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Novi Evropski vodič za prevenciju tromboembolizma kod A Fib

CHA_2DS_2-VASc skor za procjenu rizika od tromboembolizma kod A Fib!

Risk factor-based point-based scoring system - CHA_2DS_2 -VASc

Risk factor	Score
Congestive heart failure/LV dysfunction	1
Hypertension	1
Age ≥75	2
Diabetes mellitus	1
Stroke/TIA/thrombo-embolism	2
Vascular disease*	1
Age 65–74	1
Sex category (i.e. female sex)	1
Maximum score	**9**

*Prior myocardial infarction, peripheral artery disease, aortic plaque. Actual rates of stroke in contemporary cohorts may vary from these estimates.

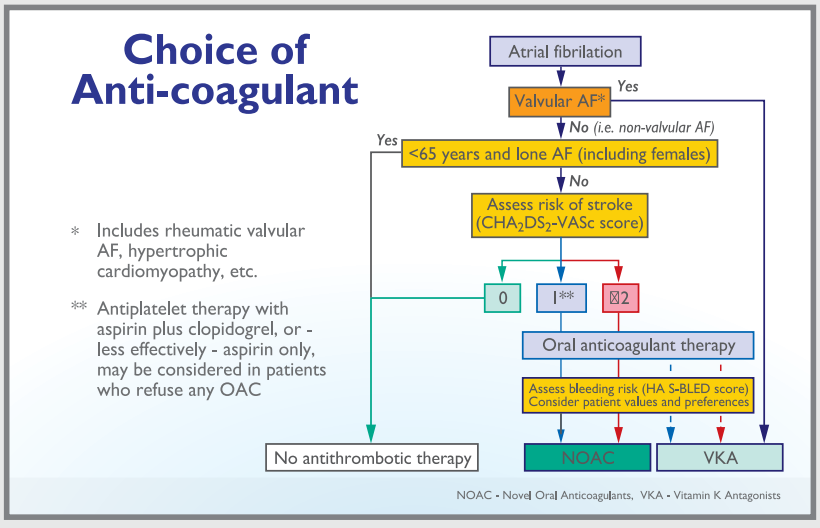
Major i non-major riziko fakori za procjenu tromboembolizma kod A Fib!

Risk factors for stroke and thrombo-embolism in non-valvular AF

Major risk factors	Clinically relevant non-major risk factors
Previous stroke	CHF or moderate to severe LV systolic dysfunction [e.g. LV EF ⊠ 40%]
TIA or systemic embolism	Hypertension
Age ⊠75 years	Diabetes mellitus
	Age 65-74 years
	Female sex
	Vascular disease

AF = atrial fibrilation; EF = ejection fraction (as documented by echocardiography, radio nudide ventriculography, cardiac catheterization, cardiac magnetic resonance imaging, etc.); LV = left venticular; TIA = trasient ischaemic attack.

Algoritam antikoagulantne terapije nakon procjene CHA_2DS_2VASc i major risk faktora!



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AIMS AND SCOPE

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Content

Original articles

Professional articles

Case reports

Characteristics of isolated and combined hand injuries in relation to etiological factors, fracture level and age distribution

Karakteristike izolovanih i kombinovanih povreda šake u odnosu na etiološke faktore, nivo frakturne lezije i dobnu distribuciju

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ABSTRACT

Introduction: acute hand trauma, as a significant segment of traumatology, carries the potential for combined bone and soft tissue lesions, which complicates reconstruction and affects the quality of postoperative recovery. Aim: to assess the presence of combined bone-soft tissue lesions of the hand to etiological factors, fracture level, and age distribution. Materials and methods: we assessed 138 cases of complex acute hand trauma treated at the Clinic of Reconstructive and Plastic Surgery, Clinical Center University of Sarajevo, in the period from 2015 to 2020, with the evaluation of etiological factors, the prevalence of combined bone-soft tissue lesions, their presence concerning to the fracture level and estimated age groups. Results: the most common etiological factor was machines (67 cases; 48.5%). Evaluation of isolated and combined hand fractures to estimated anatomical levels confirmed statistically significant differences (p=0.229; p=0.007). No statistically significant difference was found in terms of fracture levels and the pr esence of associated injuries (p=0.312). Statistical significance was not determined in the estimated age groups according to etiological factors and isolated or combined fractures (p=0.256). Conclusion: combined bone-soft tissue lesions of the hand are a potentially more complex problem from the reconstructive aspect. Clinical evaluation should be based on the assessment of the extent of tissue destruction concerning the etiological factor, and the definition of clinical guidelines for the selection of the reconstructive modality. Age distribution related to the predominance of specific etiological factors creates a greater possibility of potential exposure to certain etiological factors in the working-age population.

Keywords: hand injuries, etiologic factors, associated injury, age group

SAŽETAK

Uvod: akutna trauma šake kao značajan segment traumatologije nosi potencijalnu mogućnost kombinovanih koštano-mekotkivnih lezija, što usložnjava rekonstrukciju i utječe na kvalitet postoperativnog oporavka. Cilj: procijeniti prisutnost kombiniranih koštano-mekotkivnih lezija šake u odnosu na etiološke faktore, frakturni nivo i dobnu distribuciju. Materijal i metode: Evaluirali smo 138 slučajeva složene akutne traume šake, liječenih na Klinici za rekonstruktivnu i plastičnu kirurgiju Kliničkog centra Univerziteta u Sarajevu, u razdoblju od 2015. do 2020. godine, sa evaluacijom etiološkog faktora, zastupljenosti kombiniranih koštano-mekotikