develops in 13.4% to 19.1% of individuals, depending on the population sample (5, 6). However, approximately 2.3% - 9% of people who get infected with COVID-19 need hospital treatment, while respiratory failure requiring invasive mechanical ventilation (IMV) is reported among 2.3% - 33% of all hospitalized patients (7, 8). Despite the efforts to preserve lung function, cumulative mortality from COVID-19 among people on IMV ranges from 30% to 97% (9, 10). This can be partially attributed to systemic inflammation, multiorgan failure, and other complications associated with COVID-19, as well as to complications related to the IMV itself (9). Major complications of IMV include ventilator-induced lung injury (VILI) and ventilator-associated pneumonia (VAP) (9).

VAP accounts for the most common infection acquired in ICUs among patients with COVID-19, with an estimated cumulative incidence of 5% - 40% (11). Based on the time of the onset (within the first 4 days or after 4 days of hospitalization), two VAP entities were defined: an early onset ventilator-associated pneumonia (EVAP) and a late onset ventilator-associated pneumonia (LVAP) (10). The EVAP typically has milder clinical features and a more favorable prognosis, while LVAP is often attributed to multidrug-resistant microorganisms (10). Of multidrug-resistant (MDR) bacteria, *Acinetobacter baumannii* (*A. baumannii*) is often associated with the onset of VAP (12). In fact, carbapenem-resistant *A. baumannii* (CRAB) has been listed by the Centers for Disease Control and Prevention (CDC) as an urgent antimicrobial resistance threat in the United States just before the onset of the COVID-19 pandemic (13). Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) for 2020 underscored an exceptionally high prevalence of invasive carbapenem-resistant *Acinetobacter spp.* (exceeding 80% of all *A. baumannii* isolates in hospitals) in the Balkan countries (13): Turkey (93.1%), Greece (94.6%), North Macedonia (97.4%), Bulgaria (82.9%), Romania (93.3%) Serbia (98.6%), Montenegro (100%), Bosnia and Herzegovina (97.9%) and Croatia (96.4%). This prevalence was about 2.5 times higher than those observed within the European economic area (38%), suggesting that the Balkan region may be an *Acinetobacter spp.* hotspot (14).

The purpose of this review is to summarize the existing scientific literature on epidemiological characteristics, clinical presentation, therapeutic challenges of *A. baumannii*- induced VAP during the COVID-19 pandemic.

EPIDEMIOLOGAL CHARACTERISTICS

The presence of *A. baumannii* in hospital settings is reported worldwide. A combined incidence rate of *A. baumannii* among all hospital-acquired infections in Europe, Eastern Mediterranean region and Africa is estimated at 25.1 (95% CI 12.8-48.5) per 1,000 patients (15). A systematic review and meta-analysis of bacterial coinfection and secondary infection in patients with COVID-19 reported an *A. baumannii* prevalence of 3.5% and 14.3%, respectively (16, 17). A study conducted in hospitals in Belgrade (Serbia) reported that Gram-negative bacteria, including *Acinetobacter spp.*, *Pseudomonas aeruginosa*, and *Klebsiella/Enterobacter spp.* complex, were the most frequently isolated pathogens in patients with EVAP (39.7%, 13.8%, and 12.1%, respectively), thereby contributing to more than one-half of all early pneumonias (18). Similarly, in LVAP, *Acinetobacter spp.* and *Pseudomonas aeruginosa* were the most commonly isolated biological agents (18). Furthermore, in a study from the neighboring Croatia, during the COVID-19 pandemic, it was observed that the most common isolates in the ICU were those of *A. baumannii*, however, its frequency decreased in the post-pandemic period (19).

A study in Turkey reported almost a double increase in the prevalence of *A. baumannii* in the ICU during the COVID-19 pandemic compared to the pre-pandemic period, albeit the case-fatality ratio was similar (20). These pieces of evidence are compelling because the study included patients over a period of 27 months before and 27 months during the pandemic (20). One literature review indicated that *A. baumannii* in VAP during the COVID-19 pandemic was confirmed by deep bronchoalveolar lavage (BAL) in approximately 35%-45% of cases and endotracheal aspirate in about 40%-60% of cases (21, 22).

Community-acquired infections caused by *A. baumannii*, including pneumonia and bacteremia, are rare, even though they are associated with a relatively high mortality (23). Nevertheless, *A. baumannii* infections have become increasingly relevant as nosocomial infections, particularly in patients on IMV, because infected and colonized patients are major reservoirs of *A. baumannii* and spread with ease in hospital environment (24). More importantly, *A. baumannii* has the ability to survive on surfaces, especially plastic, in dry conditions, which allows for its ubiquitous presence in hospitals worldwide (25). The average duration of viability for sporadic *A. baumannii* strains is estimated at 27 days (range 21 to 32 days), whereas the average duration of viability for outbreak strains is 26 days (range from 21 to 33 days) (26). For this reason, the hygiene of stethoscopes, hands, and uniforms of healthcare professionals and medical stu-

dents is essential, not only during the COVID-19 pandemic, but at all times. Evidence shows that the incidence rate of MDR *A. baumannii* was 315.4 per 1000 ICU patient-days, with cumulative mortality rate being 52%-66% among those who are infected (27). A study by Novović et al. indicated that 64 isolates of *A. baumannii* were identified from COVID-19 patients admitted to the ICU in a local general hospital in Serbia, all of them requiring IMV and having poor COVID-19 outcomes (28).

Several hypotheses have been proposed to explain the increase in incidence of *A. baumannii*-induced VAP during the COVID-19 pandemic. Factors such as hospital overcrowding, a shortage of healthcare workers, and irrational use of antibiotics and immunosuppressants may be the underlying reasons (29). Thom et al. found that healthcare workers caring for patients known to be infected or colonized with *A. baumannii* carry this pathogen on their hands or gloves 30% of the time spent in hospital (30). In other studies *A. baumannii* was isolated from different sources and objects, including medical instruments, sinks, and toilet bowls, and protective equipment of healthcare professionals (31, 32). Interestingly, *A. baumannii* was identified in a swab from a plastic ventilator of the air conditioning unit 2.5 m above patient beds in an ICU of 40 m^2 in size, suggesting that it may be transmitted via airflow in addition to direct and indirect contact (19).

PATHOPHYSIOLOGICAL MECHANISMS

A major risk factor for the development of *A. baumannii* VAP is the presence of an endotracheal tube (33). In this way, infectious agents can enter the tracheobronchial system through the formation of a biofilm on the inner surface of the tube or via microaspiration around the balloon (33). As COVID-19 damages the cells of the respiratory tract, alters mucus production, and reduces cilial mobility, this cellular damage can modulate the upregulation of bacterial adhesion proteins (34). The disruption of tight junctions between cells results in lesions of the epithelial barrier, facilitating bacterial adhesion to the respiratory system cells and their paracellular migration (35). Furthermore, the altered immune function in COVID-19, coupled with changes in microbiota of the respiratory and gastrointestinal systems, further facilitates the susceptibility of COVID-19 infected patients to bacterial superinfections (36).

It has been observed that *A. baumannii* has a wide range of virulence factors, such as versatile survival mechanisms which help to evade the immune response of the host, as well as the efficient interference capacity to bind, internalize, and induce apoptosis in host cells (37). Integral to these mechanisms are the outer membrane proteins (OMPs), such as the OmpA, which serve as the key facilitator in binding and internalization of *A. baumannii* in the host epithelial cells. Beside these interactions, OmpA initiates a cascade of apoptotic factors within the cells of the host which trigger cell death (38). The capsular exopolysaccharides of *A. baumannii* operate as a protective mechanism, shielding it from both environmental and host-related defense mechanisms (38). The degree of virulence of *A. baumannii* is linked to the structure of these exopolysaccharides, which acts as a dynamic resistance system (39).

CLINICAL PRESENTATION AND DIAGNOSIS

When a patient develops a new or progressive infiltrate on chest radiography, leukocytosis, and purulent tracheobronchial secretions VAP is suspected (40). A comparison of histological analysis and culture findings of lung samples obtained immediately after death suggested that the presence of a new and persistent (>48 h) infiltrate on chest radiography along with two or more of the following criteria: 1) fever >38.3°C, 2) leukocytosis >12 × 10^9/ml, and/or 3) purulent tracheobronchial secretions, had 69% chances of confirming the VAP diagnosis (sensitivity of 69% and specificity of 75%) (41). Sensitivity of these clinical criteria for VAP diagnosis is lower in patients with acute respiratory distress syndrome (ARDS) in critical COVID-19, making it challenging to detect new radiographic infiltrates (42). With regards to the ARDS, Bell et al. (43) reported a false-negative rate of 46% for clinical diagnosis of VAP. Consequently, suspected VAP in ARDS as a complication of COVID-19 could be high.

Systemic signs of pneumonia, such as fever, tachycardia, and leukocytosis, are non-specific and could result from COVID-19, VAP or any other condition in which the release of cytokines, such as interleukin-1, interleukin-6, tumor necrosis factor-alpha, and gamma interferon, is the primary immune response (44). In cases of purulent sputum, positive sputum cultures, fever, and leukocytosis without a new pulmonary infiltrate, a diagnosis of hospital-acquired tracheobronchitis should be considered, except when the ARDS-associated VAP is suspected (45).

While a normal chest X-ray is rarely associated with VAP, a study on surgical patients found that 26% of opacities were detected through computed tomography (CT), but not on portable chest X-ray (40). Furthermore, asymmetric lung infiltrates consistent with VAP can result from various non-infectious disorders, including atelectasis, chemical pneumonitis, asymmetric cardiogenic pulmonary edema, pulmonary embolism, cryptogenic organizing pneumonia, lung contusion, and drug-induced lung reactions (46). The overall radiographic specificity of lung opacities consistent with pneumonia is only 27% to 35% (40).

Accurate laboratory identification of causative agents is crucial, but remains controversial because of the challenges when differentiating between rapid bacterial col-

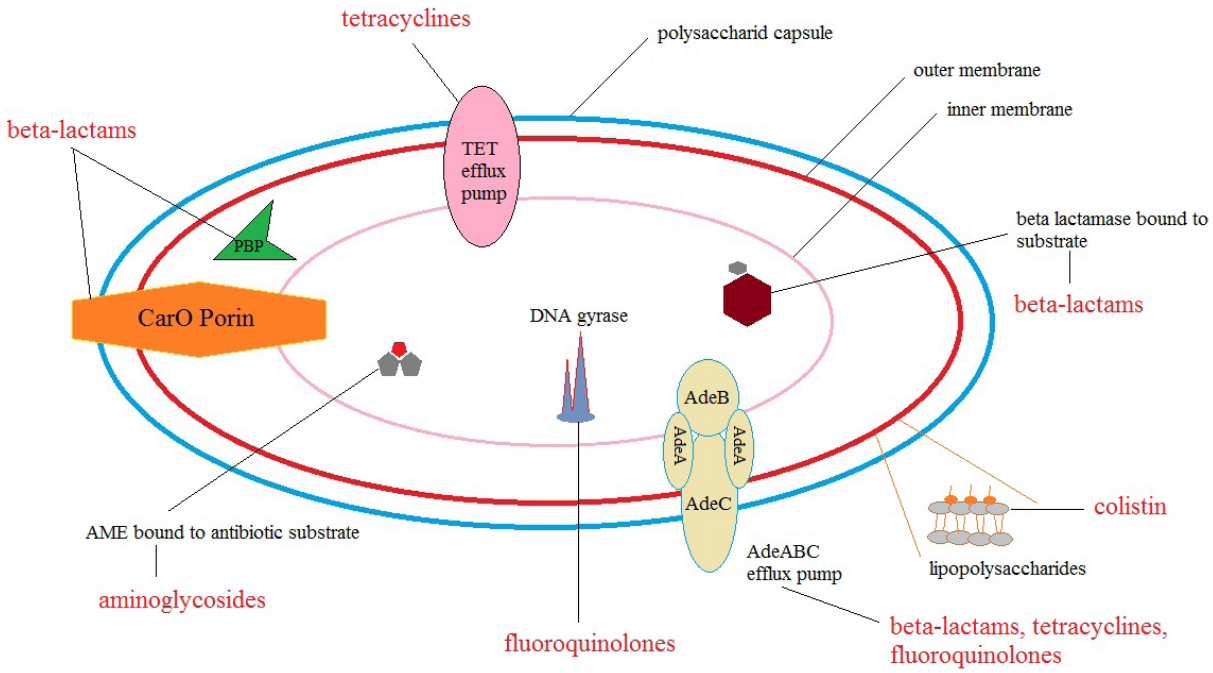


Figure 1. Resistance mechanisms of *A. baumannii* (PBP-penicillin binding protein, AME-aminoglycoside modifying enzymes)

onization in ventilated patients, coinfection, and VAP (47). Literature data support the sampling of respiratory secretions, tissues, blood, and pleural fluid to confirm VAP. In less than 10% of VAP cases, the infection spreads into the bloodstream or pleural space (46). Therefore, the experts recommend obtaining two sets of blood cultures and performing thoracentesis for non-loculated pleural effusions (≥10 mm in diameter) on chest X-rays with lateral decubitus as part of a suspected VAP (48). For loculated effusions, ultrasound-guided aspiration may be necessary (40). However, it is important to note that the sensitivity of blood cultures for VAP diagnosis is lower than 25% (49). Even when positive, infectious agents may originate from extrapulmonary sites of the infection in up to 64% of cases, despite the presence of VAP (49).

Gram staining and non-quantitative or semi-quantitative cultures of tracheal secretions have advantages, including reproducibility and minimal technical requirements, without the need for a specialized equipment (50). The semi-quantitative scoring of Gram stain was established based on the number of bacteria per high-power (×1,000) oil immersion field, utilizing the following criteria: 0 = absence of bacteria per field; 1+ = fewer than one bacterium per field; 2+ = 1–5 bacteria per field; 3+ = 6–30 bacteria per field; and 4+ = more than 30 bacteria per field (51). However, these studies contribute minimally to the sensitivity and specificity of the clinical diagnosis of VAP, as the upper respiratory tract quickly becomes colonized by lung pathogens within hours of intubation, even in the absence of pneumonia (52). In a study involving 48 patients with respiratory failure, the concordance between non-quantitative tracheal cultures and lung tissue cultures obtained through an open lung biopsy was only 40% (53). In that study, in patients with histologically confirmed pneumonia, endotracheal aspirates (ETA) exhibited sensitivity of 82%, but specificity of only 27%. Prior antibiotics use can result in a false-negative rate of 10 to 40% as well (54).

To potentially improve the specificity of VAP diagnosis and prevent the unnecessary use of antibiotics, some studies explored the role of quantitative cultures of respiratory secretions (40). These include non-bronchoscopic methods, such as quantitative cultures of endotracheal aspirates (QEA) and blind sampling of secretions from distal airways through the endobronchial catheter (40).

CHALLENGES OF MUTLI-DRUG RESISTANCE

Mechanisms of antibiotic resistance of *A. baumannii* can be categorized into three groups: (1) reduction of membrane permeability or increase in efflux of the antibiotic; (2) genetic mutation or post-translational modification; (3) hydrolysis or modification (55). Visual representation of the key resistance mechanisms of A. baumannii is provided in Figure 1. Based on these mechanisms, it can be concluded that *A. baumannii* has a remarkable ability to acquire antimicrobial resistance. It has been previously identified that *A. baumannii* is resistant to penicillins, macrolides, trimethoprim, and fosfomycin (56). Resistance to cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones has also been documented in *A. baumannii* strains (57). Infections caused by the CRAB result in a prolonged hospital stay, poor outcomes, and increase in healthcare costs compared to the infections caused by carbapenem-susceptible strains (58).

Expectedly, *A. baumannii* is resistant to a wide spectrum of antibiotics, such as penicillins, macrolides, tri-

methoprim, and fosfomycin (59-61). Moreover, this bacterium displays an extraordinary capacity to develop antimicrobial resistance to cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones, as evidenced by previous data (59). The remaining challenge focuses around the increasing resistance to carbapenems, the last line of defense against infectious agents caused by multidrug-resistant Gram-negative bacteria. Although tigecycline represents one of the last resort therapies for MDR *A. baumannii*, resistance to this antibiotic has been reported already (62). A study from Serbia emphasized that the primary mechanism behind tigecycline resistance in *A. baumannii* isolated in the Balkan hospitals was the overexpression of antibiotics efflux pump (63).

Resistance to carbapenem in *Acinetobacter spp.* is often linked to the acquired production of carbapenemases, with class D beta-lactamases, or oxacillinases (OKSAs), having a major role in this process (64). At the present moment, distinct groups of OKSA-type carbapenemases found in *A. baumannii* include OKSA-23-like, OKSA-24/40-like, OKSA-58-like, OKSA-143-like, OKSA-235-like groups, alongside an intrinsic chromosomal group similar to OKSA-51 (64-66). In addition, acquired resistance to aminoglycosides and fluoroquinolones has been observed in *A. baumannii* strains that produce carbapenemases (67).

In a recent study from Serbia, mutations in the PmrB protein were identified as the main factor in colistin resistance (68). The finding that chromosomal mutations were the culprit for colistin resistance (ColR) in Serbia may have implications for other global high-risk clonal lineages (68). This challenges a previous belief that resistant strains disseminated and subsequently proliferated locally (68). Research by Strateva et al. suggested that a rise of OXA-72 increases the risk of horizontal transfer of antibiotic resistance genes (ARGs) in nosocomial *A. baumannii* isolates and other Gram-negative bacteria through plasmids and transposable mobile genetic elements in Bulgaria (69). The increasing resistance of ColR *A. baumannii* clones suggests that there is an urgent need for a comprehensive global surveillance system of AMR (69).

THERAPEUTIC APPROACHES

General principles in treatment of VAP caused by *A. baumannii* while having COVID-19 include an isolation of a causative agent, identification of sensitivity and resistance patterns, rational antibiotic dispensing, and justification for de-escalation or discontinuation of antibiotics usage (70). Irrational antibiotics usage is especially relevant in the Serbian hospital setting as increased use of many antibiotics has been recorded over the COVID-19 pandemic (71, 72). A study of trends in antibiotics use pre- and during the COVID-19 pandemic in Serbia documented a significant increase in the use of antibiotics labelled as "Watch" and "Reserve" drugs (72). Moreover, more than one-half of children treated for COVID-19 in the first pandemic wave in Serbia received antimicrobial agents without clear indication of bacterial superinfection (71).

The treatment of CRAB depends on the antibiogram and severity of clinical presentation, but generally involves colistin administration, either alone or in a combination with other antibiotics (75). Treatment of carbapenem-sensitive strains is carried out as a monotherapy using imipenem or meropenem (75). Most commonly used antibiotics in combination with colistin for CRAB are ampicillin/sulbactam, fosfomycin, tigecycline, and meropenem (75). A combination of colistin with meropenem has shown to be either equally as effective or less effective compared to colistin alone and it is not being used if the minimum inhibitory concentrations of *A. baumannii* are high (75). Combinations of colistin with other antibiotics (ampicillin/sulbactam, fosfomycin, tigecycline) have also been more effective than colistin alone (72). Given that intravenous administration of colistin does not provide sufficient drug concentrations in lung tissue, inhalation of colistin may be a better option for VAP caused by CRAB, in addition to the previously described intravenous antibiotics administration (76).

NEW THERAPEUTIC POSSIBILITIES

Sulbactam/durlobactam (KSACDURO®) represents a co-formulated antibacterial compound to manage infections arising from the *A. baumannii-calcoaceticus* complex (ABC) (77, 78). Concurrent administration of durlobactam, a potent serine β-lactamase inhibitor exhibiting broad-spectrum activity, in conjunction with sulbactam, a well-established class A β-lactamase inhibitor effective against *A. baumannii*, prevents enzymatic degradation of sulbactam by β-lactamases produced by ABC (78). Sulbactam/durlobactam was also approved in the United States in 2023 for adult patients who had hospital-acquired bacterial pneumonia and VAP caused by ABC (77).

Zosurabalpin represents a novel category of macrocyclic peptide antibiotics (MPA) highly efficient against *Acinetobacter spp.*, specifically targeting CRAB-calcoaceticus strains (79). The outer membrane of Gram-negative bacteria is composed of an asymmetrical double layer with phospholipids in the inner layer and lipopolysaccharide (LPS) in the outer layer (79). The synthesis of LPS is finalized within the cell at the inner membrane. To facilitate the formation of the outer membrane, components of the LPS transporter in the inner membrane assemble into a subcomplex that extracts LPS from the double layer (79).

Progress in the field of reverse vaccinology, proteomics, and genomics has certainly improved vaccine development. Despite the success of experimental vaccines against *A. baumannii* on animals, their application in humans is still limited. This is, in part, attributed to a rela-

tively small number of vaccine antigen targets identified thus far, which does not allow for satisfactory vaccine effectiveness (80). Difficulties with purification and limited safety profile warrant more research in this field (80).

CONCLUSION

Epidemiological data suggest that the incidence of *A. baumannii* in ICUs during the pandemic has been on the rise. It is potentially linked to hospital overcrowding and antibiotic misuse. The complexity of clinical presentation and differential diagnosis requires a more accurate identification methods to differentiate VAP from other conditions. Effective managing *A. baumannii* VAP while having COVID-19 involves several principles: identification of the bacterium in biological specimen, understanding local resistance patterns, implementing a rational antibiotic regimen, and justifying antibiotic de-escalation or cessation. It is critical to eliminate risk factors such as endotracheal and nasogastric tubes, tracheostomy, reintubation, enteral nutrition, corticosteroid use, stomach pH modifiers, supine positioning, prior antibiotics, poor infection control, and contaminated equipment. The rise of multidrug resistance poses a significant challenge, with limited options for an effective treatment. Novel therapeutic possibilities, such as sulbactam/durlobactam and zosurabalpin, offer promising results, but research in vaccine development could potentially become a long-term solution for *A. baumannii* VAP prevention.

REFERENCES

1. Gazibara T, Maksimovic N, Dotlic J, Jeremic Stojkovic V, Cvjetkovic S, Milic M. Experiences and aftermath of the COVID-19 lockdown among community-dwelling older people in Serbia: A qualitative study. J Eval Clin Pract 2022;28(4):631-640.
2. Gazibara T, Cvjetkovic S, Milic M, Dotlic J, Maksimovic N, Jovanovic V, et al. Preferences of COVID-19 Vaccines in the General Population in Belgrade, Serbia: A Qualitative Study. Behav Med. 2022 in press doi: 10.1080/08964289.2022.2085652.
3. Maksimovic N, Gazibara T, Dotlic J, Milic M, Jeremic Stojkovic V, Cvjetkovic S, et al. "It Bothered Me": The Mental Burden of COVID-19 Media Reports on Community-Dwelling Elderly People. Medicina (Kaunas) 2023;59(11):e2011.
4. Mehta OP, Bhandari P, Raut A, Kacimi SEO, Huy NT. Coronavirus Disease (COVID-19): Comprehensive Review of Clinical Presentation. Front Public Health 2021;8:5829-32.
5. Paunic M, Filipovic S, Nieuwenhuis M, Paunic A, Pesic M, Tomasevic M, et al. Severity of COVID-19 Symptoms among University of Belgrade Students during the July–September 2021 Pandemic Wave: Implications for Vaccination. Med Princ Pract 2022;31(2):165-173.
6. Shahid Z, Kalayanamitra R, McClafferty B, Kepko D, Ramgobin D, Patel R, et al. COVID-19 and Older Adults: What We Know. J Am Geriatr Soc 2020;68(5):926-929.
7. Almeshari A, Alobaidi Y, Al Asmri M, Alhuthail E, Alshehri Z, Alenezi F, et al. Mechanical ventilation utilization in COVID-19: a systematic review and meta-analysis. MedRxiv 2020;2020-2006.
8. Ouyang L, Yu M, Zhu Y, Gong J. Respiratory supports of COVID-19 patients in intensive care unit: A systematic review. Heliyon 2021;7(4):e06813.
9. Potalivo A, Montomoli J, Facondini F, Sanson G, Lazzari Agli LA, Perin T, et al. Sixty-day mortality among 520 Italian hospitalized COVID-19 patients according to the adopted ventilatory strategy in the context of an integrated multidisciplinary clinical organization: a population-based cohort study. Clin Epidemiol 2020;12:1421-1431.
10. Zochios V, Brodie D, Shekar K, Schultz MJ, Parhar KKS. Invasive mechanical ventilation in patients with acute respiratory distress syndrome receiving extracorporeal support: a narrative review of strategies to mitigate lung injury. Anaesthesia 2022;77(10):1137-1151.
11. Kalanuria AA, Ziai W, Mirski M. Ventilator-associated pneumonia in the ICU. Crit Care 2014;18(2):208.
12. Huang Y, Zhou Q, Wang W, Huang Q, Liao J, Li J, et al. Acinetobacter baumannii Ventilator-Associated Pneumonia: Clinical Efficacy of Combined Antimicrobial Therapy and in vitro Drug Sensitivity Test Results. Front Pharmacol. 2019;10:92.
13. Centers for Disease Control and Prevention (CDC). Antibiotic Resistance Threats in the United States 2019. Available from: https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf (accessed on 8 January 2024).
14. European Centre for Disease Prevention and Control (ECDC). Antimicrobial Resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report for 2020. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/AER-EARS-Net-2020.pdf (accessed on 8 January 2024).
15. Ayobami O, Willrich N, Harder T, Okeke IN, Eckmanns T, Markwart R. The incidence and prevalence of hospital-acquired (carbapenem-resistant) Acinetobacter baumannii in Europe, Eastern Mediterranean and Africa: a systematic review and meta-analysis. Emerg Microbes Infect 2019;8(1):1747-1759.
16. Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect 2020;26(12):1622-1629.
17. Cox MJ, Loman N, Bogaert D, O'Grady J. Co-infections: potentially lethal and unexplored in COVID-19. Lancet Microbe 2020;1(1):e11.
18. Jovanović B. Risk factors for ventilator associated pneumonia in intensive care units. 2016. Doctoral thesis. Available from: https://nardus.mpn.gov.rs/bitstream/handle/123456789/7356/ Disertacija.pdf?sequence=6&isAllowed=y (accessed on 8 January 2024).
19. Pustijanac E, Hrenović J, Vranić-Ladavac M, Močenić M, Karčić N, Lazarić Stefanović L, et al. Dissemination of Clinical Acinetobacter baumannii Isolate to Hospital Environment during the COVID-19 Pandemic. Pathogens 2023;12(3):410.
20. Boral J, Genç Z, Pınarlık F, Ekinci G, Kuskucu MA, İrkören P, et al. The association between Acinetobacter baumannii infections and the COVID-19 pandemic in an intensive care unit. Sci Rep 2022;12(1):20808.
21. Bahçe YG, Acer Ö, Özüdoğru O. Evaluation of bacterial agents isolated from endotracheal aspirate cultures of COVID-19 general intensive care patients and their antibiotic resistance profiles compared to pre-pandemic conditions. Microb Pathog 2022;164:105409.
22. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and Fungal Coinfection in Individuals With Coronavirus: A Rapid Review To Support COVID-19 Antimicrobial Prescribing. Clin Infect Dis 2020;71(9):2459-2468.
23. Chen CT, Wang YC, Kuo SC, Shih FH, Chen TL, How CK, et al. Community-acquired bloodstream infections caused by Acinetobacter baumannii: A matched case-control study. J Microbiol Immunol Infect 2018;51(5):629-635.
24. Fahy S, O'Connor JA, Lucey B, Sleator RD. Hospital Reservoirs of Multidrug Resistant Acinetobacter Species-The Elephant in the Room! Br J Biomed Sci 2023;80:e11098.
25. Cavallo I, Oliva A, Pages R, Sivori F, Truglio M, Fabrizio G, et al. Acinetobacter baumannii in the critically ill: complex infections get complicated. Front Microbiol 2023; 14:11967-11974.

26. Jawad A, Seifert H, Snelling AM, Heritage J, Hawkey PM. Survival of Acinetobacter baumannii on dry surfaces: comparison of outbreak and sporadic isolates. J Clin Microbiol 1998;36(7):1938-1941.
27. Kanafani ZA, Zahreddine N, Tayyar R, Sfeir J, Araj GF, Matar GM, et al. Multi-drug resistant Acinetobacter species: a seven-year experience from a tertiary care center in Lebanon. Antimicrob Resist Infect Control 2018;7:9.
28. Novović K, Kuzmanović Nedeljković S, Poledica M, Nikolić G, Grujić B, Jovčić B, et al. Virulence potential of multidrug-resistant Acinetobacter baumannii isolates from COVID-19 patients on mechanical ventilation: The first report from Serbia. Front Microbiol 2023; 14:1094184.
29. Seneghini M, Rüfenacht S, Babouee-Flury B, Flury D, Schlegel M, Kuster SP, et al. It is complicated: Potential short- and long-term impact of coronavirus disease 2019 (COVID-19) on antimicrobial resistance-An expert review. Antimicrob Steward Healthc Epidemiol 2022;2(1):e27.
30. Thom KA, Rock C, Jackson SS, Johnson JK, Srinivasan A, Magder LS, et al. Factors Leading to Transmission Risk of Acinetobacter baumannii. Crit Care Med 2017;45(7):e633-e639.
31. 31. Shamsizadeh Z, Nikaeen M, Nasr Esfahani B, Mirhoseini SH, Hatamzadeh M, Hassanzadeh A. Detection of antibiotic resistant Acinetobacter baumannii in various hospital environments: potential sources for transmission of Acinetobacter infections. Environ Health Prev Med 2017;22(1):44.
32. Howard A, O'Donoghue M, Feeney A, Sleator RD. Acinetobacter baumannii: an emerging opportunistic pathogen. Virulence 2012;3(3):243-250.
33. Alp E, Voss A. Ventilator associated pneumonia and infection control. Ann Clin Microbiol Antimicrob 2006;5:7.
34. Hoque MN, Akter S, Mishu ID, Islam MR, Rahman MS, Akhter M, et al. Microbial co-infections in COVID-19: Associated microbiota and underlying mechanisms of pathogenesis. Microb Pathog 2021;156:104941.
35. Zamani Rarani F, Zamani Rarani M, Hamblin MR, Rashidi B, Hashemian SMR, Mirzaei H. Comprehensive overview of COVID-19-related respiratory failure: focus on cellular interactions. Cell Mol Biol Lett 2022;27(1):63.
36. Zhang F, Lau RI, Liu Q, Su Q, Chan FKL, Ng SC. Gut microbiota in COVID-19: key microbial changes, potential mechanisms and clinical applications. Nat Rev Gastroenterol Hepatol 2023;20(5):323-337.
37. Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of Acinetobacter baumannii virulence. Nat. Rev. Microbiol 2018;16(2):91-102.
38. Rumbo C, Tomás M, Fernández Moreira E, Soares NC, Carvajal M, Santillana E, et al. The Acinetobacter baumannii Omp33-36 porin is a virulence factor that induces apoptosis and modulates autophagy in human cells. Infect Immun 2014; 82:4666-4680.
39. Ayoub Moubareck C, Hammoudi Halat D. Insights into Acinetobacter baumannii: A Review of Microbiological, Virulence, and Resistance Traits in a Threatening Nosocomial Pathogen. Antibiotics (Basel) 2020;9(3):119.
40. Koenig SM, Truwit JD. Ventilator-associated pneumonia: diagnosis, treatment, and prevention. Clin Microbiol Rev 2006;19(4):637-57.
41. Fàbregas N, Ewig S, Torres A, El-Ebiary M, Ramirez J, de La Bellacasa JP, et al. Clinical diagnosis of ventilator associated pneumonia revisited: comparative validation using immediate post-mortem lung biopsies. Thorax 1999;54(10):867-873.
42. François B, Laterre PF, Luyt CE, Chastre J. The challenge of ventilator-associated pneumonia diagnosis in COVID-19 patients. Crit Care 2020;24(1):289.
43. Bell RC, Coalson JJ, Smith JD, Johanson WG Jr. Multiple organ system failure and infection in adult respiratory distress syndrome. Ann Intern Med 1983;99(3):293-298.
44. Chollet-Martin S, Montravers P, Gibert C, Elbim C, Desmonts JM, Fagon JY, et al. High levels of interleukin-8 in the blood and alveolar spaces of patients with pneumonia and adult respiratory distress syndrome. Infect Immun 1993;61(11):4553-4559.
45. Nseir S, Di Pompeo C, Pronnier P, Beague S, Onimus T, Saulnier F, et al. Nosocomial tracheobronchitis in mechanically ventilated patients: incidence, aetiology and outcome. Eur Respir J 2002;20(6):1483-1489.
46. Meduri GU, Mauldin GL, Wunderink RG, Leeper KV Jr, Jones CB, Tolley E, et al. Causes of fever and pulmonary densities in patients with clinical manifestations of ventilator-associated pneumonia. Chest 1994;106(1):221-235.
47. Berton DC, Kalil AC, Teixeira PJZ. Quantitative versus qualitative cultures of respiratory secretions for clinical outcomes in patients with ventilator-associated pneumonia. Cochrane Database Syst Rev 2014;(10):CD006482.
48. Chastre J, Fagon JY. Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002;165(7):867-903.
49. Luna CM, Videla A, Mattera J, Vay C, Famiglietti A, Vujacich P, et al. Blood cultures have limited value in predicting severity of illness and as a diagnostic tool in ventilator-associated pneumonia. Chest 1999;116(4):1075-1084.
50. Hayon J, Figliolini C, Combes A, Trouillet JL, Kassis N, Dombret MC, et al. Role of serial routine microbiologic culture results in the initial management of ventilator-associated pneumonia. Am J Respir Crit Care Med 2002;165(1):41-46.
51. Hashimoto S, Shime N. Evaluation of semi-quantitative scoring of Gram staining or semi-quantitative culture for the diagnosis of ventilator-associated pneumonia: a retrospective comparison with quantitative culture. J Intensive Care 2013;1(1):2.
52. Wałaszek M, Serwacki P, Cholewa Z, Kosiarska A, Świątek-Kwapniewska W, Kołpa M, et al. Ventilator-associated pneumonia in Polish intensive care unit dedicated to COVID-19 patients. BMC Pulm Med 2023;23(1):443.
53. Hill JD, Ratliff JL, Parrott JC, Lamy M, Fallat RJ, Koeniger E, et al. Pulmonary pathology in acute respiratory insufficiency: lung biopsy as a diagnostic tool. J Thorac Cardiovasc Surg 1976;71(1):64-71.
54. Michel F, Franceschini B, Berger P, Arnal JM, Gainnier M, Sainty JM, et al. Early antibiotic treatment for BAL-confirmed ventilator-associated pneumonia: a role for routine endotracheal aspirate cultures. Chest 2005;127(2):589-597.
55. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. Acinetobacter baumannii Antibiotic Resistance Mechanisms. Pathogens 2021;10(3):373.
56. Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, et al. Biology of Acinetobacter baumannii: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options. Front Cell Infect Microbiol 2017; 7:55.
57. Doi Y, Murray GL, Peleg AY. Acinetobacter baumannii: evolution of antimicrobial resistance-treatment options. Semin Respir Crit Care Med 2015;36(1):85-98.
58. Vivo A, Fitzpatrick MA, Suda KJ, Jones MM, Perencevich EN, Rubin MA, et al. Epidemiology and outcomes associated with carbapenem-resistant Acinetobacter baumannii and carbapenem-resistant Pseudomonas aeruginosa: a retrospective cohort study. BMC Infect Dis 2022;22(1):491.
59. Zahn M, Bhamidimarri SP, Baslé A, Winterhalter M, Van Den Berg B. Structural Insights into Outer Membrane Permeability of Acinetobacter baumannii. Structure 2016; 24:221-231.
60. Zhu LJ, Chen XY, Hou PF. Mutation of CarO participates in drug resistance in imipenem-resistant Acinetobacter baumannii. J Clin Lab Anal 2019;33:e22976.
61. Mussi MA, Limansky AS, Viale AM. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of Acinetobacter baumannii: Natural insertional inactivation of a gene encoding a member of a novel family of β-barrel outer membrane proteins. Antimicrob Agents Chemother 2005; 49:1432-1440.
62. Jo J, Ko KS. Tigecycline Heteroresistance and Resistance Mechanism in Clinical Isolates of Acinetobacter baumannii. Microbiol Spectr 2021; 9(2):e0101021.
63. Gajic I, Ranin L, Kekic D, Opavski N, Smitran A, Mijac V, et al. Tigecycline susceptibility of multidrug-resistant Acinetobacter bauman-

nii from intensive care units in the western Balkans. Acta Microbiol Immunol Hung 2020;67(3):176-181.

64. Vahhabi A, Hasani A, Rezaee MA, Baradaran B, Hasani A, Kafil HS, et al. Carbapenem resistance in Acinetobacter baumannii clinical isolates from northwest Iran: high prevalence of OXA genes in sync. Iran J Microbiol 2021;13(3):282-293.
65. Gupta N, Angadi K, Jadhav S. Molecular Characterization of Carbapenem-Resistant Acinetobacter baumannii with Special Reference to Carbapenemases: A Systematic Review. Infect Drug Resist 2022; 15:7631-7650.
66. Héritier C, Poirel L, Fournier PE, Claverie JM, Raoult D, Nordmann P. Characterization of the naturally occurring oxacillinase of Acinetobacter baumannii. Antimicrob Agents Chemother 2005;49(10):4174-4179.
67. Mączyńska B, Jama-Kmiecik A, Sarowska J, Woronowicz K, Choroszy-Król I, Piątek D, et al. Changes in Antibiotic Resistance of Acinetobacter baumannii and Pseudomonas aeruginosa Clinical Isolates in a Multi-Profile Hospital in Years 2017-2022 in Wroclaw, Poland. J Clin Med 2023;12(15):5020.
68. Kabic J, Novovic K, Kekic D, Trudic A, Opavski N, Dimkic I, et al. Comparative genomics and molecular epidemiology of colistin-resistant Acinetobacter baumannii. Comput Struct Biotechnol J 2022; 21:574-585.
69. Strateva TV, Sirakov I, Stoeva TJ, Stratev A, Peykov S. Phenotypic and Molecular Characteristics of Carbapenem-Resistant Acinetobacter baumannii Isolates from Bulgarian Intensive Care Unit Patients. Microorganisms 2023;11(4):875.
70. Lima WG, Brito JCM, da Cruz Nizer WS. Ventilator-associated pneumonia (VAP) caused by carbapenem-resistant Acinetobacter baumannii in patients with COVID-19: Two problems, one solution? Med Hypotheses 2020; 144:110139.
71. Prijić A, Gazibara T, Prijić S, Mandić-Rajčević S, Maksimović, N. Factors Associated with the Antibiotic Treatment of Children Hospitalized for COVID-19 during the Lockdown in Serbia. Int J Environ Res Public Health 2022;19(23):15590.
72. Filimonovic J, Ristić ZS, Gazibara T, Saponjic V, Dotlic J, Jovanovic V, et al. Trends and patterns of antibiotics use in Serbia from 2006 to 2021: Pre-COVID-19 period versus COVID-19 pandemic. Am J Infect Control 2024;52(3):293-304.
73. Li J, Fu Y, Zhang J, Zhao Y, Fan X, Yu L, et al. The efficacy of colistin monotherapy versus combination therapy with other antimicrobials against carbapenem-resistant Acinetobacter baumannii ST2 isolates. J Chemother 2020;32(7):359-367.
74. Kim YK, Lee JH, Lee HK, Chung BC, Yu SJ, Lee HY, et al. Efficacy of nebulized colistin-based therapy without concurrent intravenous colistin for ventilator-associated pneumonia caused by carbapenem-resistant Acinetobacter baumannii. J Thorac Dis 2017;9(3):555-567.
75. Keam SJ. Sulbactam/Durlobactam: First Approval. Drugs. 2023;83(13):1245-1252.
76. McLeod SM, Moussa SH, Hackel MA, Miller AA. In Vitro Activity of Sulbactam-Durlobactam against Acinetobacter baumannii-calcoaceticus Complex Isolates Collected Globally in 2016 and 2017. Antimicrob Agents Chemother 2020;64(4):e1-18.
77. Beninger P. Sulbactam/Durlobactam. Clin Ther 2024;46(1):82-83.
78. Zampaloni C, Mattei P, Bleicher K, Winther L, Thäte C, Bucher C, et al. A novel antibiotic class targeting the lipopolysaccharide transporter. Nature 2024 ;625(7995):566-571.
79. Guenther A, Millar L, Messer A, Giraudon M, Patel K, Deurloo EJ, et al. 2126. Safety, Tolerability, and Pharmacokinetics (PK) in Healthy Participants Following Single Dose Administration of Zosurabalpin, a Novel Pathogen-Specific Antibiotic for the Treatment of Serious Acinetobacter Infections. Open Forum Infect Dis 2023;10(Suppl 2):ofad500.1749.
80. Chiang MH, Sung WC, Lien SP, Chen YZ, Lo AF, Huang JH, et al. Identification of novel vaccine candidates against Acinetobacter baumannii using reverse vaccinology. Hum Vaccin Immunother. 2015;11(4):1065-73.

PNEUMONIJA PROUZROKOVANA ACINETOBACTER BAUMANII TOKOM MEHANIČKE VENTILACIJE USLED INFEKCIJE KOVIDOM 19: EPIDEMIOLOŠKE I KLINIČKE KARAKTERISTIKE I TERAPIJA

Tatjana Gazibara[1], Branko Beronja[2], Aleksandra Karan[3], Biljana Lukić[3]

Sažetak

Manje od 3% ljudi koji su zaraženi infekcijom kovid 19 imaju potrebu za bolničkim lečenjem. Međutim, jednoj trećini hospitalizovanih pacijenata sa kovidom 19 je neophodna invazivna mehanička ventilacija. Pneumonija povezana sa korišćenjem respiratora (PPKR) čiji je prouzrokovač multirezistentni *Acinetobacter baumannii* (*A. baumannii*) je nova infekcija u jedinicama intenzivne nege i može imati fatalne posledice kod osoba sa teškom formom infekcije izazvane kovidom 19. Zemlje Balkanskog poluostrva imaju izuzetno visoku prevalenciju karbapenem rezistentnih *Acinetobacter spp* u bolnicama. Dijagnoza ove pneumonije je prilično komplikovana. Štaviše, složenost lečenja nastaje usled rezistencije *A. baumannii* na više antibiotika. Novi lekovi, kao što su sulbaktam/durlobaktam i zosurabalpin pokazali su dobru efektivnost u borbi protiv PPKR izazvane *A. baumannii*. Antimikrobna rezistencija *A. baumannii* nije u potpunosti rasvetljena, iako je definisano nekoliko mehanizama. Za adekvatno lečenje PPKR važno je izolovati uzročnika, imati na umu mehanizme rezistencije, pažljivo dozirati antibiotike i identifikovati faktore rizika. U ovom preglednom radu biće razmotrene epidemiološke karakteristike, patofiziološki mehanizmi, klinička slika i dijagnostika, kao i aktuelne i nove terapijske mogućnosti PPKR izazvane *A. baumannii*.

Ključne reči: kovid 19, pneumonija povezana sa ventilacijom, Acinetobacter baumannii, multirezistencija na lekove

Primljen: 26.02.2024. I **Revizija:** 22.05.2024. I **Prihvaćen:** 22.05.2024.

Medicinska istaživanja 2024; 57(3):129-136

Medicinska istaživanja 2024; 57(3):137-146 | DOI: 10.5937/medi57-50484

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REVIEW ARTICLE

MR imaging features of primary sclerosing cholangitis: a comprehensive overview of image-based scoring systems for assessment of disease severity and prognosis

Jelena Đokić Kovač[1], Aleksandra Janković[1], Milica Mitrović-Jovanović[1], Aleksandar Bogdanović[2], Vladimir Dugalić[2], Tamara Milovanović[3]

[1] Center for Radiology and Magnetic Resonance Imaging, University Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

[2] First Surgical Clinic, University Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

[3] Clinic for Gastroenterohepatology, University Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Recived: 16 April 2024

Revised: 14 June 2024

Accepted: 14 June 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Jelena Djokić Kovač

Centre for Radiology and Magnetic Resonance Imaging, University Clinical Center of Serbia, 2 Pasterova Street, 11000 Belgrade, Serbia

E-mail: jelenadjokickovac@gmail.com

Summary

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease marked by inflammation, fibrosis, and narrowing of the bile ducts, leading to cholestasis. Magnetic resonance cholangiopancreatography (MRCP) is the gold standard for the diagnosis of PSC allowing insight into biliary duct changes. The typical presentation of PSC includes multifocal anular and short-segmental strictures alternating with normal or slightly dilatated biliary ducts. Besides cholangiographic findings, magnetic resonance (MR) allows the assessment of liver parenchymal changes which might indicate the severity of the disease. The scoring systems based on MR findings, such as the ANALI score, and new computer-based software analysis termed MRCP+, provide a prediction of the course of disease and identify high-risk patients. Thus, MR with MRCP is a promising diagnostic tool for the integrative evaluation of PSC patients allowing not only initial diagnosis and detection of complications but also has prognostic significance.

Key words: liver, primary sclerosing cholangitis, magnetic resonance cholangiopancreatography

Cite this article as: Đokić Kovač J, Janković A, Mitrović-Jovanović M, Bogdanović A, Dugalić V, Milovanović T. Mr imaging features of primary sclerosing cholangitis: a comprehensive overview of image-based scoring systems for assessment of disease severity and prognosis; Medicinska istaživanja 2024; 57(3):137-146 DOI: 10.5937/medi57-50484

INTRODUCTION

PSC is a chronic immune-mediated cholestatic liver disease characterized by inflammation and obliterative fibrosis of large bile ducts with subsequent development of multifocal biliary strictures. Even though PSC is a rare condition, typically seen in young and middle-aged men with slow progression, it eventually leads to cirrhosis and many associated complications (1, 2). There is a strong correlation between PSC and inflammatory bowel disease (IBD), particularly ulcerative colitis, but Crohn's disease can also be found (3). To date, there is no effective medical therapy for PSC and liver transplantation remains, the only proven life-extending treatment (4). The average transplant-free survival is estimated to be 20 years (5). There are a few prognostic models predominantly based on biochemical and clinical findings aiming to aid in predicting disease progression and time to transplant, but none of them has been widely accepted (7).

The precise etiopathogenesis of PSC remains unknown. Different factors such as autoimmune, genetic, and environmental contribute to the development of multifocal, patchy peribiliary inflammation pathognomonic for PSC (4). Histologically, concentric periductal fibrosis (onion skinning) in medium- and large-size bile ducts is a characteristic finding, accompanied by minimal inflammatory cells (7). However, this histological pattern is observed in less than 20% of patients and may also be present in secondary sclerosing cholangitis (7). Thus, liver biopsy alone is insufficient for PSC diagnosis and must be assessed in conjunction with clinical and imaging findings.

Diagnosis and follow-up of patients with PSC

The diagnosis of PSC is usually suspected due to elevation of cholestatic liver enzymes, alkaline phosphatase in particular (8). Nevertheless, elevated cholestatic biochemical markers are not mandatorily present, their values may spontaneously fluctuate and could also be normal (8). Transaminase levels are commonly slightly elevated, while the significant increase is seen during episodes of acute ascending cholangitis and also in patients with an overlap with AIH (6). Serum bilirubin levels are usually normal in PSC patients (6). Hyperbilirubinemia might be seen in cases of severe benign or malignant strictures or late stage of the disease with the development of hepatic dysfunction (6, 8). The diagnosis of PSC can be made only after the exclusion of all secondary causes of sclerosing cholangitis (8).

Since PSC is a disease of the biliary tract, the diagnosis relies on cholangiography findings obtained by endoscopic retrograde cholangiopancreatography (ERCP) or more commonly magnetic resonance cholangiopancreatography (MRCP) (8,9). MRCP exhibits high sensitivity (85%) and specificity (98%) for detection of bile duct irregularities in patients with PSC (9). Nowadays, MRCP is also used for surveillance of PSC patients and should be performed once a year (9). MRCP has superseded ERCP which was previously the only method for biliary duct evaluation (10). Although ERCP allows very good visualization of the biliary tract, it has serious post-procedural complications and also doesn't allow visualization of the lumen above the stenosis (9). ERCP is advised only when there is a need for therapeutic biliary stent placement (10). The diagnosis of PSC might be made on cholangiography if typical findings consisting of multiple strictures with or without dilatations are seen (11). However, the assessment of the presence of the strictures is made qualitatively, leading to high interobserver variability in MRCP interpretation (11). Therefore, there is an obvious need for a more quantitative approach in the analysis of MRCP findings.

Liver biopsy is generally unnecessary for the diagnosis of the most common type of PSC, large-bile duct PSC. In cases where the small bile duct type is suspected or PSC-AIH overlap syndrome liver biopsy should be performed (12). The small bile duct type is suspected when a patient with IBD presents with cholestatic laboratory findings, normal cholangiographic imaging features, and a negative antimitochondrial antibody profile (13). PSC is histologically characterized by the presence of an inflammatory infiltrate in a large intra- and extra-hepatic bile duct wall in conjunction with an obliterative concentric periductal fibrosis called "onion skin fibrosis" (12). As the disease progresses there is a gradual loss of small- and medium-sized bile ducts (ductopenia) (8, 12). Eventually, chronic inflammation leads to portal and periportal fibrosis and the development of biliary cirrhosis (8). Although liver biopsy may precisely depict the progression of the disease through different stages, it is not routinely used in everyday clinical practice due to its invasive nature and the possibility of sampling error (5,6).

MR IMAGING FEATURES OF PSC

The typical MRCP presentation of PSC consists of diffuse, multifocal strictures affecting both intra- and extrahepatic bile ducts in the majority of patients. The pathognomonic"beaded" appearance of the bile ducts is created by multiple, diffusely distributed, short, annular strictures associated with normal or slightly dilatated segments **(Figure 1)** (14). Isolated intrahepatic bile duct involvement is seen in 15% of patients, while only extrahepatic bile duct involvement is the rarest manifestation occurring in 8% of cases (11). The strictures in PSC are described as band-like strictures if the length is less than 2mm, segmental strictures if the length is from 2 mm up to 10 mm, and confluent strictures if the length is more than 10 mm (11). The strictures are commonly seen on bile duct bifurcations (11). In the early stages of PSC,

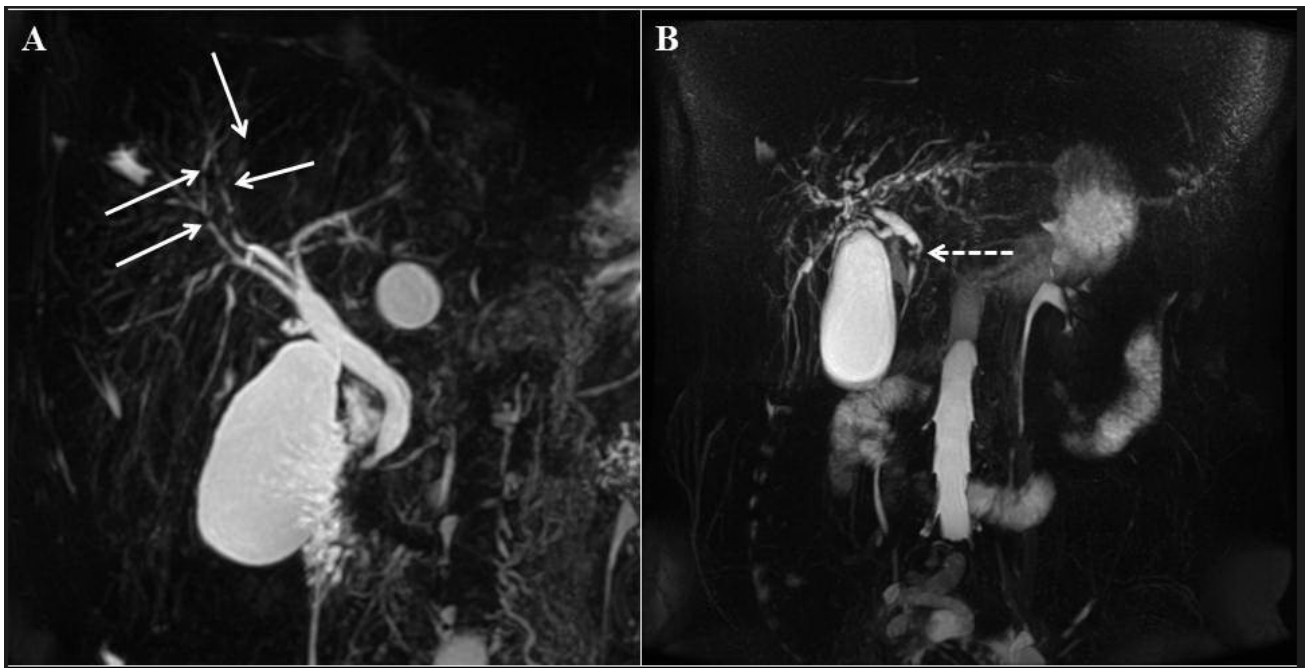


Figure 1. Primary sclerosing cholangitis in 33-year old man. (**A**) MRCP shows multiple short band-like strictures and slight luminal dilatation (*solid arrows*). In another 36-year-old patient with PSC (**B**), there is a classical "beaded appearance" of bile ducts - multiple short segmental and annular strictures with slightly dilated bile ducts between them. Note also the stricture of the middle third of the common bile duct (*dotted arrow*).

multiple strictures are seen in the absence of biliary dilatation (11, 15). With the progression of the disease, dilatation might be detected, but due to periductal fibrosis and inflammation the dilatation is rarely prominent (15).

Further disease progression may lead to complete obliteration of small peripheral ducts, resulting in a "pruned tree" appearance where only central ducts are visualized, while peripheral ducts are completely obliterated **(Figure 2)** (15). If significant dilatation is detected, complications of PSC like cholangiocarcinoma or ascending suppurative cholangitis should be suspected (16). Diverticular outpouchings are observed in up to 20% of cases. The least common feature is the presence of pigmented stones (15). Rarely, abnormalities might also be seen in the main pancreatic duct (15).

In addition to bile duct changes, MRI reveals associated parenchymal liver changes, including liver dysmorphy, confluent liver fibrosis, and parenchymal heterogeneity. A

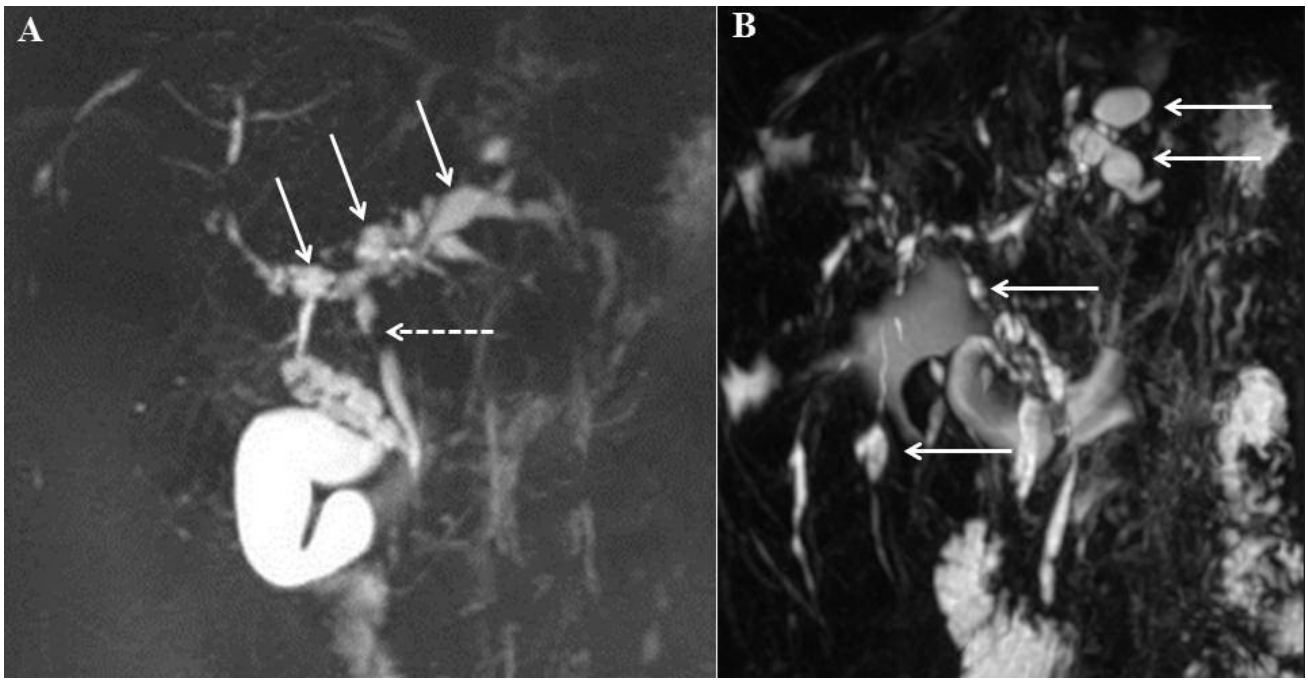


Figure 2. Advanced primary sclerosing cholangitis. (**A**) MRCP reveals obliterated peripheral bile ducts, resulting in a "pruned tree" appearance and multiple diverticular outpouchings (*solid arrows*). Moreover, note the stricture of the common hepatic duct (*dotted arrow*). In another case (**B**), MRCP in a 44-year-old patient with primary sclerosing cholangitis displays very irregular bile ducts, multiple strictures combined with diverticular biliary dilatation (*solid arrows*).

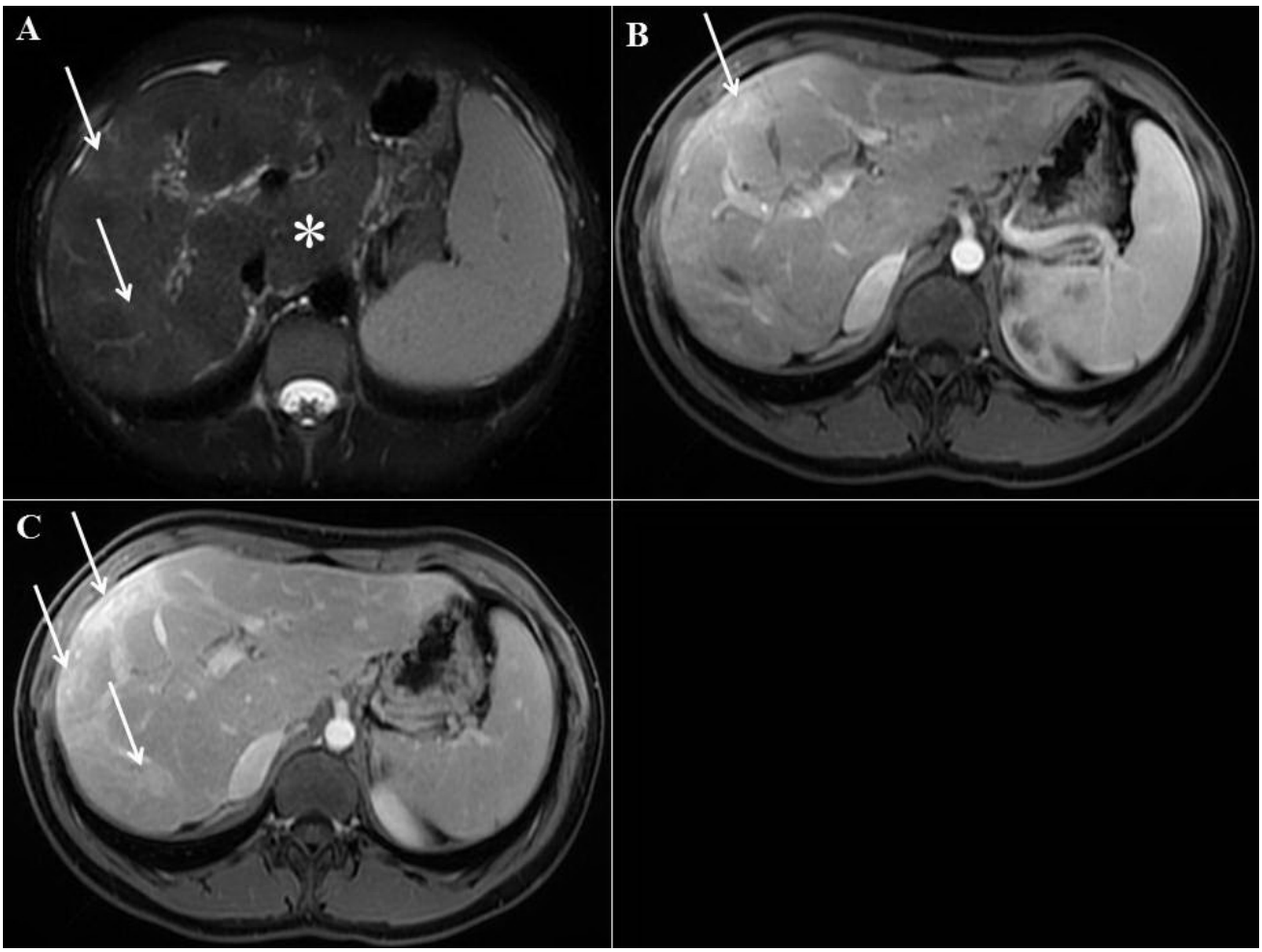


Figure 3. Primary sclerosing cholangitis in 44-year old man. **(A)** An axial T2-weighted image displays hyperintense areas in peripheral, atrophic regions within the right liver lobe, correlating with sites of parenchymal inflammation and increased water content (*solid arrows*). Additionally, observe the enlarged caudate lobe (*asterisks*). Axial T1-weighted image from the same patient, acquired after intravenous administration of gadolinium chelates during arterial **(B)** and portal-venous phase **(C)**, depicts increased enhancement of peripheral liver areas, indicative of intrahepatic perfusion abnormalities (*solid arrows*).

spherical liver shape is a typical finding for PSC and develops as the consequence of caudate lobe hypertrophy and atrophy of left lateral and right posterior segments (Figure 3A) (17). The most frequent type of liver cirrhosis in PSC patients is macronodular cirrhosis, with large regenerative nodules predominantly located in central liver parts (14, 15). This could be explained by the more severe inflammation in subcapsular regions of the liver, while central parts are partly spared and undergo hypertrophy. The severity of liver fibrosis could be assessed non-invasively with MRI using MR elastography (MRE) or diffusion-weighted MRI measurements (18). Among these two methods, liver stiffness measurement using MRE has been validated in many studies showing a significant correlation with other non-invasive markers of disease progression (8). Areas of increased T2-weighted signal intensity, wedge-shaped or reticular, with peripheral distribution are common findings in PSC (14). As this feature is explained by intense inflammation in highly fibrotic areas, increased enhancement on arterial phase contrast-enhanced MR which persists in delayed phases is also seen (Figure 3B) (14, 17). Hilar lymphadenopathy and periportal hyperintensity are also common findings, occurring due to edema and inflammation in the periportal space (14, 18). However, they are not pathognomonic for PSC as they are also seen in other cholestatic liver diseases.

Image-based scoring systems for assessment of PSC severity and prognosis

Based on clinical and laboratory findings, two prognostic risk models have been developed, including the Mayo risk model (MRS) and the Amsterdam-Oxford model (AOM) (19, 20). Both have shown great performance in estimating prognosis and survival in PSC patients. However, the results of large studies investigating the role of these scoring systems in predicting the prognosis of PSC are conflicting (20, 21). Recently, serum markers that reflect fibrosis and inflammation are increasingly being investigated in PSC patients (21). Based on these markers the enhanced liver fibrosis score was developed and evaluated in PSC (21). Additionally, the role of liver stiffness measurement using transient elastography and magnetic resonance elastography has been systematically

evaluated and was found to correlate significantly with the fibrosis stage (22).

Taking into account that PSC is the disease of bile ducts, it would be expected that cholangiographic findings should be used to stage the disease and assess its prognosis. The first scoring system based on imaging findings was developed by Li-Yeng and Goldman in 1984. and was later modified by Majoie et al. and Ponsioen et al. (23-25). This classification scheme was employed by a Dutch gastroenterologist for the development of Amsterdam cholangiographic score. Amsterdam score was developed based on ERCP features found in 174 patients with PSC (25). According to the modified Amsterdam score, all patients could be classified in three stages depending on the abnormalities of intrahepatic bile ducts: I – multiple strictures without biliary dilatation; II – multiple strictures associated with saccular dilatations and decreased arborization; III- pruning of peripheral bile ducts with good visualization of only central ducts with adequate filling pressure (24). Concerning findings in extrahepatic bile ducts patients might be divided into four stages according to modified Amsterdam score: I- slight irregularities of contour without strictures; II- the presence of segmental stricture (the stricture 3-10mm in length); III- stricture of almost whole extrahepatic bile ducts; IV – extreme irregularities of bile duct contours and the presence of diverticulum-like outpouchings (24). Using this classification system and the age of the patient at the time of initial ERCP, a prognostic model was created showing that the sum of intrahepatic and extrahepatic scoring was a significant predictor in determining the prognosis (26). Namely, patients with high overall scores had significantly lower survival rates (26). To overpass the disadvantages of the scoring system developed by Majoie et al. and Ponsioen et al. which was based on qualitative assessment and therefore had very poor interobserver agreement, Craig et al. introduced quantitative measurements of bile duct changes on ERCP performed in patients with PSC (27). The novel classification system included the following measurements: the grade of bile duct narrowing, the length of strictures, the extent of stricturing (localized or diffused), the diameter of the common bile duct, right and left main hepatic duct, and secondary intrahepatic ducts (27). Among all measurements, two variables were found to have prognostic significance. Namely, patients with high-grade intrahepatic duct strictures had a 19% decrease, while patients with diffuse intrahepatic strictures had a 16% decrease in transplant-free survival at three years follow-up (27).

Although ERCP has the potential to accurately depict the state of the biliary tract in PSC patients since biliary ducts are filled with contrast media under pressure, it is an invasive diagnostic modality and carries the risk of many possible post-procedural complications (28). That is the reason why MRCP as a non-invasive and non-ionizing diagnostic modality has largely replaced ERCP for the initial diagnosis and follow-up of patients with PSC (29). After the introduction of MRCP in the evaluation of PSC patients, efforts were made to adapt Amsterdam score to MRCP. Nevertheless, it has been shown that there was a 5% overestimation of intrahepatic biliary changes and a 10% discrepancy in staging extrahepatic disease when Amsterdam score was applied to MRCP findings (30). These discrepancies might be explained by different acquisition protocols. Thus, in ERCP biliary ducts are filled with contrast media under pressure, while in MRCP biliary tree is examined in a resting state without distension (30). Therefore, it is much more difficult to evaluate strictures on MRCP than on ERCP (31).

Considering that MRI provides not only insight into bile duct changes but also delineates morphological changes in the liver parenchyma, Ruiz et. al introduced a new score for staging and prognostic purposes in PSC, termed ANALI score (32). This classification system is based on quantitative measurements of MRCP, including the garde of intrahepatic biliary duct dilatation, length of strictures in main intrahepatic ducts and peripheral ducts, and morphological changes of liver parenchyma such as parenchymal enhancement heterogeneity, portal hypertension, and liver dysmporphy (32). Liver dysmorphy was defined as severe right or left lobe atrophy occurring as a consequence of severe biliary dilatation, or an abnormal caudate to right lobe volume ratio (32). Concerning parenchymal enhancement heterogeneity, peribiliar enhancement was assessed implying the severity of peribiliary parenchymal inflammation (32). Intrahepatic biliary duct dilatation was scored as 0 (less than 4mm), 1 (4mm), or 2 (more than 4mm), where other variables were either present or absent. Using the abovementioned variables, the following two scores were developed:

Score (MRI without gadolinium): 1 x intrahepatic bile duct dilatation + 2 x dysmorphy + 1 x portal hypertension

Score (MRI with gadolinium) = 1 x dysmorphy + 1 x parenchymal enhancement heterogeneity

Subsequent studies have shown that the ANALI score has good prognostic value in patients with PSC and that it is significantly correlated with existing biochemical and clinical scores like MRS and PRESTO (33, 34). According to a large retrospective multicenter study on 238 PSC patients, the predictive accuracy of ANALI score without and with gadolinium was 0.89% IC 95%, and 0.75% IC 95% (34). In another large study evaluating the value of MR and MRCP in the prediction of PSC progression, multivariate logistic regression analysis showed that liver dysmorphy, signs of portal hypertension, and perihepatic lymph nodes were significantly associated with transplant-free survival and adverse clinical outcomes in long-standing PSC (35). Accordingly, a modified MRCP-risk score was developed as an upgrade of the ANALI

score. Nevertheless, the evaluation of PSC changes remains a challenge for radiologists since subtle irregularities are hardly detectable and the evaluation is quite subjective (36). Taking into account the importance of detecting PSC early and close monitoring of disease progression, a more objective approach to disease staging with limited variability in reporting is necessary (37).

In the era of artificial intelligence, several deep learning models have been developed for the automatic detection of PSC-compatible cholangiography alterations (38). Ringe et al. reported a great diagnostic accuracy of these models with sensitivity, specificity, positive and negative predictive values higher than 90% (38). Recently, a quantitative biliary tree analysis software (MRCP+, Perspectum Diagnostics Limited) was developed allowing semi-automatic quantification of bile duct changes in PSC (39, 40). After uploading the 3D MRCP examination, the MRCP+ post-processing tool provides multiple measurements of biliary ducts. Among many quantitative variables that can be generated, the following metrics have been highlighted as the most important: sum of relative severity of dilatations; proportion of dilatated biliary tree; biliary tree dilatation score; and total stricture severity score (33, 34). Bile duct dilatation was considered to be present if the lumen of the bile duct is at least 1mm increased in comparison to the diameter of the closest duct (41). The dominant stricture was defined as the diameter of the common bile duct less than 1.5mm, and less than 1mm for the left and right hepatic duct (41). Currently, there are a few published studies implying the value of the new computer-based system suggesting its high diagnostic accuracy in the diagnosis of PSC (41-44). Among different variables obtained by the 3D-biliary analysis tool in PSC patients, increased gallbladder volume and higher dilatation metrics were found to be significant predictors of survival (43). Distension of the gallbladder in PSC patients has previously been described as a characteristic finding, occurring probably due to lower levels of hydrophobic serum bile acids (45). It has been hypothesized that gallbladder has a protective role in PSC, as PSC patients who had cholecystectomy develop more severe cholangiographic findings (45). Furthermore, Selvaraj et al. have shown that dilatation metrics had a significant correlation with non-invasive biochemical markers of disease severity and were also significantly higher in the high-risk PSC group defined by MRS (42). In contrast to dilatation variables, stricture metrics did not differ significantly among high-risk and low-risk PSC groups. No significant correlation between biliary stricture severity score and advanced stages of liver fibrosis in PSC patients was also found in the study by Song et al. (46). Similar results were reported by another group that tested the correlation between intrahepatic stricture severity and disease stage assessed by magnetic resonance elastography (47). In opposite, Ismail et al. reported that the stricture severity index was significantly correlated with biochemical prognostic scores indicating that also stricture metrics derived from MRCP+ may have a prognostic purpose (44). The generally low sensitivity of stricture metrics might be explained by technical limitations of MRCP which is unable to assess the severity of strictures due to examining non-distended biliary ducts. Furthermore, MRCP images often have artifacts disabling adequate measurement of the bile duct lumen (9). It should be pointed out that even with ERCP, the prognostic significance of stricture severity and dominant stricture is questionable as no correlation with biochemical markers of cholestasis was demonstrated (48). On the other hand, the importance of biliary dilatation was also stressed by Ruiz et al. who incorporated this parameter in the ANALI score (32). Opposite to the index of biliary dilation severity, biliary tree volume obtained from MRCP+ analysis did not show an association with bad outcomes in PSC patients (33, 34). It could be explained by the fact that biliary tree volume is increased in an intermediate stage of disease but it often decreases in advanced disease due to reduction of peripheral ducts and atrophy of liver segments with severe dilatation (49). Nevertheless, although the most important cholangiographic finding obtained by the computer-based 3-dimensional model of the biliary tree is the severity of biliary dilatation, other metrics might also have a role in the evaluation of disease severity (42).

DIFFERENTIAL DIAGNOSIS OF PSC

Differential diagnosis of primary sclerosing cholangitis includes IgG4-sclerosing cholangitis (IgG4-SC), primary biliary cirrhosis, ischemic cholangitis, and AIDS cholangitis (11, 15). Characteristic cholangiographic findings, young age, male patients, and association with IBD favor the diagnosis of PSC (11, 14). IgG4-SC, which is more commonly seen in elderly men, is characterized by long segmental strictures with prestenotic dilation (50). Moreover, it is frequently associated with autoimmune pancreatitis and other disorders from the IgG4 disease spectrum (50). If classical cholangiographic findings are absent and papillary stenosis is the predominant imaging feature in cholangiography, AIDS cholangitis should be considered (51). Further correlation with clinical and laboratory findings allows the correct differential diagnosis among these two types of cholangitis. Ischemic cholangitis is typically seen in posttransplant patients due to ischemic injury and is characterized by strictures of the middle third of the common bile duct and the hilar part of the biliary tree (52). Together with PSC, primary biliary cirrhosis comprises a spectrum of primary cholestatic liver diseases (53). In contrast to PSC, PBS is typically seen in middle-aged women without characteristic cholangiographic findings. Furthermore, laboratory data adds additional information necessary for distinguishing among these two entities (53).

Complications of PSC

Cholangiocellular carcinoma (CCC) represents the most severe complication arising from long-standing PSC, and affecting approximately 10-14% of patients (54). The exact pathogenesis of cholangiocarcinoma in PSC patients is still not elucidated, but chronic inflammation probably plays the most important role. The detection of cholangiocarcinoma in the early stage is a challenge for radiologists as there are no pathognomonic cholangiographic features (55). The majority of cholangiocarcinomas originate in the perihilar area, but some develop in intrahepatic bile ducts. The tumor is usually seen as an indistinct hypovascular mass with progressive delayed enhancement in imaging studies (Figure 4) (56, 57). However, findings may be more discrete, and a stricture accompanied by prominent wall thickening, alongside significant proximal biliary dilatation, might be the only indicator of CCC (58). To facilitate early CCC detection, regular screening of PSC patients is advocated, involving CA 19-9 measurements every six months and MRCP annually. Elevated CA 19-9 levels have high diagnostic accuracy, with a sensitivity of 75% and specificity of 80% for a cutoff value of 100 U/ml (59). From a clinical standpoint of view sudden worsening of symptoms in PSC patients, characterized by cholestasis and weight loss, should raise the suspicion of CCC. Unfortunately, CCC development in the context of PSC carries a poor prognosis, even after resection or liver transplantation, with 3-year survival rates ranging from 0% to 42% (58, 60). The radiologist should bear in mind that the progression of the dominant stricture with subsequent biliary dilatation does not necessarily mean the development of CCC, but it can also occur due to the worsening of inflammation (15). In doubtful cases further check-out with cholangioscopy if available is recommended.

Another complication in long-standing PSC is acute ascending cholangitis with superimposed biliary sepsis (54). It usually occurs in patients with severe strictures of the common bile duct, left or right hepatic duct. In such cases, after medicamentous treatment of acute infection, therapeutic endoscopic dilatation should be performed.

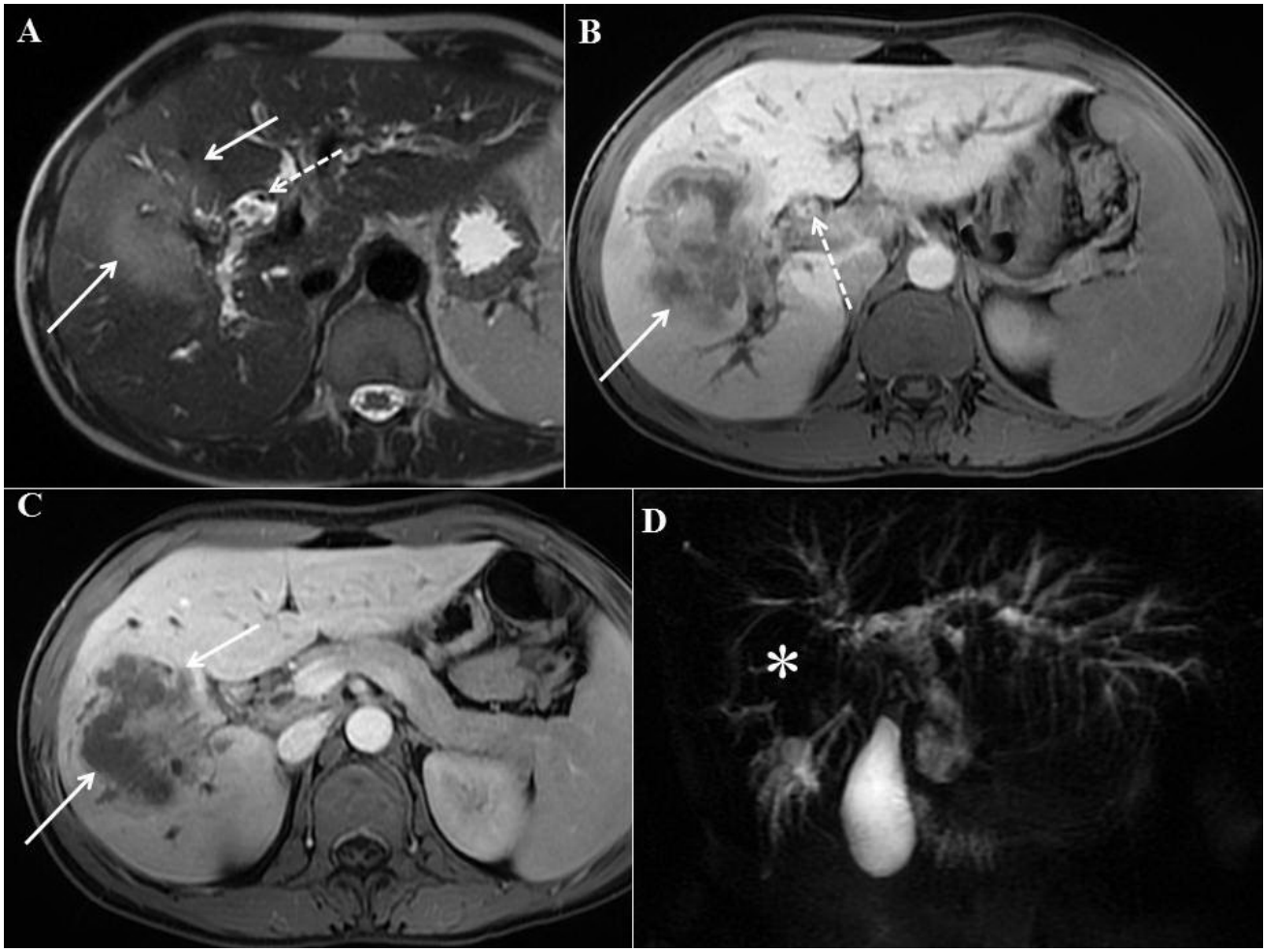


Figure 4. Cholangiocellular carcinoma complicating long-standing primary sclerosing cholangitis in a 52-year-old male patient. **(A)** An axial T2-weighted image displays a large irregular, moderately hyperintense mass in the right liver (*solid arrows*). Bile ducts are irregularly dilated in both lobes due to primary disease. Multiple intrahepatic calculi are also present (*dotted arrows*). **(B)** An axial T1-weighted fat-saturated image reveals a hypointense mass with a central necrotic part (*solid arrow*). Intrahepatic calculi are also visible as hyperintense lesions within dilated bile ducts (*dotted arrow*). **(C)** On the portal-venous phase T1-weighted fat-saturated image, the tumor appears as a hypovascular lesion (*solid arrows*). **(D)** A thick slab MRCP demonstrates multifocal alternating strictures of intrahepatic bile ducts with loss of bile duct visualization in segment V corresponding to tumor infiltration (*asterisks*).

Medical therapy for PSC has limited value, and liver transplantation remains the only life-expanding treatment, offering a 75-85% five-year post-transplant survival (61). However, recurrence of PSC after transplantation is observed in up to 25% of cases, necessitating careful differential diagnosis due to various potential causes of post-transplant biliary strictures (61). Diagnosing recurrent PSC after liver transplantation is quite difficult due to the various causes responsible for post-transplant biliary strictures, such as ischemia, rejection, allograft reperfusion injury, recurrent biliary sepsis, ABO incompatibility, or technical issues with biliary reconstruction (61, 62). To make a diagnosis of recurrent PSC the non-anastomotic strictures occurring three months post-transplantation must be detected (62). However, distinguishing between chronic rejection and recurrence remains challenging. Certain imaging features have been previously described to aid in this differentiation (62). An additional feature that favors the diagnosis of recurrent PSC instead of chronic rejection is an enlarged liver with a slightly nodular contour, whereas, in chronic rejection, it typically maintains a normal size (62). Furthermore, MRCP findings in recurrent disease often reveal multiple non-anastomotic strictures with mildly dilated bile ducts, while cholangiograms in chronic rejection patients show peripheral arterial insufficiency-induced reduction of the peripheral biliary tree (61, 62). A liver biopsy is recommended when a non-invasive differential diagnosis cannot be established (62).

CONCLUSION

In conclusion, the role of MR and MRCP in evaluating patients with PSC is evolving. Although there are a few disadvantages of MRCP in comparison to ERCP, its non-invasive nature, and the possibility of repeating examinations without adverse effects make it the gold standard for the diagnosis and follow-up in PSC patients. In addition to cholangiography findings, MRCP allows the assessment of liver parenchymal changes which might indicate the severity of the disease. The scoring systems based on MR findings, such as the ANALI score, and new computer-based software analysis termed MRCP+, provide a prediction of the course of disease and identify high-risk patients. Thus, MR with MRCP is a promising diagnostic tool for the integrative evaluation of PSC patients allowing not only initial diagnosis and detection of complications but also has prognostic significance.

REFERENCES

1. Lewin M, Vilgrain V, Ozenne V, Lemoine M, Wendum D, Paradis V, et al. Prevalence of sclerosing cholangitis in adults with autoimmune hepatitis: a prospective magnetic resonance imaging and histological study. Hepatology 2009; 50(2): 528-537. doi: 10.1002/hep.23024.
2. Sohal A, Kayani S, Kowdley KV. Primary Sclerosing Cholangitis: Epidemiology, Diagnosis, and Presentation. Clin Liver Dis 2024; 28(1): 129-141. doi: 10.1016/j.cld.2023.07.005.
3. Eksteen B. The Gut-Liver Axis in Primary Sclerosing Cholangitis. Clin Liver Dis 2016; 20(1): 1-14. doi: 10.1016/j.cld.2015.08.012.
4. Cullen S, Chapman R. Aetiopathogenesis of primary sclerosing cholangitis. Best Pract Res Clin Gastroenterol 2001; 15: 577-589. doi: 10.1053/bega.2001.0206.
5. Silveira MG, Lindor KD. Primary sclerosing cholangitis. Can J Gastroenterol 2008; 22(8): 689-98. doi: 10.1155/2008/824168.
6. Lazaridis KN, LaRusso NF. Primary Sclerosing Cholangitis. N Engl J Med 2016; 375(25): 2501-2502. doi: 10.1056/NEJMc1613273.
7. Yu S, Vidal B, Peric M, Rosenbaum MW, Cates JMM, Gonzalez RS. Comparative histologic features among liver biopsies with biliary-pattern injury and confirmed clinical diagnoses. Hum Pathol 2024: 146: 8-14. doi: 10.1016/j.humpath.2024.03.003.
8. Tow CY, Chung E, Kaul B, Bhalla A, Fortune BE. Diagnostic Tests in Primary Sclerosing Cholangitis: Serology, Elastography, Imaging, and Histology. Clin Liver Dis 2024; 28(1): 157-169. doi: 10.1016/j.cld.2023.07.007.
9. Dave M, Elmunzer BJ, Dwamena BA, Higgins PD. Primary sclerosing cholangitis: meta-analysis of diagnostic performance of MR cholangiopancreatography. Radiology 2010; 256: 387-396. doi: 10.1148/radiol.10091953.
10. Aabakken L, Karlsen TH, Albert J, Arvanitakis M, Chazouilleres O, Dumonceau J-M, et al. Role of endoscopy in primary sclerosing cholangitis: European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) Clinical Guideline. Endoscopy 2017; 49(6): 588-608. doi: 10.1055/s-0043-107029.
11. Pria HD, Torres US, Faria SC, Velloni FG, Caiado AHM, Tiferes DA, et al. Practical Guide for Radiological Diagnosis of Primary and Secondary Sclerosing Cholangitis. Semin Ultrasound CT MR 2022; 43(6): 490-509. doi: 10.1053/j.sult.2022.06.007.
12. Sarcognato S, Sacchi D, Grillo F, Cazzagon N, Fabris L, Cadamuro M, et al. Autoimmune biliary diseases: Primary biliary cholangitis and primary sclerosing cholangitis. Pathol J Ital Soc Anat Pathol Diagn Cytopathol 2021; 113: 170–184. doi: 10.32074/1591-951X-245.
13. Björnsson E, Olsson R, Bergquist A, Lindgren S, Braden B, Chapman RW, et al. The natural history of small-duct primary sclerosing cholangitis. Gastroenterology 2008; 134: 975-980. doi: 10.1053/j.gastro.2008.01.042.
14. Kovač JD, Ješić R, Stanisavljević D, Kovač B, Maksimovic R. MR imaging of primary sclerosing cholangitis: additional value of diffusion-weighted imaging and ADC measurement. Acta Radiol 2013; 54(3): 242-248. doi: 10.1177/0284185112471792.
15. O'Brien C, Malik M, Jhaveri K. MR Imaging in Primary Sclerosing Cholangitis and Other Cholangitis. Radiol Clin North Am 2022; 60(5): 843-856. doi: 10.1016/j.rcl.2022.05.007.
16. Kovač JD, Janković A, Đikić-Rom A, Grubor N, Antić A, Dugalić V. Imaging Spectrum of Intrahepatic Mass-Forming Cholangiocarcinoma and Its Mimickers: How to Differentiate Them Using MRI. Curr Oncol 2022; 29(2): 698-723. doi: 10.3390/curroncol29020061.
17. Düşünceli E, Erden A, Erden I, Karayalçin S. Primary sclerosing cholangitis: MR cholangiopancreatography and T2-weighted MR imaging findings. Diagn Interv Radiol 2005; 11: 213-218. PMID: 16320228.
18. Kovač JD, Daković M, Stanisavljević D, Alempijević T, Ješić R, Seferović P, et al. Diffusion-weighted MRI versus transient elastography in quantification of liver fibrosis in patients with chronic cholestatic liver diseases. Eur J Radiol 2012; 81(10): 2500-2506. doi: 10.1016/j.ejrad.2011.10.024.
19. Kim WR, Therneau TM, Wiesner RH, Poterucha JJ, Benson JT, Malinchoc M, et al. A revised natural history model for primary sclerosing cholangitis. Mayo Clin Proc 2000; 75: 688-94. doi: 10.4065/75.7.688.

20. de Vries EM, Wang J, Williamson KD, Leeflang MM, Boonstra K, Weersma RK, *et al*. A novel prognostic model for transplant-free survival in primary sclerosing cholangitis. Gut 2018; 67: 1864-1869. doi: 10.1136/gutjnl-2016-313681.
21. Vesterhus M, Hov JR, Holm A, Schrumpf E, Nygard S, Godang K, et al. Enhanced liver fibrosis score predicts transplant-free survival in primary sclerosing cholangitis. Hepatology 2015; 62: 188-197. doi: 10.1002/hep.27825.
22. Corpechot C, Gaouar F, El Naggar A, Kemgang A, Wendum D, Poupon R, et al. Baseline values and changes in liver stiffness measured by transient elastography are associated with severity of fibrosis and outcomes of patients with primary sclerosing cholangitis. Gastroenterology 2014; 146: 970-979. doi: 10.1053/j.gastro.2013.12.030.
23. Chen LY, Goldberg HI. Sclerosing cholangitis: Broad spectrum of radiographic features. Gastrointest Radiol 1984; 9: 39-47. doi: 10.1007/BF01887799.
24. Majoie CB, Reeders JW, Sanders JB, Huibregtse K, Jansen PL. Primary sclerosing cholangitis: A modified classification of cholangiographic findings. AJR Am J Roentgenol 1991; 157: 495-497. doi: 10.2214/ajr.157.3.1651643.
25. Ponsioen CY, Vrouenraets SM, Prawirodirdjo W, Rajaram R, RauwsEA, Mulder CJ, et al. Natural history of primary sclerosing cholangitis and prognostic value of cholangiography in a Dutch population. Gut 2002; 51: 562-566. doi: 10.1136/gut.51.4.562.
26. Ponsioen CY, Reitsma JB, Boberg KM, Aabakken L, Rauws EA, Schrumpf E. Validation of a cholangiographic prognostic model in primary sclerosing cholangitis. Endoscopy 2010; 42: 742-747. doi: 10.1055/s-0030-1255527.
27. Craig DA, MacCarty RL, Wiesner RH, Grambsch PM, LaRusso NF. Primary sclerosing cholangitis: Value of cholangiography in determining the prognosis. AJR Am J Roentgenol 1991; 157: 959-964. doi: 10.2214/ajr.157.5.1927817.
28. Aabakken L, Karlsen TH, Albert J, Arvanitakis M, Chazouilleres O, Dumonceau J-M, et al. Role of endoscopy in primary sclerosing cholangitis: European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) Clinical Guideline. Endoscopy 2017; 49: 588–608. doi: 10.1055/s-0043-107029.
29. Chazouilleres O, Beuers U, Bergquist A, Karlsen TH, Levy C, Samyn M, et al. EASL Clinical Practice Guidelines on sclerosing cholangitis. J Hepatol 2022; 77: 761–806. doi: 10.1016/j.jhep.2022.05.011.
30. Tenca A, Mustonen H, Lind K, Lantto E, Kolho KL, Boyd S, et al. The role of magnetic resonance imaging and endoscopic retrograde cholangiography in the evaluation of disease activity and severity in primary sclerosing cholangitis. Liver Int 2018; 38(12): 2329-2339. doi: 10.1111/liv.13899.
31. Moff SL, Kamel IR, Eustace J, Lawler LP, Kantsevoy S, Kalloo AN, et al. Diagnosis of primary sclerosing cholangitis: a blinded comparative study using magnetic resonance cholangiography and endoscopic retrograde cholangiography. Gastrointest Endosc 2006; 64(2): 219-223. doi: 10.1016/j.gie.2005.12.034.
32. Ruiz A, Lemoinne S, Carrat F, Corpechot C, Chazouilleres O, Arrive L. Radiologic course of primary sclerosing cholangitis: Assessment by three-dimensional magnetic resonance cholangiography and predictive features of progression. Hepatology 2014; 59: 242-250. doi: 10.1002/hep.26620.
33. Trivedi PJ, Arndtz K, Abbas N, Telford A, Young L, Banerjee R, et al. Quantitative MRCP and metrics of bile duct disease over time in patients with primary sclerosing cholangitis: A prospective study. Aliment Pharmacol Ther 2024. doi: 10.1111/apt.17944.
34. Cazzagon N, Lemoinne S, El Mouhadi S, Trivedi P, Dohan A, Fankem AK, et al. Two simple magnetic resonance scores are able to predict survival in patients with primary sclerosing cholangitis. Hepatology 2018; 68(Suppl 1): 29A-30A. doi: 10.1007/s00261-023-04051-4.
35. Muir AJ. Is Magnetic Resonance Cholangiopancreatography Worth a Thousand Words in Determining Prognoses of Patients With Primary Sclerosing Cholangitis? Clin Gastroenterol Hepatol 2019; 17(13): 2654-2656. doi: 10.1016/j.cgh.2019.04.067.
36. Zenouzi R, Liwinski T, Yamamura J, Weiler-Normann C, Sebode M, Keller S, et al. Follow-up magnetic resonance imaging/3D-magnetic resonance cholangiopancreatography in patients with primary sclerosing cholangitis: challenging for experts to interpret. Alimentary pharmacology & therapeutics 2018; 48(2): 169-178. doi: 10.1111/apt.14797.
37. Schramm C, Eaton J, Ringe KI, Venkatesh S, Yamamura J; MRI working group of the IPSCSG. Recommendations on the use of magnetic resonance imaging in PSC-A position statement from the International PSC Study Group. Hepatology 2017; 66(5): 1675-1688. doi: 10.1002/hep.29293.
38. Ringe KI, Vo Chieu VD, Wacker F, Lenzen H, Manns MP, Hundt C, et al. Fully automated detection of primary sclerosing cholangitis (PSC)-compatible bile duct changes based on 3D magnetic resonance cholangiopancreatography using machine learning. Eur Radiol 2021; 31(4): 2482-2489. doi: 10.1007/s00330-020-07323-5.
39. Cazzagon N, El Mouhadi S, Vanderbecq Q, Ferreira C, Finnegan S, Lemoinne S, et al. Quantitative magnetic resonance cholangiopancreatography metrics are associated with disease severity and outcomes in people with primary sclerosing cholangitis. JHEP Reports 2022; 4(11):100577. doi: 10.1016/j.jhepr.2022.100577.
40. Cristoferi L, Porta M, Bernasconi DP, Leonardi F, Gerussi A, Mulinacci G, et al. A quantitative MRCP-derived score for medium-term outcome prediction in primary sclerosing cholangitis. Digestive and Liver Disease. 2023; 55(3): 373-380. doi: 10.1016/j.dld.2022.10.015.
41. McCrary J, Trout AT, Mahalingam N, Singh R, Rojas CC, Miethke AG, et al. Associations Between Quantitative MRI Metrics and Clinical Risk Scores in Children and Young Adults With Autoimmune Liver Disease. AJR Am J Roentgenol 2022; 219(1): 142-150. doi: 10.2214/AJR.21.27204.
42. Selvaraj EA, Culver EL, Bungay H, Bailey A, Chapman RW, Pavlides M. Evolving role of magnetic resonance techniques in primary sclerosing cholangitis. World J Gastroenterol. 2019; 25(6): 644-658. doi: 10.3748/wjg.v25.i6.644.
43. Goldfinger MH, Ridgway GR, Ferreira C, Langford CR, Cheng L, Kazimianec A, et al. Quantitative MRCP Imaging: Accuracy, Repeatability, Reproducibility, and Cohort-Derived Normative Ranges. J Magn Reson Imaging 2020; 52(3): 807-820. doi: 10.1002/jmri.27113.
44. Ismail MF, Hirschfield GM, Hansen B, Tafur M, Elbanna KY, Goldfinger MH, et al. Evaluation of quantitative MRCP (MRCP+) for risk stratification of primary sclerosing cholangitis: comparison with morphological MRCP, MR elastography, and biochemical risk scores. Eur Radiol 2022; 32(1): 67-77. doi: 10.1007/s00330-021-08142-y.
45. Cazzagon N, Gonzalez-Sanchez E, El-Mourabit H, Wendum D, Rainteau D, Humbert L, et al. Protective potential of the gallbladder in primary sclerosing cholangitis. JHEP Reports 2023; 5(4): 100649. doi: 10.1016/j.jhepr.2022.100649.
46. Song C, Lewis S, Kamath A, Hectors S, Putra J, Kihira S, et al. Primary sclerosing cholangitis: diagnostic performance of MRI compared to blood tests and clinical scoring systems for the evaluation of histopathological severity of disease. Abdom Radiol (NY) 2020; 45: 354-364. doi: 10.1007/s00261-019-02366-9.
47. Grigoriadis A, Morsbach F, Voulgarakis N, Said K, Bergquist A, Kartalis N. Inter-reader agreement of interpretation of radiological course of bile duct changes between serial follow-up magnetic resonance imaging/3D magnetic resonance cholangiopancreatography of patients with primary sclerosing cholangitis. Scand J Gastroenterol 2020; 55(2): 228-235. doi: 10.1080/00365521.2020.1720281.
48. Björnsson E, Lindqvist-Ottosson J, Asztely M, Olsson R. Dominant strictures in patients with primary sclerosing cholangitis. Am J Gastroenterol 2004; 99(3): 502-508. doi: 10.1111/j.1572-0241.2004.04106.x.
49. Schulze J, Lenzen H, Hinrichs JB, Ringe B, Manns MP, Wacker F, et al. An imaging biomarker for assessing hepatic function in patients with primary sclerosing cholangitis. Clin Gastroenterol Hepatol 2019; 17: 192–199. doi: 10.1016/j.cgh.2018.05.011.
50. Tokala A, Khalili K, Menezes R, Hirschfield G, Jhaveri KS. Comparative MRI analysis of morphologic patterns of bile duct disease in IgG4-related systemic disease versus primary sclerosing cholangitis. Am J Roentgenol 2014; 202(3): 536–543. doi: 10.2214/AJR.12.10360.

51. Tonolini M, Bianco R. HIV-related/AIDS cholangiopathy: pictorial review with emphasis on MRCP findings and differential diagnosis. Clin Imaging 2013; 37(2): 219-226. doi: 10.1016/j.clinimag.2012.03.008.
52. Katabathina VS, Dasyam AK, Dasyam N, Hosseinzadeh K. Adult bile duct strictures: role of MR imaging and MR cholangiopancreatography in characterization. Radiographics 2014; 34(3): 565-586. doi: 10.1148/rg.343125211.
53. Kovač JD, Ješić R, Stanisavljević D, Kovač B, Banko B, Seferović P, et al. Integrative role of MRI in the evaluation of primary biliary cirrhosis. Eur Radiol 2012; 22(3): 688-694. doi: 10.1007/s00330-011-2296-y.
54. Azizi L, Raynal M, Cazejust J, Ruiz A, Menu Y, Arrivé L. MR Imaging of sclerosing cholangitis. Clin Res Hepatol Gastroenterol 2012; 36(2): 130-138. doi: 10.1016/j.clinre.2011.11.011.
55. Bowlus CL, Arrivé L, Bergquist A, Deneau M, Forman L, Ilyas SI, et al. AASLD practice guidance on primary sclerosing cholangitis and cholangiocarcinoma. Hepatology 2023; 77(2): 659-702. doi: 10.1002/hep.32771.
56. Kovač JD, Galun D, Đurić-Stefanović A, Lilić G, Vasin D, Lazić L, et al. Intrahepatic mass-forming cholangiocarcinoma and solitary hypovascular liver metastases: is the differential diagnosis using diffusion-weighted MRI possible? Acta Radiol 2017; 58(12): 1417-1426. doi: 10.1177/0284185117695666.
57. Kovač JD, Daković M, Janković A, Mitrović M, Dugalić V, Galun D, et al. The role of quantitative diffusion-weighted imaging in characterization of hypovascular liver lesions: A prospective comparison of intravoxel incoherent motion derived parameters and apparent diffusion coefficient. PLoS One 2021; 16(2): e0247301. doi: 10.1371/journal.pone.0247301.
58. Saca D, Flamm SL. Cholangiocarcinoma Surveillance Recommendations in Patients with Primary Sclerosing Cholangitis. Clin Liver Dis 2024; 28(1): 183-192. doi: 10.1016/j.cld.2023.07.010.
59. Charatcharoenwitthaya P, Enders FB, Halling KC, Lindor KD. Utility of serum tumor markers, imaging, and biliary cytology for detecting cholangiocarcinoma in primary sclerosing cholangitis. Hepatology 2008; 48(4): 1106-1117. doi: 10.1002/hep.22441.
60. Bonato G, Cristoferi L, Strazzabosco M, Fabris L. Malignancies in Primary Sclerosing Cholangitis--A Continuing Threat Dig Dis 2015; 33: 140-148. doi: 10.1159/000440826.
61. Prokopič M, Beuers U. Management of primary sclerosing cholangitis and its complications: an algorithmic approach. Hepatol Int 2021; 15(1): 6-20. doi: 10.1007/s12072-020-10118-x.
62. Henson JB, King LY. Post-Transplant Management and Complications of Autoimmune Hepatitis, Primary Biliary Cholangitis, and Primary Sclerosing Cholangitis including Disease Recurrence. Clin Liver Dis 2024; 28(1): 193-207. doi: 10.1016/j.cld.2023.07.009.

MR KARAKTERISTIKE PRIMARNOG SKLEROZIRAJUĆEG HOLANGITISA: PREGLED SKORING SISTEMA ZASNOVANIH NA IMIDŽING KARAKTERISTIKAMA KOJI SE KORISTE ZA PROCENU TEŽINE I PROGNOZE

Jelena Đokić Kovač[1], Aleksandra Janković[1], Milica Mitrović-Jovanović[1], Aleksandar Bogdanović[2], Vladimir Dugalić[2], Tamara Milovanović[3]

Sažetak

Primarni sklerozirajući holangitis (PSC) je hronično, holestatsko oboljenje jetre koje se odlikuje hroničnom inflamacijom, fibrozom i stenozom žučnih puteva. Magnetno rezonantna holangiopankreatiografija (MRCP) predstavlja zlatni standard za dijagnozu PSC-a i omogućava uvid u promene na bilijarnom stablu. Tipična radiološka slika PSC-a obuhvata multifokalne anularne i kratke-segmente strikture u kombinaciji sa normalnim ili lako dilatiranim žučnim vodovima. Pored holangiografskog prikaza, magnetna rezonanca (MR) pruža uvid i u morfološke promene parenhima jetre koje mogu ukazivati na težinu bolesti. Savremeni skoring sistemi zasnovani na MR nalazima, uključuju ANALI skor i novu softversku analizu zvanu MRCP+, omogućavaju procenu težine bolesti i identifikovanje visoko-rizičnih pacijenata. Stoga, MR sa MRCP-om predstavlja dijagnostičku metodu koja pruža sveobuhvatnu evaluaciju pacijenata sa PSC-om, uključujući inicijalnu dijagnozu, praćenje pacijenata radi rane detekcije komplikacija uz prognostički značaj.

Ključne reči: jetra, primarni sklerozirajući holangitis, magnetno rezonantna holangiopankreatografija

Primljen: 16.04.2024. I **Revizija:** 14.06.2024. I **Prihvaćen:** 14.06.2024.

Medicinska istaživanja 2024; 57(3):137-146

Medicinska istaživanja 2024; 57(3):147-150 | DOI: 10.5937/medi57-50916

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REVIEW ARTICLE

Anesthesia for awake craniotomy – how do we perform it?

Tijana Nastasović[1,2], Nevena Savić[2], Igor Lazić[1,2], Tjaša Ivošević[2,3], Ivan Bogdanović[2,4], Rosanda Ilić[2,4], Filip Milisavljević[4]

[1] University Clinical Center of Serbia, Department of Anesthesiology and Resuscitation, Neurosurgery Clinic, Belgrade, Serbia

[2] University of Belgrade, Faculty of Medicine, Belgrade, Serbia

[3] University Clinical Center of Serbia, Department of Anesthesiology and Resuscitation, ENT and Maxillofacial Clinic, Belgrade, Serbia

[4] University Clinical Center of Serbia, Neurosurgery Clinic, Belgrade, Serbia

Recived: 11 May 2024

Revised: 16 July 2024

Accepted: 25 July 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Tijana Nastasović

University Clinical Center of Serbia, Department of Anesthesiology and Resuscitation, Neurosurgery Clinic, 11000 Belgrade, Serbia

E-mail: tijananastasovic@yahoo.com

Summary

Awake craniotomy is a neurosurgical procedure that is used for the safe removal of intracranial lesions near Broca's and Wernicke's speech areas. During this procedure, speech mapping is done. Its use demands anesthesia for awake craniotomy, which includes different possibilities for the management of anesthesia with awake patients at the moment of tumor removal. There are two widely accepted concepts: "awake-awake-awake", when the patient is consciously sedated ("awake") throughout surgery, with lighter or deeper sedation (monitored anesthesia care), or "asleep-awake-asleep", when the patient is introduced into general endotracheal anesthesia while opening the skull, but is awake during lesion removal and asleep during closure. Our protocol is a form of "asleep-awake" concept, with general endotracheal anesthesia for craniotomy and awake patients during and after lesion removal (including skull closure).

Keywords: awake craniotomy, anesthesia, speech mapping, "awake-awake-awake", "asleep-awake-asleep"

Cite this article as: Nastasović T, Savić N, Lazić I, Ivošević T, Bogdanović I, Ilić R, Milisavljević F. Anesthesia for awake craniotomy – how do we perform it? Medicinska istaživanja 2024; 57(3):147-150 DOI: 10.5937/medi57-50916

INTRODUCTION

Awake craniotomy is a neurosurgical procedure that is used for the removal of intracranial lesions (tumors, arterio-venous malformations, epileptogenic foci) near speech areas (1). During removal, speech mapping is performed to preserve these areas (Broca's and Wernicke's areas) (2). For these procedures, it is necessary to perform anesthesia for awake craniotomy (3,4).

Anesthesiologists have several options to perform this procedure. Monitored anesthesia care ("awake-awake-awake") is an anesthetic approach in which the patient experiences varying levels of anesthesia depth during a neurosurgical procedure. During speech mapping, anesthesia must be lightened or paused to allow the patient to fully awaken (5). "Asleep-awake-asleep" is an anesthetic protocol in which the patient is introduced into general endotracheal anesthesia as usual (6,7). After craniotomy and dura mater incision, anesthesia is lightened, and the patient wakes up. The patient is fully awake during the speech mapping procedure. After tumor removal, the patient remains awake or lightly sedated ("asleep-awake") (1) or can be reintroduced to general anesthesia (6,7).

Benefits of awake craniotomy

Compared to neurosurgical interventions in general endotracheal anesthesia, awake craniotomy provides complete tumor removal with preserved neurological functions (3,8). Additionally, these patients have fewer ICU days, shorter intrahospital stays, and better survival (1-3).

Contraindications to awake craniotomy

Contraindications to awake craniotomy are different. The main contraindication is an uncooperative patient and the patient's rejection of the procedure (1,3). Relative contraindications include the presence of comorbidities such as uncontrolled hypertension, comorbidities related to safe airways (chronic obstructive lung disease, sleep apnea, morbid obesity), and tumor characteristics (large and highly vascular tumors) (9,10).

AWAKE CRANIOTOMY PROTOCOL

At Neurosurgery Clinic, University Clinical Center of Serbia, Belgrade, awake craniotomy intervention was started in 2017. We developed our protocol for anesthesia in these patients. This protocol can be classified as "asleep-awake" with induction into general endotracheal anesthesia, awakening before tumor removal, and awakening during tumor removal and skull closure or Dexmedotimidine use for agitated and restless patients (7,9).

After a neurologist and neuropsychologist prepare the patient for intraoperative speech testing (speech mapping) (10,11), he/she is admitted to our clinic.

Premedication

On the day of the neurosurgical intervention, the patient will get premedication before the transfer to the operating theater. It consists of benzodiazepine, Diazepam, in a dose of 10mg intramuscularly (IM). Atropine in a dose of 0.5mg IM should be considered because of side effects, but it has antisialogogue which is desirable for awake craniotomy. The antibiotic for antimicrobe prophylaxis which we use is a cephalosporin belonging to the 1st or the 3rd generation (Cefazolin, Ceftriaxone) intravenously (IV). Patients should also take their oral antiepileptics.

After admission to the operating theater, the patient is prepared for induction into general endotracheal anesthesia (GA) (10,12). One intravenous cannula is placed into the arm's peripheral vein. ECG monitoring, non-invasive blood pressure measurement, and pulse oximetry were initiated. Bispectral index (BIS) monitoring for the level of consciousness follow-up is started (1,2,4).

Before induction, Ondansetron 4mg IV (antiemetic action), Metilprednizolone 40-80mg IV (anti-inflammatory action), Pantoprazole 40mg IV (decreased risk of regurgitation), and Lidocaine 40mg IV (blunted response on endotracheal intubation) are administered.

Induction

After 3-minute preoxygenation with a mask, induction into GA is initiated with the following doses of anesthetics and relaxants: Midazolam 2.5-5mg IV, Fentanyl 50-100μg IV, Propofol 1.5-2mg/kg IV, Rocuronium 50-70mg IV. When conditions for endotracheal intubation are met, the patient is intubated with an armored tube of the appropriate size. Controlled mechanical ventilation is initiated with capnography. After the fixation of the tube with sticky tapes, the throat pack is performed for additional securing of the tube position. Before starting the intervention, intraoperative heating of the patient is initiated to prevent hypothermia and intraoperative shivering (13,14).

Maintenance

When the patient is induced into GA, an additional intravenous cannula is placed together with an arterial cannula into the radial artery and a urinary catheter. BIS value is maintained between 40 and 60.

The patient is positioned on the right (in most cases) or the left side, with the head fixed by Mayfield pins. Intubation in the case of failed emergence should be possible in that position (1-3). The eyes are protected with pads. The skin points of pin placement are infiltrated by sub-

cutaneous 2% Lidocaine injections (2ml for every point). A bilateral scalp block with 0.25% Bupivacaine and Epinephrine 1: 200 000 is done (with a total of 30 ml of solution), then a Mayfield frame is placed for head fixation (15). Seven nerves on either side of the scalp are infiltrated with local anesthetic: supraorbital nerve, supratrochlear nerve, zygomaticotemporal nerve, auriculotemporal nerve, lesser occipital nerve, greater occipital nerve, and greater auricular nerve (15). After infiltration, the operative field is cleaned and disinfected, and the head is covered with sterile drapes.

Anesthesia is maintained with a continuous infusion of Propofol at 75-200 μg/kg/min and Remifentanil at 0.2-0.3 μg/kg/min, with a BIS value of 40-60, from the skin incision until the skull is fully opened and the dura mater is incised (16,17). Prior to incision, the dura mater is soaked with 2% Lidocaine solution (1). Once the brain cortex is exposed, the patient is prepared for awakening

Emergence

Emergence (awakening) is the most complex part of anesthesia in these patients. The infusion of Propofol and Remifentanil is discontinued. We wait for an increase in BIS value to 80, then the dose of Flumazenil 0.2 mg IV (reversion of residual Midazolam action) is administered. If the patient exhibits insufficient breathing, Naloxone is administered intravenously at a dose of 0.2 mg. When the patient is awake, obeys commands, and breaths sufficiently, extubating is performed. Following extubation, the patient is oxygenated using a nasal cannula, while capnography is simultaneously monitored with Capnostream (16). Hemodynamic stability is maintained, as well as appropriate oxygenation.

If emergence fails, we should consider intubation of the patient by video laryngoscope. The operation is continued without speech mapping.

After tumor removal and during the closure of the skull, the patient stays awake or can fall into spontaneous sleep untill the end of the intervention. If the patient is agitated or disturbed, continuous infusion of Dexmedetomidine (2,7,9) can be initiated in a dose of 0.2-0.7 μg/kg/h. If the patient is hypertensive, a bolus of 12.5 mg IV Urapidil can be administered followed by continuous infusion of an average dose of 9 mg/h (1-3). If seizures occur after awakening, cold normal saline irrigation of the brain should be done. If cold saline irrigation is ineffective for seizure cessation, boluses of Propofol 10-20 mg IV or Midazolam 1-2 mg IV should be administered (1). Endotracheal intubation should be considered in these cases.

After the speech test is finished and extirpation of the intracranial lesion is completed, the closure of the skull is initiated. Additional dura mater irrigation with 2% Lidocaine can be done. In case of pain, a Remifentanil infusion at doses of 0.01-0.05 μg/kg/min can be initiated, and nonsteroidal anti-inflammatory analgesics or Tramadol 50 mg IV may also be administered. After dura mater closure, antiepileptic Phenobarbitone 220 mg IM is administered. At the end of the operation, the patient is transferred to the intensive care unit for postoperative monitoring.

CONCLUSION

Anesthesia for awake craniotomy can be managed in different ways. The "asleep-awake" protocol provides good conditions for neurosurgical intervention (awake and cooperative patient for speech mapping) and can influence functional outcomes.

Acknowledgments: None

Author contribution: TN and NS made the concept of the work and wrote the manuscript, IL and RI did the acquisition of the data, IB and RI reviewed the manuscript and prepared the draft, and FM interpreted the revised version of the manuscript.

REFERENCES

1. Singh K, Dua A. Anesthesia for Awake Craniotomy. [Updated 2023 Jul 6]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK572053/
2. Prontera A, Baroni S, Marudi A, Valzania F, Feletti A, Benuzzi F, et al. Awake craniotomy anesthetic management using dexmedetomidine, propofol, and remifentanil. Drug Des Dev Ther. 2017; 11:593–598. doi: 10.2147/DDDT.S124736 PMID: 28424537
3. Zhang K, Gelb AW. Awake craniotomy: indications, benefits, and techniques. Colomb J Anesthesiol. 2018;46(Supp.):46–51. doi:10.1097/CJ9.0000000000000045
4. Burnand C, Sebastian J. Anaesthesia for awake craniotomy. Continuing Education in Anaesthesia, Critical Care and Pain 2014;14(1):6–11. https://doi.org/10.1093/bjaceaccp/mkt024
5. Picht T, Kombos T, Gramm HJ, Brock M, Suess O. Multimodal protocol for awake craniotomy in language cortex tumor surgery. Acta Neurochir. (Wien) 2006; 148:127–138. doi: 10.1007/s00701-005-0706-0 PMID: 16374563
6. Piccioni F, Fanzio M. Management of anesthesia in awake craniotomy. Minerva Anestesiol. 2008;74(7–8):393–408. PMID: 18612268
7. Ard Jr. JL, Bekker AY, Doyle WK. Dexmedetomidine in awake craniotomy: a technical note. Surg Neurol. 2005;63(2):114–117. doi: 10.1016/j.surneu.2004.02.029 PMID: 15680647
8. Bulsara KR, Johnson J, Villavicencio AT. Improvements in brain tumor surgery: the modern history of awake craniotomies. Neurosurg Focus 2005;18(4):e5 doi: 10.3171/foc.2005.18.4.6. PMID: 15844868
9. Rozet I. Anesthesia for functional neurosurgery: the role of dexmedetomidine. Curr Opin Anaesthesiol. 2008;21(5):537-43. DOI: 10.1097/ACO.0b013e32830edafd PMID: 18784476
10. Eseonu CI, ReFaey K, Garcia O, John A, Quiñones-Hinojosa A, Tripathi P. Awake craniotomy anesthesia: a comparison of the monitored anesthesia care and asleep-awake-asleep techniques. World Neurosurg. 2017 Aug; 104:679-686. doi: 10.1016/j.wneu.2017.05.053 PMID: 28532922
11. Berkenstadt H, Perel A, Hadani M, Unofrievich I, Ram Z. Monitored anesthesia care using remifentanil and propofol for

awake craniotomy. J Neurosurg Anesthesiol. 2001; 13:246–249. doi:10.1097/00008506-200107000-00013 PMID:11426102

12. Dilmen OK, Akcil EF, Oguz A, Vehid H, Tunali Y. Comparison of conscious sedation and asleep-awake-asleep techniques for awake craniotomy. J Clin Neurosci. 2017; 35:30-34. DOI: 10.1016/j.jocn.2016.10.007 PMID: 27771234
13. Sarang A, Dinsmore J. Anaesthesia for awake craniotomy–the evolution of a technique that facilitates awake neurological testing. Br J Anaesth. 2003;90(2):161–165. doi: 10.1093/bja/aeg037 PMID:12538371
14. Whittle IR, Midgley S, Georges H, Pringle A-M, Taylor R. Patient perceptions of "awake" brain tumor surgery. Acta Neurochir. (Wien) 2005;147(3):275–277. doi: 10.1007/s00701-004-0445-7 PMID: 15627921
15. Huncke K, Van de Wiele B, Fried I, Rubinstein EH. The asleep-awake-asleep anesthetic technique for intraoperative language mapping. Neurosurgery 1998;42(6):1312–1316. doi: 10.1097/00006123-199806000-00069 PMID: 9632190
16. D'Onofrio G, Izzi A, Manuali A, Bisceglia G, Tancredi A, Marchello V, Recchia A, Tonti MP, Icolaro N, Fazzari E, et al. Anesthetic Management for Awake Craniotomy Applied to Neurosurgery. *Brain Sciences*. 2023; 13(7):1031. https://doi.org/10.3390/brainsci13071031
17. Duffau H. The reliability of asleep-awake-asleep protocol for intraoperative functional mapping and cognitive monitoring in glioma surgery. Acta Neurochirur. (Wien) 2013;155(10):1803–1804. doi: 10.1007/s00701-013-1807-9 PMID: 23851600

ANESTEZIJA ZA KRANIOTOMIJU U BUDNOM STANJU- PRINCIPI IZVOĐENJA

Tijana Nastasović[1,2], Nevena Savić[2], Igor Lazić[1,2], Tjaša Ivošević[2,3], Ivan Bogdanović[2,4], Rosanda Ilić[2,4], Filip Milisavljević[4]

Sažetak

Kraniotomija u budnom stanju je intervencija koja se koristi tokom operacije intrakranijalnih tumora blizu Brokine i Vernikeove govorne zone. Tokom ove intervencije vrši se mapiranje govornih zona. Kraniotomija u budnom stanju zahteva anesteziju za kraniotomiju u budnom stanju koja uključuje različite mogućnosti za njeno izvođenje, pri čemu je pacijent budan tokom ekstirpacije tumora. Postoje dva opšte prihvaćena koncepta za izvođenje anestezije: engl. *awake-awake-awake,* pri čemu je pacijent budan ali u plićoj ili dubljoj sedaciji sve veme tokom hirurške intervencije (engl. *monitored anesthesia care*) ili engl. *asleep-awake-asleep*, kada je pacijent uveden u opštu endotrahealnu anesteziju za samu kraniotomiju (otvaranje) ali je budan tokom same ekstirpacije tumora, a u anesteziji je tokom zatvaranja. Naš protokol je oblik engl. *asleep-awake* koncepta, sa opštom anestezijom tokom kraniotomije i budnim pacijentom tokom i posle ekstirpacije tumora i zatvaranja lobanje.

Ključne reči: kranitomija u budnom stanju, anestezija, mapiranje govora, awake-awake-awake, asleep-awake-asleep

Primljen: 11.05.2024. I **Revizija:** 16.07.2024. I **Prihvaćen:** 25.07.2024.

Medicinska istaživanja 2024; 57(3):147-150

Medicinska istaživanja 2024; 57(3):151-160 | DOI: 10.5937/medi57-50990

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REVIEW ARTICLE

Nutrition in critically ill adult patients

Ivan Palibrk[1,2], Marija Đukanović[1,2], Dona Stefanović[2]

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

[2] Center for Anesthesiology and Reanimatology, Department of Anaesthesiology and Intensive Care, Hospital for Digestive Surgery, University Clinical Centre of Serbia, Belgrade, Serbia

Recived: 12 May 2024

Revised: 16 July 2024

Accepted: 25 July 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Ivan Palibrk

Center for Anesthesiology and Reanimatology, Department of Anaesthesiology and Intensive Care, Hospital for Digestive Surgery, University Clinical Centre of Serbia

6 Dr Koste Todorovića Street, 11000 Belgrade, Serbia

E-mail: ivanpalibrk@yahoo.com

Summary

Nutrition is a crucial component of critically ill patients' treatment. The key questions to address are when to initiate nutrition therapy, how to determine the optimal route, the appropriate amounts of macro and micronutrients, and the precise energy requirements for each patient.

Critical illness has three phases: early period (previously "ebb phase") lasting 1-2 days; late period (previously "flow" phase) lasting for 3-7 days; late phase (Phase Rehabilitation or Chronic Phase). Each of the above-mentioned phases has its characteristics. During the first phase, severe catabolism is increased, and it gradually proceeds to anabolism during the following 3 to 4 days. The recommendations for critically ill patients' nutrition have been formed based on these phases.

Early nutrition therapy, especially early parenteral nutrition with high energy and protein intake, should be avoided in the first three days of critical illness. Reaching the nutritional goal should be initiated only 3 to 4 days upon the onset of critical illness. According to ESPEN recommendations, daily calorie intake should be initiated at 20-25 kcal/kg/day, while daily protein intake should be initiated at 0.8g/kg/day with a gradual increase to 1.3 g/kg/day. On the other hand, ASPEN recommends 12-25kcal/kg/day of daily calorie intake with daily protein intake at 1.2-2 g/kg/day. The optimal route of feeding is enteral whenever possible. Alternatively, parenteral route should be used. Indirect calorimetry serves as a basis for determining nutritional needs in critically ill patients.

Conclusion: Nutritional therapy is essential for critically ill patients. Nutrition should be obtained through enteral route whenever possible. Energy and protein intake should be gradually introduced in critically ill patients' treatment. Nutritional therapy prescription should be adapted to the patients' needs.

Keywords: critically ill, enteral nutrition, intensive care, parenteral nutrition

Cite this article as: Palibrk I, Đukanović M, Stefanović D. Nutrition in critically ill adult patients; Medicinska istaživanja 2024; 57(3):151-160 DOI: 10.5937/medi57-50990

INTRODUCTION

It has been widely accepted that nutrition not only serves as support, but is also a therapy on its own, one that evidently affects patients' treatment outcome. Similar to other treatment methods, such as antibiotics or vasopressors, nutritional therapy has specific indications and contraindications that are dependent on the type of formula, its components and ingredients. The dosage of micro and macronutrients in various types of formulas is well known. Nutrition therapy applied to critically ill patients has surpassed a universal prescription. Nowadays nutritional therapy is particularly prescribed according to the patient's diagnosis, stage of the disease and nutritional status. It can be described as being individually tailored. The precise moment of the induction of nutritional therapy, its duration and the interval of implementation are individually determined. All these components have a significant impact on morbidity, mortality, quality of life, time of recovery and, finally, to the positive economic impact (1-3).

How do we define a critically ill patient? The definition is certainly hard to find. It is a patient who has developed or is under the risk of developing an acute severe or multiple organ failure. Likewise, it can also be a patient with other comorbidities and under the risk of further deterioration. A common denominator among all the above is that they are patients requiring constant monitoring, whether invasive or non-invasive, with 24-hour care and comprehensive individualized treatment. For example, such a patient would require invasive or non-invasive respiratory support, as well as the need for vasoactive drugs, nutritional therapy, continuous renal replacement therapy, and/or purification processes **(Figure 1)**.

Organ dysfunction can be monitored using the Sequential Organ Failure Assessment (SOFA) score, a scoring system that also provides insights into the level of inflammation and organ impairment. SOFA score enables us to trace the degree of respiratory system dysfunction based on the PaO_2/FiO_2 ratio. Furthermore, we can detect the coagulation system dysfunction based on platelet count, the liver dysfunction based on bilirubin values, the cardiovascular system dysfunction based on the values of mean arterial pressure and/or the use of vasoactive drugs, the central nervous system dysfunction based on Glasgow Coma Score (GCS), and renal dysfunction based on the values of creatinine and/or diuresis (4, 5).

EPIDEMIOLOGY

A significant number of post-surgery patients have been hospitalized after experiencing substantial weight loss

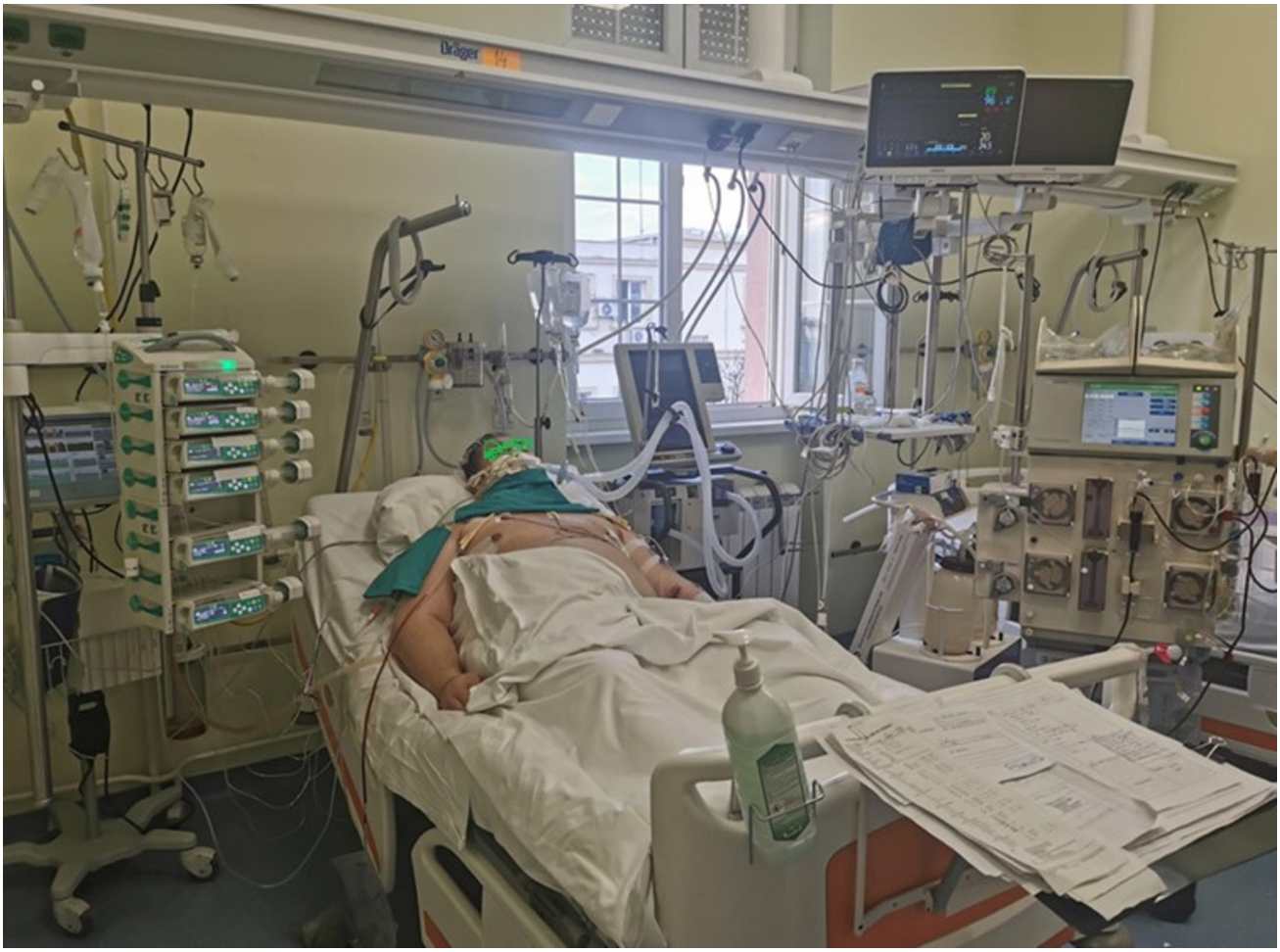


Figure 1. Critically ill patient in the intensive care unit (author's personal archive)

(over 10 kg) within the past six months. At the Clinic for Digestive Surgery, University Clinical Centre of Serbia (UCCS), this percentage amounted to 53.3% in 2008, while in Europe, in 2006, it reached 33%. The starvation trend continues after hospital admission, because some patients suffered from loss of appetite (28.95%), 12.28% had difficulties swallowing food, while an astonishing 45.6% did not receive food orally due to diagnostic interventions and preparations for upcoming surgical procedures. Hospitalized patients evidentially have problems with food intake, which places them in need of nutritional therapy. The Clinic for Digestive Surgery UCCS has, therefore, made the nutritional therapy an integral component of the patients' treatment during their preoperative preparation and other procedures. As a result, there were 26.31% of patients on parenteral nutrition in 2008, 13,51% of patients on enteral nutrition, and 2.63% of patients who received specialized nutrition with the introduced protein supplements amounting to 0.88% cases. This systemic assessment of nutritional problems has resulted in the formation of a nutritional therapy team and the evaluation of nutritional risk following patients' admission into hospital, through the implementation of Nutritional Risk Screening 2002(NRS 2002) (6-8).

NUTRITIONAL RISK ASSESSMENT

Nutritional risk assessment is a critical matter. The implementation of it, in practice, enables us to identify patients that are under a greater risk of complications due to prolonged fasting. Two most common scoring systems that have been used in the Intensive Care Unit (ICU) are mNUTRIC (modified Nutrition Risk in Critically Ill Score) and NRS 2002 (Nutritional Risk Screening). The mNUTRIC takes the following into account: the age, APACHE II (Acute Physiology and Chronic Health Evaluation II), SOFA score (Sepsis-Related Organ Failure Assessment), comorbidities and days since the time of hospital admission and to the ICU. Score ≤ 4 is defined as low risk, whereas score ≥ 5 is defined as high risk. NRS 2002 is composed of age, BMI (body mass index), percentage of weight loss, energy intake compared with energy requirement and severity of disease. Nutrition risk classification is scored as: < 3: No risk, ≥ 3: At risk, ≥ 5: High risk. Nutritional risk assessment in critically ill patients could be very challenging, since those patients are at a great nutritional risk considering the fact that they are in a critical condition as soon as they enter the ICU, being in severe catabolism and inflammation and requiring nutritional therapy. As a result, the scoring systems mentioned above could not be applied to critically ill patients. It is considered that the most useful tool to assess the nutritional risk of patients in the ICU is NUTRIC (Nutrition Risk in Critically Ill Score), that has all the same parameters as the mNUTRIC with the exception of interleukin-6; score ≤ 5 is considered as low risk; score ≥ 6 is considered as high risk (9). Patients with the NRS score ≥ 3 have a significantly longer hospital stay (24 vs 14 days) and a significantly higher number of complications (57.1% vs. 13.3%) compared to patients with an NRS score <3. Postoperative morbidity was increased 3.84 times for each positive response to the initial four screening questions (10).

EFFECTS OF NUTRITION

Nutrition is certainly a crucial part of therapy for critically ill patients. The impact of nutrition on reducing morbidity and mortality in critical illness has been confirmed. Alberda's multicentre study reached interesting conclusions (11). Critically ill patients received amounts of energy (15,0-30,9 kcal/kg/day) and protein (1,0-1,4 g/kg/day) intake based on their BMI. 69.0% of patients received enteral nutrition (EN), 8.0% received parenteral nutrition (PN), 17.6% received EN plus PN, and 5.4% received neither EN nor PN. The results indicated that increased energy and protein intake improved the treatment outcome of critically ill patients, especially those with BMI below 25 and greater than 35. On the other hand, the increased energy and protein intake did not enhance the outcome of patients with BMI 25-35 (11). In a multicentre study, Compher et al. divided critically ill patients into two groups: patients with NUTrition Risk score < 5 and patients with NUTrition Risk score ≥ 5. By analyzing mortality on the 4th day of critical illness, it was observed that there was no change in odd ratio when it comes to mortality or an increase in energy intake for the group of patients with high nutritional risk. However, by analyzing interaction between NUTRIC category, energy intake and mortality on the 12th day of critical illness, the study noted that the odds of death were significantly reduced by 11.6% with each 10% increase in delivery of goal energy intake but not in the low-risk patients. A similar result was obtained in relation to the goal protein intake. Increasing protein intake on the 4th day of critical illness does not reduce the odds of mortality in patients with a high Nutrition Risk, but on the 12th day of critical illness in patients in the group with an increased Nutrition Risk, the odds of death decreased significantly by 10.1% with each 10% increase in protein intake relative to goal but not significantly in the low-risk patients. In conclusion, patients with higher nutritional risk at the ICU admission may benefit from greater protein and energy intake, especially during longer ICU stays, while patients with lower nutritional risk have no benefit from greater intake (12).

NUTRITIONAL RECOMMENDATIONS

Should one start nutritional therapy in critically ill patients? And if so, what would be the right time for

introducing it? There is no doubt that nutrition proved beneficial during critically ill patients' therapy. Studies have shown that nutrition improves the outcome of the treatment and a proper timing is crucial. Determining the right moment for the beginning of nutritional therapy and the right amount of energy and nutrient intake are crucial. The European Society for Clinical Nutrition and Metabolism (ESPEN) has given the following recommendations: every critically ill patient is considered to be at risk of malnutrition if the length of stay in the ICU is longer than 48 hours. Oral intake of food and water is preferable. However, as this is often not feasible in critically ill septic patients with the intact gastrointestinal tract, enteral nutrition is recommended whenever possible, especially within the first 72 hours. Furthermore, if there are any cases in which enteral nutrition is not achievable while the patient is at a high nutritional risk (NRS score greater than 5), parenteral nutrition should be introduced gradually, taking into account all the risks it carries (occurrence of overfeeding and refeeding). Early full EN and PN are to be prescribed within three to seven days. The recommendation is to use indirect calorimetry to assess the energy requirements of critically ill patients, especially in cases involving mechanical ventilation. If predictive equations are used to determine the energy requirement of a critically ill patient (20 to 25 kcal/kg/day), a hypocaloric intake (below 70% of the calculated needs) is recommended. This recommendation should be used during the first seven days of critical illness (13-15). American Society for Parenteral and Enteral Nutrition (ASPEN) states that there is no significant difference in the applied amount of energy in the first days of critical illness, but they recommended the intake of 12 to 25 kcal/kg/day during the first days of critical illness (8, 16).

What is the appropriate protein intake for a critically ill patient? This is yet another important question that requires our attention, and it is as crucial as the issue of energy-per-day parameters which are connected to the critically ill patients' treatment outcome. According to ESPEN recommendations, daily protein intake should be initiated at 0,8g/kg/day with a gradual increase to 1.3 g/kg/day, while ASPEN recommends 1.3 g/kg/day past 24-48h, upon hemodynamic stabilization. The amount of protein intake depends on the severity of critical illness. For instance, in patients with burns, additional protein intake should be administered (up to 2g/kg/day) (8-14).

How did we establish these recommendations and their undeniable necessity for an individual approach to the patients' needs? While the exact amount of nutrients and energy intake is well known, it should be pointed out that the "breaking point" for reaching the nutritional goal comes only after the 3-4th day from the onset of critical illness. The question is why it is so.

PATHOPHYSIOLOGICAL BASIS OF THE RECOMMENDATION

Critical illness has three phases: early period (previously known as "ebb" phase) lasting for 1-2 days, late period (previously known as "flow" phase) lasting for 3-7 days, late phase (Phase Rehabilitation or Chronic Phase) (8,14,15,17,18).

Early period is characterized by hypermetabolism, a severe increase in catabolism, muscle wasting, protein and amino acid degradation, production of glucose and acute-phase proteins, insulin resistance and finally, hemodynamic instability. It is important to remember that 500 to 1400 kcal of endogenous energy is produced during this phase and that any addition of energy through nutrition will lead to overfeeding and all its negative outcomes.

Late period is characterized by organism slowly turning towards catabolism (third day) which in turn causes a decrease of endogenous energy production. In this period, we gradually increase energy along with the protein uptake.

Late phase (Phase Rehabilitation or Chronic Phase) marks a period when anabolism begins to overtake catabolism triggering the patients' rehabilitation. In certain cases, however, some patients fail to recover, leaving them in a late persistent catabolism and resulting in prolonged hospitalization.

ESTABLISHING A SET OF RECOMMENDATIONS

Over the past decades a great amount of previous studies were conducted with a goal of determining the exact protein and energy daily intake in critically ill patients, avoiding the negative impact on morbidity and mortality at the same time.

Casear et al. (2011) have published a randomized, multicentre study on the impact of early (within 48h) versus late initiation (starting 8 days later) of parenteral nutrition in critically ill patients as an addition to enteral nutrition targeted to reach the caloric goal. The results were in favor of late implementation of parenteral nutrition with an aim of reaching the caloric goal **(Table 1)**. Late parenteral nutrition initiation has proven to be superior due to patients' shortened length of stay in the ICU, as well as a lowered number of infections and renal complications. The latter indicates that the lower protein intake positively correlates with the lower renal stress. As a conclusion, late PN initiation has evidently contributed to a faster recovery, followed by a reduced number of complications as compared to the early PN initiation (19).

Heidegger (2013) published the results of a controlled, randomized study, in which a parenteral nutrition supplemented the already existing enteral nutrition with an aim of optimizing energy intake in critically ill patients. The nutritional goal was at 25kcal/kg/day of ideal bodyweight for women and 30kcal/kg/day of ideal body-

Table 1. The main results of early and late supplemental parenteral nutrition implementation (prepared according to the reference (19))

	Late initiation group (n=2328)	Early initiation group (n=2312)	p Value
Discharged alive from the ICU within 8 days no (%)	1750 (75.2)	1658 (71.7)	0.007
Death in ICU no (%)	141 (6.1)	146 (6.7)	0.76
Death in hospital no (%)	242 (10.4)	251 (10.9)	0.63
Death within 90 days upon enrolment no (%)	257 (11.2)	255(11.2)	1.00
Nutrition related complication no (%)	423 (18.2)	434(18.8)	0.62
Length of stay in the ICU	3 (2-7)	4 (2-9)	0.02
Infection no (%)	531 (22,8)	605 (26,2)	0,008
Mechanical ventilation; median duration (days)	2 (1-5)	2 (1-5)	0,02

weight for men. During the first three days, all patients received enteral nutrition after which one group continued receiving enteral nutrition, while the other group received enteral nutrition with supplemental parenteral nutrition (Figure 2). Both groups had a target of reaching the nutritional caloric goal. Those patients who received enteral nutrition exclusively were less susceptible to nosocomial infections, as they did not always manage to reach the caloric goal. Mortality was reduced in patients who received supplemental parenteral nutrition (ICU mortality 5 vs7%, p= 0.211and general mortality 13% vs 18%; p=0.119). The conclusion of this study was that the individually optimized energy supplementation with supplemental parenteral nutrition starting 4 days after ICU admission should be considered as a strategy to improve clinical outcome in patients in the ICU for whom enteral nutrition is insufficient (20). In other words, there is no need to rush with implementing of parenteral nutrition with an aim of reaching the caloric goal during the first 3-4 days of the onset of critical illness. Furthermore, enteral nutrition should be introduced as early as possible, with a gradual increase of caloric intake up to 25 kcal/kg/day. In those cases where this goal is impossible to reach, after four days supplemental parenteral nutrition should be considered (21).

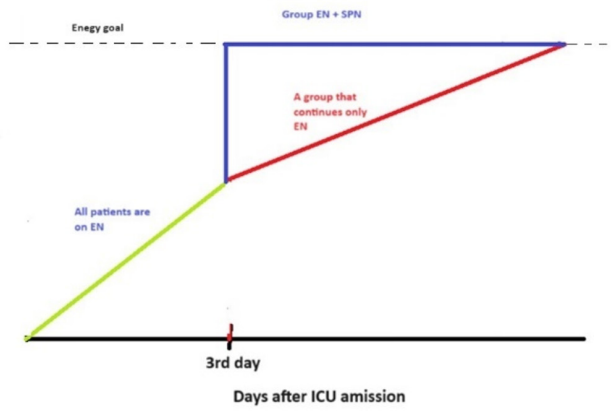


Figure 2. Study design (Prepared according to the reference (20))

Prospective cohort study published in 2012 set the initial calorie (25-30 kcal/kg/day) and protein goal (1.2 – 1.5 g/kg/day) in critically ill patients. They hypothesized that securing the protein intake improves the outcome of the critically ill patients' treatment. Ten-day mortality was the lowest in the group of patients with the highest amount of proteins (1.46+0.29 g/kg/day). The conclusion of this study suggested that the increase of proteins and amino acids figures as more important to the recovery of critically ill patients than the increased caloric intake alone (22). Weijs et al. monitored four groups of critically ill patients during their treatment with nutritional therapy. The first group included patients who did not reach either the caloric or the protein goal, while the second group reached both of these goals. The third group was comprised out of patients who had reached only the caloric goal, and the fourth group included the patients who had reached only the protein goal. The results have shown that in those patients who had only reached the protein goal within 1.2g/kg/day the 28-day mortality was lowered, as well as the hospital mortality and the ICU mortality. The best results with regards to mortality were shown in the group that had reached both the caloric and the protein goal. Reaching the caloric goal exclusively (1600 kcal/kg/day) did not impact mortality (23). Zusman et al.'s retrospective observational study showed that nutritional therapy which was commenced within the first 4 hours of the admission in the ICU had resulted in the lowest mortality rates providing that 70% of the total caloric needs (energy expenditure) were met. The protein intake was 1.3g/kg/day. Ultimately, this study excludes the thesis that 100% of calorie intake fares as necessary for the patient's recovery and highlights the indirect calorimetry as an essential factor for the assessment of patient's caloric needs (24).

In a prospective cohort study (**PROTINVENT**), critically ill patients were divided according to calorie intake in three groups: hypocaloric (less than 80% caloric needs), normocaloric (80-100% caloric needs), and hypercaloric intake (more than 110% caloric needs), with a desired protein intake from 1.5 up to 2g/kg/day. The conclusion of this study was that late medium protein and late high energy intake were associated with survival benefit in septic patient; on the other hand, early high protein intake was associated with higher 6-month mortality, while late protein intake higher than 0.8g/kg/day was proven to be beneficial (25). In an EFFORT Protein international, randomized, multicentre study the pro-

tein intake was initiated within 96 hours of ICU admission. The patients were divided into two groups; the first group was receiving high doses of proteins (at least 2.2g/kg/day, or more), while the second group was receiving the usual doses of proteins (1.2g/kg/day or less). Calorie intake was not controlled. In conclusion, it was highlighted that the protein intake of 1.2g/kg/day was reasonable and safe for critically ill patients, while delivery of higher doses of protein to mechanically ventilated critically ill patients did not improve the time-to-discharge-alive from hospital and might have worsened outcomes for patients with acute kidney injury and high organ failure scores (26). Reignier et al. hypothesized that early protein and energy restriction in critically ill patients in comparison with a standard intake could improve treatment outcome, but the results were surprising. There were fewer complications in the group with restricted intake, but without impact on mortality. The conclusion was that low caloric and protein intake (6 kcal/kg/day; 0.2 – 0.4 g/kg/day) compared to standard caloric and protein intake (25 kcal/kg/day; 1.0 -1.3 g/kg/day) during the acute phase of critical illness led to a faster recovery with fewer complications, without affecting mortality (27).

WHY LESS PROVIDES MORE?

Reviewing the above mentioned studies, it is suggested that in the first days of critical illness a reduced amount of energy and protein is needed in order to achieve the best possible results in the treatment of these patients. It could be said that less is more. This could be explained by more expressed catabolism, produced endogenous energy and muscle protein degradation in the first days of critical illness. Nutritional therapy during that period could lead to overfeeding and renal stress, which could be accompanied by further renal damage in patients with impaired renal function (18). Additionally, reduced energy intake and fasting have been shown to be the most potent physiological triggers for activating autophagy, which is essential for defending the body against microorganisms and for eliminating damaged or dead cells. Parenteral nutrition itself leads to suppression of autophagy, which has been proven in experiments on rabbits. Autophagy is greatly reduced in the liver by parenteral nutrition, which is a pillar of the body's defence against detritus and pathogenic microorganisms. Early parenteral nutrition, especially with an increased amount of amino acids and lipids, reduces the body's defence and should therefore be avoided during the early phase of critical illness. Intraoperative administration of amino acids together with lidocaine and magnesium has been shown to reduce the postoperative inflammatory response, as measured by inflammation parameters. That being said, the strategy of fasting under the first 2 to 3 days of critical illness could be justified (28-31). One of the reasons for nutritional therapy in early stages of critical illness is an attempt to salvage the muscles from catabolism, as well as to maintain muscle mass. Unfortunately, this could not be possible during a critical illness, provided that muscle strength is reduced while myofibrils are replaced by fat tissue; moreower, during an early parenteral nutrition this process is more expressed. Hence, the above mentioned could be the reason why the early parenteral nutrition should be avoided during the early stages of critical illness (32).

During a critical illness, the urea-creatinine level is increased in response to muscle wasting, which is more expressed in traumatized patients; the higher the ratio, the longer is the stay in the ICU and the higher is the mortality rate. Increased load of amino acids leads to worsening of critical illness and deterioration of renal function. With this in mind, the protein intake should be reduced in the first days of critical illness in order to reduce the urea-creatinine ratio as an indicator of catabolism. This could explain why the use of glutamine in critically ill patients has not given the expected satisfactory results, on the contrary, glutamine use led to liver overload. Glutamine is well known as the amino acid with the most potential of producing urea and glycogen, which significantly overloads the liver and kidneys (14, 33,34). Nutritional therapy is known to suppress ketogenesis, while fasting activates lipolysis and ketogenesis. Ketogenesis is of great importance in critical illness for the following reasons: ketone bodies are a great alternative to glucose in the brain and cardiomyocytes during the first days of critical illness, they have a signaling role in activating an immune response, autophagy, activating muscle regeneration, as well as having anti-inflammatory features. Therefore, ketone bodies that are produced during fasting represent an evolutionary mechanism of body defense (35). It could be concluded that early nutrition therapy, especially early parenteral nutrition with high energy and protein intake, should be avoided in the first three days of critical illness on the account of introducing overfeeding, protein, amino acid and carbohydrate overload, muscle catabolism, reduced production of ketone bodies, which all lead to higher morbidity and mortality. In short, less is more.

OTHER REQUIREMENTS

There are two key issues that nutritional therapy of critically ill patients ought to resolve – the starting time for nutrition, and the exact amounts of protein/energy intake. These have, evidently, had the most impact on the mortality and morbidity of critically ill patients. Alongside the specific energy and amino acid needs, nutritional therapy requires the implementation of glucose and lipids or fatty acids. These amounts are added into the daily energy needs of critically ill patients. Lipids are introduced intravenously, through emulsions. It is important to highlight that the daily energy intake must include

all the lipids, together with those introduced through the anesthetic propofol. Taking all these lipid (or fatty acid) sources into account, the total daily value should not exceed 1.5 g/kg/day. Daily amounts of fatty acids have to be adjusted individually, according to each patient's needs. Consequently, their triglyceride and cholesterol values require monitoring. If the values rise, the intake has to be either adjusted accordingly, or terminated completely.

Lipid emulsions used in parenteral nutrition may contain polyunsaturated fatty acids of fish oil, olive or soybean oil and their immunomodulatory role in critically ill and septic patients has been proven. Thus, soybean oil-based fatty acids (omega-6 fatty acids) should be avoided in septic patients because of their pro-inflammatory effect. Fatty acids based on fish oil (omega-3 fatty acids) have an anti-inflammatory effect, while olive oil-based fatty acids (omega-9, monounsaturated fatty acids) have a relatively neutral effect on the inflammatory response. Knowing all this, the immune response in critically ill patients could be modified by using certain fatty acids. Implementing omega-3 fatty acids in critically ill septic patients' nutrition therapy has been proven to be beneficial in some aspects, such as improved gas exchange and earlier weaning from mechanical ventilation. However, their positive impact in critical illness has not shown obvious proofs (36). On the other hand, all of the three above mentioned fatty acids are essential, meaning they are not endogenously synthetized, therefore, their intake during critical illness is crucial. Essential fatty acids are necessary for synthesizing eicosanoids, from which biologically active substances are produced (prostaglandins, leukotrienes, thromboxane). These substances modulate the coagulum production, regulating circulation and affect immune processes (14-16,27,37). Nowadays, there are lipid emulsion formulas that contain all these three essential fatty acids.

Glucose is a necessary macronutrient, as it is a basic source of energy. Daily dose of glucose should not exceed 5mg/kg/min or 150g/day, respectively. During the first two days it is necessary to monitor the glucose level every 4 hours. If the values of glucose exceed 10mmol/L, insulin should be implemented in therapy (14-16,27,37).

Vitamins (hydrophilic and lipophilic) and micronutrients have an important role in metabolic processes, immune response and as antioxidants in gene synthesis and reparation. Therefore, it is necessary to supplement them daily, either orally or parenterally (14-16).

COMPLICATIONS AND MONITORING OF NUTRITIONAL THERAPY

Nutritive therapy complications arise due to two reasons: poor planning or poor monitoring. Monitoring prevents complications that result from nutritive therapy treatment, such as metabolic, infectious or mechanic complications, as well as thrombosis. Electrolyte imbalance is particularly significant since it could lead to refeeding syndrome. For that reason, a careful daily monitoring of electrolyte and biochemical panel prevents any further electrolyte imbalance as well as increased urea and creatinine levels. Refeeding syndrome is a metabolic disorder that occurs during the transition from the fasting state to a state of food intake during the initial phase of nutritional therapy in patients who are severely malnourished or are in a state of stress due to a critical illness. That being the case, gradual intake of food with daily electrolyte monitoring should be carefully planned. Particular attention should be paid to the potassium, sodium, phosphorus and magnesium levels. Main refeeding syndrome symptoms include arrhythmias, decompensated heart failure, breathing disorders, vision disorders, and neurological disorders. Potassium, sodium, phosphorus, magnesium and thiamine supplementation should be applied from the first day of nutritional therapy in patients who are at a risk of developing refeeding syndrome. Thiamine is an essential vitamin that has a role as a coenzyme in the Krebs cycle and is necessary for the conversion of glucose into adenosine triphosphate (ATP). Along with other vitamins, thiamine should be given 200 to 300 mg per os, or intravenously daily (8,31,37-39). During the fasting phase, a decomposition of cells begins since there is no energy that would activate the Na-K pump whose role maintains osmotic equilibrium between intracellular and extracellular space. Once the Na-K pump ceases to function, cellular death occurs. The beginning of food intake, whether enterally or parenterally, the organism receives the necessary nutrients for cell reconstruction and energy for activating Na-K pump. In order to create energy, sufficient amounts of thiamine and phosphorus are needed. As a result, phosphorus starts building up in this energetic carrier, as well as in cell membranes, all of which leads to hypophosphatemia. With the appearance of ATP a large number of Na-K pumps are activated, with the aim of maintaining the newly created cells that pump potassium into the cell. This results in hypokalaemia and hypomagnesemia.

Hyperbilirubinemia and acalculous cholecystitis are frequent complications that occur after a long-term application of parenteral nutrition, although these complications can arise shortly after implementing the therapy. It is a result of certain components' toxicity, as well as overfeeding. The main symptoms are abdominal pain and discomfort followed by elevated transaminase and lactate dehydrogenase levels. Furthermore, elevated levels of bilirubin have been recorded. Acalculous cholecystitis is presented as a gallbladder dysfunction and its etiological factors may include critical illness, fasting, weight loss, and total parenteral nutrition. Best course of action is to terminate parenteral nutrition and make a transition to enteral nutrition or at least to exclude fatty acids from the therapy. Hyperbilirubinemia and acalculous cholecystitis can, however, also occur in case of enteral nutrition (40, 41).

Nutritive therapy monitoring is based on the laboratory and biochemical panel monitoring, as well as energy needs and protein catabolism monitoring. The optimal practice for energy needs monitoring of patients is indirect calorimetry. If such an approach is not available, the nitrogen balance offers the best way to find out if the patient's energy needs are met. This practice allows us to calculate the ratio of nitrogen input versus output. If nitrogen output is greater than the input, the patient's energy needs have not been met yet, leaving the organism to consume its proteins for energy production. When nitrogen input is greater than the output, the patient has met their energy needs and the organism no longer needs to consume its proteins for energy production (8, 15,37,39).

CONCLUSION

Nutritional therapy is essential for critically ill patients. The optimal route of feeding is enteral whenever possible. Alternatively, parenteral route should be used. The basis of nutritional therapy prescription is to determine the necessary energy and protein amounts. Hypocaloric and hypoproteinemic intake during the first 2 to 3 days of critical illness is recommended. Additionally, indirect calorimetry serves as a basis for determining nutritional needs in critically ill patients.

Author Contributions

Conception, design of the manuscript; final approval of the version to be published, analysis, interpretation of data: Ivan Palibrk

Analysis, interpretation of data, Marija Đukanović

Drafting, revising manuscript critically: Dona Stefanović

Co-first author: Marija Đukanović and Dona Stefanović have contributed equally to this work and they share first authorship.

REFERENCES

1. Muscaritoli M, Krznaric Z , Barazzoni R, Cederholm T, Golay A, Van Gossum A, et al. Effectiveness and efficacy of nutritional therapy e A cochrane systematic review. Clin Nutr 2017;36(4):939-957.doi: 10.1016/j.clnu.2016.06.022. PMID: 27448948.
2. Lambell KJ Babet O T, Chapple L, Gantner D, Ridley EJ..Nutrition therapy in critical illness: a review of the literature for clinicians. Crit Care. 2020;4;24(1):35. doi: 10.1186/s13054-020-2739-4. PMID: 32019607.
3. Palibrk I, Veličković J, Pantić Palibrk V, Arsenijević V, Tanović B, Ugrinović Đ. Preoperative preparation for patients with nutritional disorders. Acta Chir Iugosl. 2011;58(2):163-168. DOI: 10.2298/aci1102163p. PMID: 21879667.
4. Vincent, J.-L., Creteur, J. The Critically Ill Patient.In: Critical Care Nephrology.3rd Ed.Elseviaer;2019. doi:10.1016/b978-0-323-44942-7.00001-7
5. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. TheThirdInternationalConsensusDefinitions for Sepsis andSepticShock(Sepsis-3). JAMA.2016;315(8):801-810. doi:10.1001/jama.2016.0287. PMID: 26903338.
6. Palibrk I, Matić M, Peško P, Ranković V, Tomašević Lj, Milenković M, Nenadić B, Maširević V. Nutrition day in Serbia. 30th Congress of ESPEN, Florence, Italy September 13-16, 2008. Clinical Nutrition.2008;3 Suppl 1:P 110. doi.org/10.1016/S1744-1161(08)70172-8
7. Palibrk I, Pantić-Palibrk V, Matić M, Milenković M, Maširević V, Ranković V. Nutrition day 2009 in one hospital in Serbia – what should we do? 31th Congress of ESPEN, Vienna, Austria. August 29-01 September, 2009. Clinical Nutrition.2009;4 Suppl 2:P 069. doi.org/10.1016/S1744-1161(09)70119-X
8. Palibrk I, Pantić Palibrk V, Đukanović M, Ivošević T, Đurašić Lj, Lađević N. NUTRITION SUPPORT IN INTENSIVE CARE UNIT. SJAIT2013;3-4:141-152. Nutrition support in intensive care unit (ceon.rs)
9. Cattani A, Eckert IC, Brito JC, Tartari RF, Silva FM. Nutritional risk in critically ill patients: how it is assessed, its prevalence and prognostic value: a systematic review. Nutr Rev. 2020;78(12):1052-1068. doi: 10.1093/nutrit/nuaa031. PMID: 32529226.
10. Veličkovic J, Palibrk I, Žnidaršič M, Zec S, Marnic M, Đukanovic M. SUN-P198 NUTRITIONAL RISK ASSESSMENT IN PATIENTS UNDERGOING GASTROINTESTINAL SURGERY: CAN NRS 2002 SCORE BE SIMPLIFIED? Clinical Nutrition.2017;36 Suppl 1:S127.doi.org/10.1016/S0261-5614(17)30430-2
11. Alberda C, Gramlich L, Jones N, Jeejeebhoy K, Day AG, Dhaliwal R, Heyland DK. The relationship between nutritional intake and clinical outcomes in critically ill patients: results of an international multicenter observational study. Intensive Care Med. 2009;35(10):1728-37.doi: 10.1007/s00134-009-1567-4. PMID: 19572118.
12. Compher C, Chittams J, Sammarco T,Nicolo M, Heyland DK. Greater Protein and Energy Intake May Be Associated With Improved Mortality in Higher Risk Critically Ill Patients: A Multicenter, Multinational Observational Study. Critical Care Medicine 2017;45(2):156-163. DOI: 10.1097/CCM.0000000000002083. PMID: 28098623.
13. Palibrk I, Đukanović M. Osnove enteralne ishrane. SJAIT 2015;1-2:15-19. 2217-77441502015P.pdf (ceon.rs)
14. Singer P, Reintam Blaser A, Berger MM, Alhazzani W, Calder PC, Casaer MP, et al. ESPEN guideline on clinical nutrition in the intensive care unit. Clinical Nutrition 2019;38:48-79. doi.org/10.1016/j.clnu.2018.08.037. PMID: 30348463
15. Singer P, Blaser AR, Berger MM, Calder PC Michael Casaer M, Michael Hiesmayr ESPEN practical and partially revised guideline: Clinical nutrition in the intensive care unit. Clinical Nutrition 2023;42: 1671-1689. DOI: 10.1016/j.clnu.2023.07.011. PMID: 37517372
16. Compher C, Bingham AL, McCall M, Patel J, Rice TW, Braunschweig C McKeever L. Guidelines for the provision of nutrition support therapy in the adult critically ill patient: The American Society for Parenteral and EnteralNutrition. J Parenter Enteral Nutr.2022;46:12–41. doi.org/10.1002/jpen.2267. PMID: 34784064.
17. Wischmeyer PE. Nutrition Therapy in Sepsis. Crit Care Clin 2018;34(1):107-125.doi: 10.1016/j.ccc.2017.08.008 PMID: 29149933.
18. Fiaccadori E, Sabatino A, Barazzoni R, Carrero JJ, Cupisti A, De Waele E, Jonckheer J, Singer P, Cuerda C. ESPEN guideline on clinical nutrition in hospitalized patients with acute or chronic kidney disease. Clinical Nutrition 2021;40:1644-1668. doi.org/10.1016/j.clnu.2021.01.028. PMID: 33640205.
19. Casaer MP ,Mesotten D, Hermans G, Wouters PJ, Schetz M, Meyfroidt,G. Early versus late parenteral nutrition in critically ill adults. N Engl J Med. 2011;365(6):506-17. doi: 10.1056/NEJMoa1102662. Epub 2011 Jun 29. PMID: 21714640.
20. Heidegger CP , Berger MM, Graf S, Zingg W, Darmon P, Costanza MC, et al. Optimisation of energy provision with supplemental parenteral nutrition in critically ill patients: a randomised controlled clinical trial. Lancet 2013; Feb 2;381(9864):385-93. doi: 10.1016/S0140-6736(12)61351-8. PMID: 23218813

21. Vincent JL, Preiser JC. When should we add parenteral to enteral nutrition?Lancet 2013;381:354. doi: 10.1016/S0140-6736(12)61893-5. PMID: 23218814.
22. Allingstrup MJ, Esmailzadeh N, Wilkens Knudsen A, Espersen K, Jensen TH, Wiis J, et al. Provision of protein and energy in relation to measured requirements in intensive care patients. Clin Nutr. 2012 Aug;31(4):462-8.doi: 10.1016/j.clnu.2011.12.006. PMID: 22209678
23. Weijs P, Stapel S, de Groot SDW, Driessen RH, de Jong E, Girbes ARJ, et al. ptimal protein and energy nutrition decreases mortality in mechanically ventilated, critically ill patients: a prospective observational cohort study. JPEN J Parenter Enteral Nutr. 2012 Jan;36(1):60-8. doi: 10.1177/0148607111415109. PMID: 22167076.
24. Zusman O, Theilla M, Cohen J, Kagan I, Bendavid I, Singer P. Resting energy expenditure, calorie and protein consumption in critically ill patients: a retrospective cohort study. Crit Care. 2016; Nov 10;20 (1):367.doi: 10.1186/s13054-016-1538-4. PMID: 27832823.
25. de Koning MSL, Koekkoek VACK, Kars JCNHK, van Zanten ARH. Association of PROtein and CAloric Intake and Clinical Outcomes in Adult SEPTic and Non-Septic ICU Patients on Prolonged Mechanical Ventilation: The PROCASEPT Retrospective Study. JPEN J Parenter Enteral Nutr. 2020; Mar;44(3):434-443.doi: 10.1002/jpen.1663. PMID: 31172544
26. Heyland D, Patel J,Compher C, Rice TW, Bear DE, Lee ZY,et al. The effect of higher protein dosing in critically ill patients with high nutritional risk (EFFORT Protein): an international, multicentre, pragmatic, registry-based randomised trial. Lancet 2023 Feb 18;401(10376):568-576.doi: 10.1016/S0140-6736(22)02469-2. PMID: 36708732
27. Reignier J, Plantefeve G, Mira JP, Argaud L , AsfarP, Aissaoui N. et al. Low versus standard calorie and protein feeding in ventilated adults with shock: a randomised, controlled, multicentre, open-label, parallel-group trial (NUTRIREA-3). Lancet Respir Med. 2023 Jul;11(7):602-612. doi: 10.1016/S2213-2600(23)00092-9. PMID: 36958363.
28. Kroemer G, Marin G,Levine B. Autophagy and the Integrated Stress Response. Mol Cell 2010;40:280-93. DOI 10.1016/j.molcel.2010.09.023. PMID: 20965422
29. Derde S,Vanhorebeek I,Güiza F, Derese I, Gunst J, Fahrenkrog B, et al. Early Parenteral Nutrition Evokes a Phenotype of Autophagy Deficiency in Liver and Skeletal Muscle of Critically Ill Rabbits. Endocrinology 2012; 153: 2267–2276. doi: 10.1210/en.2011-2068. PMID: 22396453.
30. Sarenac O, Lazovic R, Vukcevic B, Lazovic J, Palibrk I. Impact of Perioperatively Administered Amino Acids, Lidocaine, and Magnesium on Inflammatory Response and Pain Associated with Abdominal Surgery: A Prospective Clinical Study Med Sci Monit, 2023; 29: e939204. DOI: 10.12659/MSM.939204. PMID: 36703508
31. Ivan Palibrk. Rastvori aminokiselina i glukoze i emulzije masti. U: M. Jović, P. Stevanović, KME, Medicinski fakultet - Beograd editor. Lečenje infuzionim rastvorima. Kontinuirana medicinska edukacija, Medicinski fakultet, Univerziteta u Beogradu, DAS anesteziološke sveske No VI;2008.p. 97-111.ISBN 978-86-904891-4-5
32. Hermans G, Casaer MP, Clerckx B, Güiza F, Vanhullebusch T ,Derde S, Meersseman P,et al.Effect of tolerating macronutrient defi cit on the development of intensive-care unit acquired weakness:a subanalysis of the EPaNIC trial.Lancet Respir Med 2013; Oct1(8):621-629. DOI: 10.1016/S2213-2600(13)70183-8. PMID: 24461665.
33. Haines RW, Zolfaghari P, Wan Y, Pearse RM, Puthucheary Z, Prowle JR.. Elevated urea-to-creatinine ratio provides a biochemical signature of muscle catabolism and persistent critical illness after major trauma. Intensive Care Med 2019; Dec;45(12):1718-1731.doi: 10.1007/s00134-019-05760-5. PMID: 31531715.
34. Heyland DK, Elke G, Cook D, Berger MM, Wischmeyer PE, Albert M, et al. Glutamine and Antioxidants in the Critically Ill Patient: A Post Hoc Analysis of a Large-Scale Randomized Trial.JPEN . 2015; may39 (4):401-409. doi.org/10.1177/0148607114529994. PMID: 23594003.
35. De Bruyn A, Gunst J, Goossens C , Vander Perre S, Guerra GG, Verbruggen S, et al Effect of withholding early parenteral nutrition in PICU on ketogenesis as potential mediator of its outcome benefit. Critical Care 2020;24:536doi.org/10.1186/s13054-020-03256-z. PMID: 32867803.
36. Koekkoek WK, Panteleon V, Rh van Zanten A. Current evidence on ω-3 fatty acids in enteral nutrition in the critically ill: A systematic review and meta-analysis. Nutrition 2019; Mar:59:56-68. doi: 10.1016/j.nut.2018.07.013. PMID: 30419501.
37. Ivan Palibrk. Komplikacije: Poremećaji serumskih elektrolita i glukoze. U. P. Peško,P. Stevanović, M. Jović, V. Bumbaširević., KME, Medicinski fakultet- Beograd editor. Osnovi kliničke nutricije. Kontinuirana medicinska edukacija, Medicinski fakultet, Univerziteta u Beogradu, DAS anesteziološke sveske NoV;2007.p.343-380.ISBN 978-86-904891-3-8
38. Reber E, Friedli N, Vasiloglou MF, Schuetz P, Stanga Z Management of Refeeding Syndrome in Medical Inpatients. J Clin Med. 2019; Dec 13;8(12):2202. doi: 10.3390/jcm8122202. PMID: 31847205.
39. Palibrk I, Veličković J, Djukanović M. Ishrana hirurškog bolesnika. U Hirurgija sa anesteziologijom. Simić A, Grujičić D, Spasovski D. Ed. 383-390. Medicinski fakultet Univerziteta u Beogradu, Beograd, 2023.ISBN 978-86-7117-677-4
40. Żalikowska-Gardocka M, Przybyłkowski A. Review of parenteral nutrition-associated liver disease.Clin Exp HEPATOL 2020;6, 2: 65–73./doi.org/10.5114/ceh.2019.95528. PMID: 32728621.
41. Jones MW; Ferguson T, editors. Acalculous Cholecystitis [monograph on the Internet]. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan [cited 2024 May 9]. Available from: Acalculous Cholecystitis - StatPearls - NCBI Bookshelf (nih.gov)

ISHRANA KOD KRITIČNO OBOLELIH ODRASLIH BOLESNIKA

Ivan Palibrk[1,2], Marija Đukanović[1,2], Dona Stefanović[2]

Sažetak

Ishrana je sastavni deo terapije kritično obolelog bolesnika. Postavlja se pitanje kada je započeti, na koji način i koje količine makro i mikronutrienata treba primeniti, a svakako je najvažnije pitanje koja količina energije je potrebna takvom bolesniku.

Kritična bolest ima tri faze: *early* period (ranije *ebb*) u trajanju od 1 do 2 dana, *late* period (ranije *flow phase*) u trajanju od 3 do 7 dana i *late phase* (*phase rehabilitation* ili *chronic phase*). Svaka faza ima svoje karakteristike. U prvoj fazi je izražen jak katabolizam, koji od 3. do 4. dana polako prelazi u anabolizam. Na osnovu toga su napravljene i preporuke za ishranu kritično obolelog.

Prvih dana kritične bolesti ne preporučuje se pun energetski i proteinski unos hrane. Dosezanje nutritivnog cilja treba započeti tek posle 3-4 dana od početka kritične bolesti. Energetski unos treba da se kreće od 20 do 25 kcal/kg/dan, a proteinski od 0,8g/kg/dan uz postepeno povećanje do 1,3 g/kg/dan po ESPEN-u. Po ASPEN-u energetski unos treba da je od 12 do 25 kcal/kg/dan , a proteinski od 1,2 do 2 g/kg/dan. Preporučuje se enteralni unos hrane kada god je moguće, a ako on nije moguć onda se primenjuje parenteralni način ishrane.

Rani, puni energetski i proteinski unos može rezultovati povećanim morbiditetom i mortalitetom kod kritično obolelog.

Za određivanje energetskih potreba kritično obolelog bolesnika preporučuje se korišćenje indirektne kalorimetrije.

Zaključak: Kritično obolelog treba hraniti. Koliko god je moguće ishrana treba da bude enteralnim putem. Bilo da je ona enteralna ili parenteralna, unos energije i proteina treba da je postepen. Preskripcija nutritivne terapije treba da je individualna, prilagođena potrebama bolesnika.

Ključne reči: kritična bolest, enteralna ishrana, intenzivno lečenje, parenteralna ishrana

Primljen: 12.05.2024. I **Revizija:** 28.05.2024. I **Prihvaćen:** 26.07.2024.

Medicinska istaživanja 2024; 57(3):151-160

Medicinska istaživanja 2024; 57(3):161-165 | DOI: 10.5937/medi57-48275

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

CASE REPORT

Using integra® dermal regeneration template in electrical burn injury defect reconstruction – a case report

Marko Jović[1, 2], Branko Sudecki[1], Biljana Ćertić[1], Jelena Isaković Subotić[1], Milana Jurišić[1], Milana Marinković[1], Milan Jovanović[1, 2], Milan Stojičić[1, 2], Jelena Jeremić[1, 2]

[1]Clinic for Burns, Plastic and Reconstructive Surgery, University Clinical Center of Serbia, Belgrade, Serbia
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Recived: 16 December 2023
Revised: 08 April 2024
Accepted: 30 May 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Marko Jović
Clinic for Burns, Plastic and Reconstructive Surgery, University Clinical Center of Serbia
2, Pasterova Street, 11000 Belgrade, Serbia
Email: marko.jovic@med.bg.ac.rs

Summary

Introduction: Electrical burn injuries present a major public health issue in industrialized countries, and unlike electrical arc flash burns, they often result in deeper and more extensive injuries, especially in hands and feet. When choosing from the range of reconstructive modalities, prompt coverage of exposed defects is imperative, ensuring a better functional and cosmetic outcome.

Patient review: A 26-year-old male was admitted into our facility's Burns Unit following a high-voltage electrical burn injuries sustained on the same day. After several debridement sessions, the left foot was left with a 100 square centimeter dorsal surface defect with the involvement of ankle joint region. The defect was covered using Integra® Dermal Regeneration Template (IDRT) with a 100% success rate both with the template and later split-thickness skin graft (STSG) application. There were no reported perioperative complications, with satisfactory cosmetic and functional results at the 6-month follow-up.

Conclusion: IDRT can be considered a good surgical choice for effective defect coverage with satisfactory results. Nevertheless, the final outcome and patient recovery also often depend on a multitude of other factors, such as the defect size and the involvement of underlying structures.

Key words: electrical burn injuries, foot defect, Integra® Dermal Regeneration Template, skin graft

Cite this article as: Jović M, Sudecki B, Ćertić B, Isaković Subotić J, Jurišić M, Marinković M, Jovanović M, Stojičić M, Jeremić J. Using integra® dermal regeneration template in electrical burn injury defect reconstruction – a case report; Medicinska istaživanja 2024; 57(3):161-165 DOI: 10.5937/medi57-48275

INTRODUCTION

Electrical burn injuries present a major public health issue in industrialized societies due to the serious outcomes they can have and large socioeconomic burden they present. Although they account for only 0.04-5% of all Burns Unit admissions in developed countries, the incidence can go as high as 27% in developing countries (1). Unlike electrical arc flash burns in which there is no current passage through the body, in electrical burns the current passes through the body often resulting in deeper and more extensive injuries with therefore higher morbidity and mortality (2). The hands are the most common entry point, followed by the head, while the feet are usually the ground point (3). With these types of tissue loss in extremities the primary wound closure is often unsuccessful and wounds are left to heal by secondary intention which involves granulation tissue filling the defect and marginal epidermal cells spreading from the wound edges, covering the tissue and forming a scar. These types of scars have a much higher contraction rate and, in most cases, adhere much more to the underlying structures, thus jeopardizing later extremity function (4). Using various reconstructive procedures does not only restore skin integrity more quickly, but it also shortens healing time, resulting in better functional and cosmetic outcomes (5). One of the many tools in plastic surgeon's arsenal are the acellular dermal matrices (6).

Integra Dermal Regeneration Template (IDRT) is an acellular bilayer membrane consisting of a cross-linked bovine tendon collagen and glycosaminoglycan matrix covered by a semipermeable silicone layer. This design ensures immediate coverage of large surface defects of different etiology (burns, trauma, tumors), followed by dermal regeneration which goes through four distinct histologic stages: imbibition, fibroblast migration, neovascularization, and remodeling and maturation (7,8). During dermal regeneration the silicone layer provides flexible but adherent coverage of the wound while increasing the tear strength of the template, ensuring moisture control, and serving as an infection barrier (7). IDRT based reconstruction is typically two-staged, with the second stage taking place in 2-3 weeks and involving coverage of formed neodermis using split-thickness skin grafts (STSG) (8). In this article we present a case of a large electrical burn injury dorsal foot defect involving the ankle joint region managed by using IDRT.

CASE REPORT

We present a case of a 26-year-old male admitted to our facility's Burns Unit following high-voltage electrical burn injuries of multiple body parts sustained on the same day while performing reparations on a transformation station. The patient was initially admitted to the local hospital and then promptly referred to our facility for further treatment. Upon arrival, the patient underwent a thorough assessment and stabilization of his hemodynamic status. In later course multiple debridement sessions of devitalized tissue were performed. Since the injury distribution included the perianal region, a colostomy bag was derived. Dressing changes were performed regularly every 2 days. The patient was also treated using Hyperbaric Oxygen Therapy (HBOT). After final debridement session the foot was left with a defect that measured 100 square centimeters in size and covered the proximal part of the dorsum and ankle joint, with the underlying joint capsule remaining intact **(Figure 1a)**. On the post-admission day 15, after surgical wound-bed preparation, Integra® Dermal Regeneration Template (IDRT) was placed and fixated using non-absorbable single sutures. Regular dressing changes were performed every 2-3 days, in correlation with the template's local status, and 18 days later the outer silicone layer was removed **(Figure 1b)**, and wound bed covered using split-thickness skin graft (0.2 mm) harvested from the right calf region. Due to the extent of burn injuries, the 5th toe of the left foot was partially amputated with the wound closed directly using single non-absorbable sutures. No postoperative complications

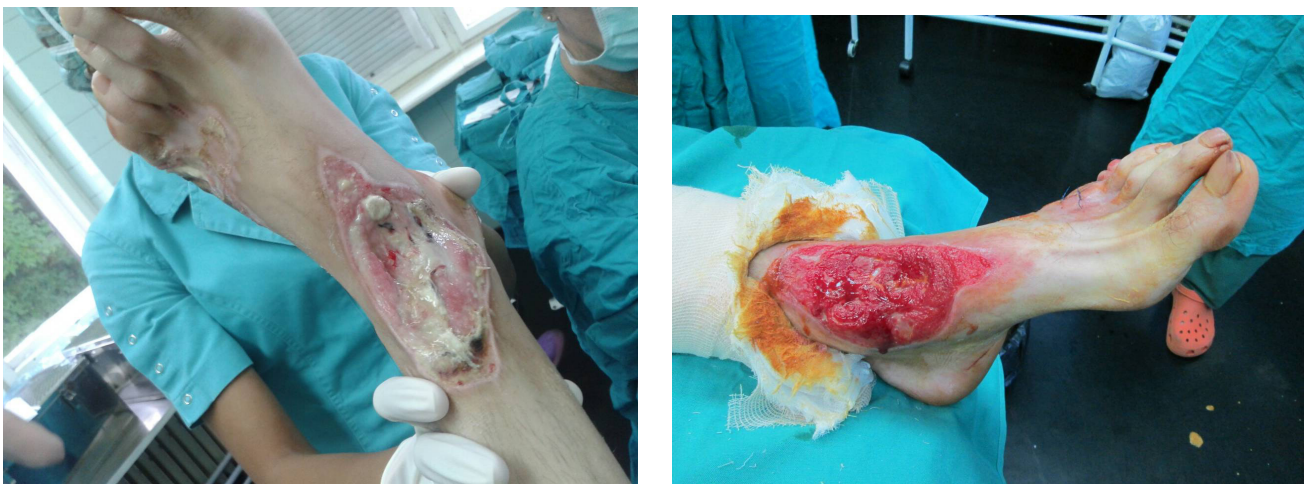


Figure 1. Surgical treatment of the exposed foot defect: (a) the defect size after a number of debridement sessions; (b) neodermal wound bed after the removal of IDRT's silicone layer.

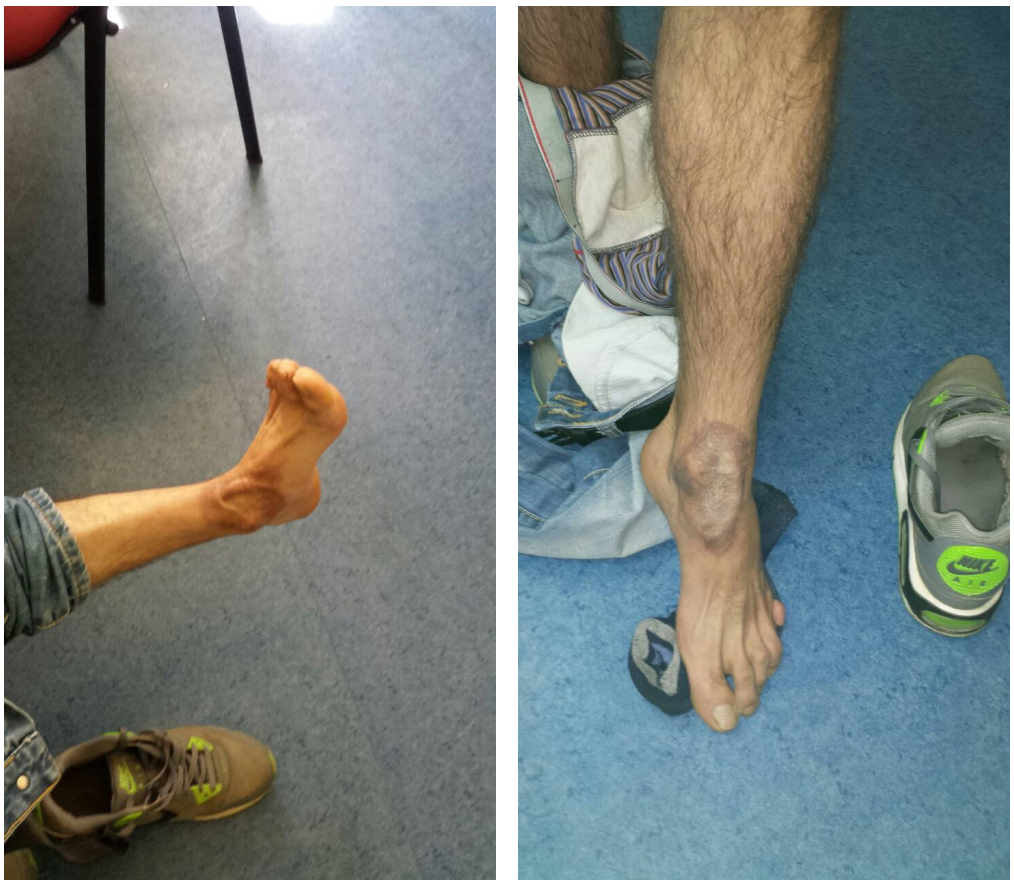


Figure 2. Functional and aesthetic outcomes at the 6-month follow-up session. Intact dorsal and plantar flexion can be noted.

were observed, with both IDRT and STSG acceptance rates at 100%. Due to the extent of his injuries and the requirement of other reconstructive procedures, the patient was hospitalized for additional 43 days, after which he was discharged and later followed by outpatient visits. No STSG failure or additional skin disruption was observed during a 6-month outpatient follow-up **(Figure 2)**.

DISCUSSION

The use of Integra® Dermal Regeneration Template (IDRT) has been described on multiple occasions in the literature for covering wide spectrum of injuries such as burns and trauma, and post-oncologic resection defects (6). Although it was first synthesized as a primary skin replacement in treatment of extensive burns, its many advantages showed that it can be a very good solution, especially in defects involving regions with high functional requirements such as hands and feet. Although there is a substantial number of papers regarding the use of IDRT, only a handful have described its specific use on foot defects (9,10).

Furthermore, the published articles were generally focused on post-traumatic defects or chronic wounds (5,9,10), with no references to electrical burn injuries. These types of injuries present a real surgical challenge for multiple reasons. Firstly, the body distribution of burned tissue can often be quite unpredictable, both regarding the Total Body Surface Area (TBSA) involved, as well as the depth and underlaying structures involvement. More complex procedures, such as axial or microvascular flaps require healthy blood vessels unaffected by the passing electrical current which can lead to endothelial and smooth muscle disfunction, increasing the future risk of thrombosis or stenosis in the injured region (11). For these patients, acellular dermal matrices (ADMs) offer a better chance of reducing the donor site morbidity and leaving the potential donor sites available for covering more urgent exposed regions.

Even if the IDRT does fail, no native patient tissue is lost, and donor region morbidity is avoided. The lack of donor region has also allowed this method to be especially advantageous in elderly patients, or with patients not suitable for general anesthesia involved in longer and more complex reconstructive procedures, such as free flap surgery (12). IDRT application can be done in both local or regional anesthesia, and the two-staged approach provides dressing changes being done in an outpatient setting thus reducing the hospital stay duration and costs.

Using DRT as a reconstructive tool also allows better contouring in cases of extensive tissue loss. Even if the initial resurfacing is inadequate and not bulky enough, the procedure can be repeated multiple times, with multiple layers of IDRT offering aesthetically a more pleasing scar and avoiding over bulking commonly seen with pedicle flap reconstruction (5). Opposite to flap surgery, using skin grafting as a method of choice often leads in the hollow appearance, when compared to the unaffected foot.

Shortening the grafting time allows lesser scar tissue development which improves the final aesthetic and functional outcomes. In their study, Weigert et al. reported that the use of IDRT lead to more rapid restoration of extremity function regarding everyday living activities (5).

Amplifying the IDRT take rate can also be achieved using Negative Pressure Wound Therapy (NPWT) as a bolster, which strengthens the template's adherence to the wound bed. In a study conducted on a series of 16 combat-related wounds, Helgeson et al. reported an overall success rate of 83% while using this method (10), while Park et al. reported a 100 % success rate, with no complications during a 3-month follow-up period (13).

Some disadvantages of IDRT use are the need for a two-stage reconstruction with regular dressing changes done by individual versed in IDRT-based reconstruction management, the risk of infection or hematoma resulting in DRT failure, and its high price per square centimeter which makes it unavailable in limited resources clinical settings. The infection could be avoided by providing a clean well prepared wound bed, using meticulous wound handling techniques during and after the surgery, using preventive dressing options such as nanocrystalline silver dressings, and antibiotic prophylaxis (14).

Regardless of the reconstruction method used, surgeons should fully inform the patients of the primary goals of the procedure (defect closure), and the impact of the initial trauma damage on later functional recovery.

CONCLUSION

Integra® Dermal Regeneration Template can be a valuable tool for covering a wide spectrum of foot defects, often resulting in very satisfactory functional and aesthetic results. Nevertheless, the patient should always be fully informed that final results also vary and depend on multiple factors, such as the initial defect size and the underlying structures involved.

Acknowledgments

None.

Conflicts of interest

None to declare.

References:

1. Shih JG, Shahrokhi S, Jeschke MG. Review of Adult Electrical Burn Injury Outcomes Worldwide: An Analysis of Low-Voltage vs High-Voltage Electrical Injury. J Burn Care Res. 2017;38(1):e293–8.
2. Ding H, Huang M, Li D, Lin Y, Qian W. Epidemiology of electrical burns: a 10-year retrospective analysis of 376 cases at a burn centre in South China. J Int Med Res. 2020 Mar;48(3):300060519891325.
3. Bounds EJ, Khan M, Kok SJ. Electrical Burns. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Nov 4]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK519514/
4. Demmer W, Sorg H, Steiert A, Hauser J, Tilkorn DJ. Wound Healing and Therapy in Soft Tissue Defects of the Hand and Foot from a Surgical Point of View. Med Sci (Basel). 2021 Nov 13;9(4):71.
5. Weigert R, Leclere FM, Delia G, De Luca L, Al Mutairi K, Casoli V. Long-term patient-reported functional and cosmetic outcomes following severe traumatic foot and ankle wound reconstruction with acellular dermal matrix. Journal of Cosmetic and Laser Therapy. 2015;17(6):321–9.
6. Petrie K, Cox CT, Becker BC, MacKay BJ. Clinical applications of acellular dermal matrices: A review. Scars Burn Heal. 2022 Jan 19;8:20595131211038313.
7. Burke JF, Yannas IV, Quinby WC, Bondoc CC, Jung WK. Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. Ann Surg. 1981 Oct;194(4):413–28.
8. Taupin P, Gandhi A, Saini S. Integra® Dermal Regeneration Template: From Design to Clinical Use. Cureus. 2023 May;15(5):e38608.
9. Hamad M, Abulezz T, Elsayed G. Different Modalities Used for Reconstruction of Soft Tissue Defects of the Foot in Sohag University Hospital. Egyptian Journal of Plast Reconstruct Surg. 2017 Jul 1;41.
10. Helgeson MD, Potter BK, Evans KN, Shawen SB. Bioartificial dermal substitute: a preliminary report on its use for the management of complex combat-related soft tissue wounds. J Orthop Trauma. 2007 Jul;21(6):394–9.
11. Park KH, Park WJ, Kim MK, Kim HS, Kim SH, Cho GY, et al. Alterations in arterial function after high-voltage electrical injury. Critical Care. 2012 Feb 12;16(1):R25.
12. Levin LS. Foot and ankle soft-tissue deficiencies: who needs a flap? Am J Orthop (Belle Mead NJ). 2006 Jan;35(1):11–9.
13. Park CA, Defranzo AJ, Marks MW, Molnar JA. Outpatient reconstruction using integra* and subatmospheric pressure. Ann Plast Surg. 2009 Feb;62(2):164–9.
14. Gonzalez SR, Wolter KG, Yuen JC. Infectious Complications Associated with the Use of Integra: A Systematic Review of the Literature. Plast Reconstr Surg Glob Open. 2020 Jul 15;8(7):e2869.

UPOTREBA INTEGRA® DERMALNE REGENERATIVNE PLOČE U REKONSTRUKCIJI DEFEKTA STOPALA ZAOSTALOG NAKON ELEKTROKUCIJE- PRIKAZ SLUČAJA

Marko Jović[1, 2], Branko Suđecki[1], Biljana Ćertić[1], Jelena Isaković Subotić[1], Milana Jurišić[1], Milana Marinković[1], Milan Jovanović[1, 2], Milan Stojičić[1, 2], Jelena Jeremić[1, 2]

Sažetak

Uvod: Elektrokucije predstavljaju veliki javno zdravstveni problem u industrijski razvijenim zemljama jer za razliku od elektrokombustija izazvanih voltinim lukom često rezultuju dubljim i ekstenzivnijim povredama, posebno šaka i stopala. Prilikom odabira rekonstruktivnih opcija, promptno pokrivanje defekta ima prioritet jer obezbeđuje pacijentima bolji funkcionalni i estetski rezultat.

Prikaz slučaja: Muškarac star 26 godina primljen je u Jedinicu intenzivnog lečenja odeljenja opekotina naše ustanove radi lečenja povreda zadobijenih visokovoltažnom elektrokucijom istog dana. Nakon više sukcesivnih nekrektomija devitalizovanog tkiva, zaostao je defekt veličine oko 100 cm^2 koji zahvata skočni zglob i dorzum levog stopala. Defekt je rekonstruisan u dva akta, upotrebom Integra® Dermalne Regenerativne Ploče (IDRP), sa stoprocentnim stepenom prihvatanja kako ploče, tako i autotransplantata kože parcijalne debljine. Postoperativni tok pacijenta protekao je bez komplikacija uz postignute zadovoljavajuće funkcionalne i estetske rezultate na šestomesečnom kontrolnom pregledu.

Zaključak: IDRP predstavlja dobar izbor za efektivno pokrivanje defekta uz postizanje zadovoljavajućih rezultata. Međutim, finalni ishod lečenja i stepen oporavka često zavise i od mnogobrojnih drugih faktora kao što su veličina defekta i zahvaćenost dubljih struktura povredom.

Ključne reči: elektrokucije, opekotinske povrede, stopalo, Integra® Dermalna Regenerativna Ploča, autotransplantat

Primljen: 16.12.2023. I **Revizija:** 08.04.2024. I **Prihvaćen:** 30.05.2024.

Medicinska istaživanja 2024; 57(3):161-165

Medicinska istaživanja 2024; 57(3):167-171 | DOI: 10.5937/medi57-50143

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

CASE REPORT

Sudden death in anorexia nervosa: exploring the mechanism of death

Bogdanović Milenko[1], Popović Vesna[1], Atanasijević Tatjana[1], Radnić Bojana[1], Petrović Tijana[1], Milošević Veljko[1]

1 Institute of Forensic Medicine "Milovan Milovanović", University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Recived: 30 March 2024

Revised: 13 May 2024

Accepted: 15 May 2024

Check for updates

Funding information:
This work was supported by the Ministry of Science of the Republic of Serbia, Grant No. 175093

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Milošević Veljko
Institute of Forensic Medicine "Milovan Milovanović", University of Belgrade, Faculty of Medicine
31a Deligradska Street. 11000 Belgrade, Serbia
E-mail: veljkomilosevic97@gmail.com

Summary

Introduction: Anorexia nervosa (AN) is a severe chronic psychiatric disorder with often underestimated prevalence. Patients suffering from anorexia nervosa can die from natural causes, such as severe heart problems, organ failure, or malnutrition, as well as from unnatural causes, such as suicide. However, the mechanism of death in anorexia nervosa is poorly understood.

Patient review: Our paper describes a case of sudden death in a 22-year-old woman suffering from anorexia nervosa. The autopsy showed severe malnutrition. Upon opening the abdominal cavity, extremely dilated stomach (without signs of wall necrosis or rupture) was noted. Biochemical findings showed that the vitreous chloride, sodium and potassium levels were markedly low, suggesting antemortem significant hypokalemia. There were no biochemical signs of severe hypoglycemia and dehydration. As cardiac disease is a significant cause of death in AN, aggravation caused by concurrent biochemical derangement must be taken into account. It is possible that gastric dilatation has a role in the occurrence of death, but this remains questionable.

Conclusion: In this case, the possible mechanism of death could have been a disruption of heart rhythm in an arrhythmogenic state due to an electrolyte imbalance or vagal activation related to gastric dilatation.

Key words: anorexia nervosa, gastric dilatation, arrhythmia, sudden death, autopsy

Cite this article as: Bogdanović M, Popović V, Atanasijević T, Radnić B, Petrović T, Milošević V. Sudden death in anorexia nervosa: exploring the mechanism of death; Medicinska istaživanja 2024; 57(3):167-171 DOI: 10.5937/medi57-50143

INTRODUCTION

Anorexia nervosa (AN) is a severe chronic psychiatric disorder with often underestimated prevalence. The mortality rate of the patients suffering from AN from all causes of death is higher than that of the general population (1).

AN patients can die from natural causes, such as severe heart problems, organ failure, or malnutrition, as well as from unnatural causes, such as suicide. However, mechanism of death in AN is poorly understood (2, 3). Many patients with AN struggle with various digestive symptoms. In this case, we noticed gastric dilatation in autopsy. Since the first description of acute gastric dilatation in 1833, there has been an ample amount of cases documented in literature (4). Generally, the cause of gastric dilatation is difficult to pinpoint (5). This condition may be the result of mechanical and/or neurogenic factors and may appear in individuals with physical and mental impairment (AN). In addition to eating disorders, gastric dilatation has been reported to result from superior mesenteric artery syndrome, volvulus of hiatal hernias, trauma resuscitation, medications, air swallowing due to neuromuscular incoordination, diabetes mellitus, and other conditions (3,6,7,8,).

In this paper, we shall present a case of a young anorectic woman who died suddenly and discuss possible mechanisms of death.

CASE REPORT

A 22-year-old female was found dead in her bedroom bed. According to heteroanamnestic data, she had suffered from anorexia nervosa for five years. That day, according to her mother's statement, she had a meal for the first time in two days. She rapidly consumed porridge (approximately 400 ml) and one apple and she drank about 600 ml of tea. A few minutes later (without history of any symptoms) the woman lost consciousness and died. The autopsy was performed two days later.

The deceased was extremely underweight – she weighed only 23 kg and was 161 cm tall (BMI 8.9 kg/m2) **(Figure 1)**. There were no signs of external or internal injuries. The abdomen was not distended. Upon opening the abdominal cavity, extremely dilated stomach was noted **(Figure 2)**. It completely filled the abdominal and partially the pelvic cavity (extending from the xiphoid to the bladder). Its vertical diameter was around 40 cm, while its horizontal diameter amounted to around 30 cm. When the stomach was opened, it revealed around 1.2 l of undigested content. The gastric wall was of usual color, without necrosis. The gallbladder was highly distended. The intestines contained a small amount of semisolid fecal content. There were no obstructions, ascites, peritonitis or depositions of fibrin in the digestive tract. The diaphragm was not elevated. All organs were small in size, yet without obvious signs of dehydration.

Figure 1. The patient's body was poorly nourished with body mass of only 21 kg (BMI 8,6 kg/m^2).

Toxicological testing was negative for alcohol and drugs. Biochemical findings showed vitreous chloride, sodium and potassium in the following concentrations: 78 mmol/l, 116 mmol/l and 9.86 mmol/l, respectively. The vitreous glucose, and urea levels were 4.1 mmol/l and 28 mmol/l, respectively; no ketones were found.

Finally, the underlying cause of death was AN, and the possible mechanism of death could have been a disruption of heart rhythm due to an electrolyte imbalance or vagal activation related to gastric dilatation.

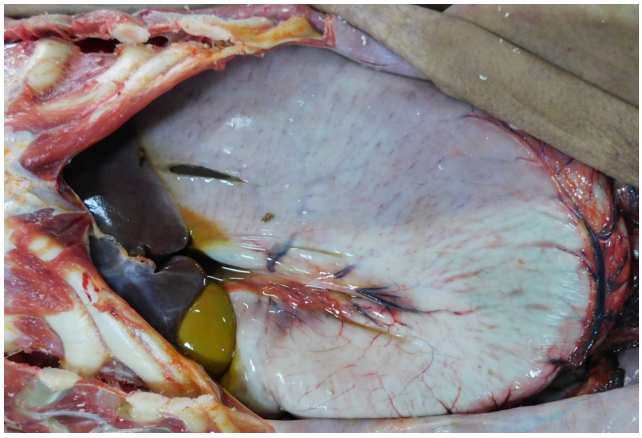


Figure 2. Abnormally distended stomach.

DISCUSSION

Long-term complications of AN include cardiovascular, gastrointestinal, endocrinological, hematological, psychiatric, fertility and pregnancy disorders (9). Recognized cardiovascular complications in these patients include numerous structural, hemodynamic, conduction, repolarization and peripheral vascular changes (9). Although ischemic heart disease and acute coronary syndrome are sparsely described in AN patients, definitive data in support of an increased risk of atherosclerotic vascular disease in anorexia nervosa population are lacking. Literature does suggest that anorexia nervosa could be associated with sudden death (8, 11). As precise etiology behind the increased risk of sudden cardiac death in anorexia nervosa remains unclear, the prolongation of the QT interval has been suggested as a potential cause because of its well-known association with torsade de pointes (12, 13). However, more recently it has been reported that anorexia nervosa might not be inherently associated with QT prolongation; rather, when it is present on electrocardiography, it should point towards secondary causes, such as electrolyte aberrations (hypokalemia, hypomagnesemia), or medications known to prolong the QT interval (9). Therefore, cardiac disease is a significant cause of death in AN, aggravated by concurrent biochemical derangement due to poor food intake and/or purging. That being said, arrhythmogenic complications due to severe hypokalemia that was proven in this case cannot be excluded and, may have, indeed, played a significant role in causing the young woman's death. Furthermore, autonomic dysfunction in AN has been in the focus of numerous studies. Some researchers showed that the cardiac vagal tone was higher in AN patients compared to the healthy control group (14). However, autonomic dysfunction and heart rhythm disorders cannot be proven in a postmortem examination.

In case of starvation, the immediate cause of death could be dehydration. Hypoglycemic coma is also reported in literature as one of the immediate causes of death (15). The vitreous levels of sodium, chloride and potassium, glucose level and ketones were measured and, even though it is well-known that the concentrations of many natural chemical substances in the corpse are rapidly distorted by postmortem autolysis (16), certain postmortem values of electrolytes might be indicative of their antemortem levels. The concentrations of sodium and chloride decrease after death, while the potassium level rises. Elevated vitreous levels of chloride (>135 mmol/l) and sodium (>155mmol/l) are indicative of antemortem dehydration, which is not the case in this paper. The level of potassium was low, even for a 2-day postmortem period, suggesting antemortem hypokalemia that, as we have previously stated, could have been the cause of arrhythmia. Vitreous glucose level was 4.1 mmol/l, while the analysis showed no ketones. In relation to hypoglycemia, vitreous glucose level below 1.4 mmol/l was taken by Sturner et all. to be an indication of low antemortem glucose level (16).

Anemia, leukopenia and thrombocytopenia are common findings in AN patients. In those patients, the diagnosis of infection could be delayed due to the absence of inflammatory and febrile response. In our case, we did not find any signs of infection (17).

In this case, the finding of gastric dilatation was very interesting. Acute gastric dilatation is recognized and described as gastrointestinal complication of AN (8). The symptoms of gastric dilatation can be vague, patients often present with emesis and gradual abdominal distention with pain, but in this case there were no data about prominent gastrointestinal symptomatology.

Acute gastric dilatation is defined as a condition in which the gastric wall rapidly loses its tension, and the gastric lumen is filled with gas and secretions, leading to rapid distention in the absence of structural obstruction in the stomach and/or duodenum (18). In the presented case, there was no structural obstruction in the stomach and/or duodenum. Literature describes deaths related to severe stomach dilatation followed by wall necrosis. In the described cases, high intragastric pressure overwhelms gastric venous pressure, producing ischemia, necrosis and finally the perforation of the gastric wall (8,11). In the presented case, there were no such complications. Fatalities following acute gastric dilatation without wall necrosis and rupture are rarely described and discussed, which is why the question is raised about the cause of death in those cases. Acute gastric dilatation accompanied by in-

creased intra-abdominal pressure and compression of the inferior vena cava may result in the congestion of bilateral lower limbs. In the case reported by Sincina, the inferior vena cava was compressed between dilated duodenum and vertebral bodies (19). Overextension of the gastric wall could induce peritoneal stimulation and vasovagal reflex, contributing to neurogenic shock (11, 19, 20). This is an example of distributive shock resulting from imbalance of sympathetic and parasympathetic regulation of vascular smooth muscles and heart rate. Also, acute gastric distension accompanied with increased intra-abdominal pressure could lead to diaphragmatic elevation and consequent limitation of respiratory movement. Space-restrictive disturbances in the thoracic cavity may also lead to the fatal outcome (6, 21). Therefore, all of the above suggests that acute gastric dilatation could be considered the cause of sudden death. However, the patient presented in this paper did not have any of the previously described complications. The role of gastric dilatation in the occurrence of death remains questionable.

The cause and mode of death in the presented case can be discussed. The absence of peritoneal irritation, ischemia and necrosis of the stomach wall, as well as fibrin deposits associated with liquid blood, suggest rapid death. It is possible that the previous heavy cachexia contributed to faster death; therefore, "there was not enough time" for the development of intestine necrosis and subsequent perforation and peritonitis.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Science of the Republic of Serbia, Grant No. 175093.

CONCLUSION

Finally, even though abnormalities in the heart rhythm cannot be proven postmortem, arrhythmogenic state caused by hypokalemia, could be considered as the mechanism of death in the presented case. Although the role of gastric dilatation in this case remains unclear, it could theoretically contribute to the occurrence of death. This possibility should be considered when determining the cause and manner of death in similar cases in the future.

Ethical approval

This article does not include any studies involving human participants or animals conducted by the authors.

Author contributions

All authors have evenly contributed to the conception of the work, interpretation of data and preparing the draft of the manuscript or interpretation of revised version of the manuscript.

REFERENCES

1. Meczekalski B, Podfigurna-Stopa A, Katulski K. Long-term consequences of anorexia nervosa. Maturitas. 2013 Jul;75(3):215-20. doi: 10.1016/j.maturitas.2013.04.014. PMID: 23706279.
2. Derman T, Szabo CP. Why do individuals with anorexia die? A case of sudden death. Int J Eat Disord. 2006 Apr;39(3):260-2. doi: 10.1002/eat.20229. PMID: 16485270.
3. Norris ML, Harrison ME, Isserlin L, Robinson A, Feder S, Sampson M. Gastrointestinal complications associated with anorexia nervosa: A systematic review. Int J Eat Disord. 2016 Mar;49(3):216-37. doi: 10.1002/eat.22462. PMID: 26407541.
4. Byard RW, Couper RT, Cohle S. Gastric distension, cerebral palsy and unexpected death. J Clin Forensic Med. 2001 Jun;8(2):81-5. doi: 10.1054/jcfm.2001.0478. PMID: 16083676.
5. Omond KJ, Byard RW. Lethal mechanisms in gastric volvulus. Med Sci Law. 2017 Jan;57(1):39-41. doi: 10.1177/0025802416689764. PMID: 28094603.
6. Steen S, Lamont J, Petrey L. Acute gastric dilation and ischemia secondary to small bowel obstruction. Proc (Bayl Univ Med Cent). 2008 Jan;21(1):15-7. doi: 10.1080/08998280.2008.11928348. PMID: 18209748; PMCID: PMC2190544.
7. Liu TH, Mehall JR, Mercer DW. Image of the month. Acute gastric dilation with necrosis. Arch Surg. 2001 Dec;136(12):1437-8. doi: 10.1001/archsurg.136.12.1437. PMID: 11735876.
8. Todd SR, Marshall GT, Tyroch AH. Acute gastric dilatation revisited. Am Surg. 2000 Aug;66(8):709-10. PMID: 10966022.
9. Sachs KV, Harnke B, Mehler PS, Krantz MJ. Cardiovascular complications of anorexia nervosa: A systematic review. Int J Eat Disord. 2016 Mar;49(3):238-48. doi: 10.1002/eat.22481. PMID: 26710932.
10. Bohnert M. Death as a Result of Starvation: Diagnostic Criteria. In: Tsokos M, editor. Forensic pathology reviews, Vol. 2. Totowa, NJ: Humana Press, 2005;3–27.
11. Watanabe S, Terazawa K, Asari M, Matsubara K, Shiono H, Shimizu K. An autopsy case of sudden death due to acute gastric dilatation without rupture. Forensic Sci Int. 2008 Sep 18;180(2-3):e6-e10. doi: 10.1016/j.forsciint.2008.07.005. PMID: 18757145.
12. Neumärker KJ. Mortality and sudden death in anorexia nervosa. Int J Eat Disord. 1997 Apr;21(3):205-12. doi: 10.1002/(sici)1098-108x(199704)21:3<205::aid-eat1>3.0.co;2-o. PMID: 9097194.
13. Isager T, Brinch M, Kreiner S, Tolstrup K. Death and relapse in anorexia nervosa: survival analysis of 151 cases. J Psychiatr Res. 1985;19(2-3):515-21. doi: 10.1016/0022-3956(85)90061-5. PMID: 4045762.
14. Kollai M, Bonyhay I, Jokkel G, Szonyi L. Cardiac vagal hyperactivity in adolescent anorexia nervosa. Eur Heart J. 1994 Aug;15(8):1113-8. doi: 10.1093/oxfordjournals.eurheartj.a060636. PMID: 7988604.
15. Isner JM, Roberts WC, Heymsfield SB, Yager J. Anorexia nervosa and sudden death. Ann Intern Med. 1985 Jan;102(1):49-52. doi: 10.7326/0003-4819-102-1-49. PMID: 3966745.
16. Saukko P, Knight B. Knight's forensic pathology. 3rd ed.Oxford: Oxford University Press; 2004.
17. Westmoreland P, Krantz MJ, Mehler PS. Medical Complications of Anorexia Nervosa and Bulimia. Am J Med. 2016 Jan;129(1):30-7. doi: 10.1016/j.amjmed.2015.06.031. PMID: 26169883.
18. Murakami C, Irie W, Sasaki C, Nakamaru N, Sakamoto M, Nagato J, Satoh F. Extensive gastric necrosis secondary to acute gastric dilatation: A case report. Leg Med (Tokyo). 2019 Feb;36:85-88. doi: 10.1016/j.legalmed.2018.11.007. PMID: 30448603.

19. Sinicina I, Pankratz H, Büttner A, Mall G. Death due to neurogenic shock following gastric rupture in an anorexia nervosa patient. Forensic Sci Int. 2005 Dec 1;155(1):7-12. doi: 10.1016/j.forsciint.2004.10.021. PMID: 16216705.

20. Hmouda H, Jemni L, Jeridi G, Ernez-Hajri S, Ammar H. Unusual presentation of gastric dilatation. Dramatic complete atrioventricular block. Chest. 1994 Aug;106(2):634-6. doi: 10.1378/chest.106.2.634. PMID: 7774359.

21. Nakao A, Isozaki H, Iwagaki H, Kanagawa T, Takakura N, Tanaka N. Gastric perforation caused by a bulimic attack in an anorexia nervosa patient: report of a case. Surg Today. 2000;30(5):435-7. doi: 10.1007/s005950050618. PMID: 10819480.

NAPRASNA SMRT U SLUČAJU ANOREKSIJE NERVOZE: RAZMATRANJE MEHANIZAMA UMIRANJA

Bogdanović Milenko[1], Popović Vesna[1], Atanasijević Tatjana[1], Radnić Bojana[1], Petrović Tijana[1], Milošević Veljko[1]

Sažetak

Uvod: Anoreksija nervoza je teško hronično psihijatrijsko oboljenje čija tačna učestalost nije poznata. Pacijenti sa anoreksijom nervozom mogu umreti od prirodnih uzroka smrti, poput kardiovaskularnih bolesti, popuštanja organa, pothranjenosti, ili usled nasilnih uzroka smrti, kao što je samoubistvo. U svakom slučaju, mehanizam umiranja kod anoreksije nervoze nije do kraja istražen.

Prikaz slučaja: Opisujemo slučaj iznenadne smrti mlade žene stare 22 godine koja je bolovala od anoreksije nervoze. Na obdukciji je pokazana teška pothranjenost. Neposredno po otvaranju trbušne duplje uočeno je da je želudac izrazito dilatiran (bez postojanja znakova nekroze ili rupture). Biohemijskom analizom uzorka tečnosti staklastog tela pokazano je da su vrednosti hlorida, natrijuma i kalijuma izrazito niske, ukazujući na značajnu antemortalnu hipokalijemiju. Biohemijski nisu pokazani znaci ozbiljnije hipoglikemije i dehidratacije. Imajući u vidu da su kardiovaskularne bolesti značajan uzrok smrti kod osoba sa anoreksijom nervozom, pogoršanje kardiovaskularne bolesti je moglo biti izazvano poremećajem elektrolita što se mora uzeti u razmatranje. Potencijalno je moguć uticaj dilatacije želuca na pogoršanje kardiovaskularne bolesti i smrtni ishod i kao takvog ga treba uzeti u razmatranje, ali ovo ostaje nejasno.

Zaključak: Mogući mehanizam umiranja u ovom slučaju bi mogao biti poremećaj srčanog ritma nastao usled proaritmogenog stanja izazvanog poremećajem elektrolita ili usled vagalne aktivacije izazvane dilatiranim želucem.

Ključne reči: anoreksija nervoza, dilatacija želuca, aritmija, iznenadna smrt, obdukcija

Primljen: 30.03.2024. I **Revizija:** 13.05.2024. I **Prihvaćen:** 15.05.2024.

Medicinska istaživanja 2024; 57(3):167-171

Izdavač i vlasnik | Publisher and owner
Medicinski fakultet Univerziteta u Beogradu

Uredništvo i administracija | Editorial board and administration
11105 Beograd, Dr Subotića br. 8, soba 311

Tehnički urednik | Technical editor
Radević Vladimir

Lektor za engleski jezik | English language editor
doc. dr Danka Sinadinović

Tehnički sekretar | Technical secretary
Dragana Popović

Grafički dizajn | Graphic design
Prof. dr Slobodan Štetić
Fakultet pedagoških nauka u Jagodini Univerziteta u Kragujevcu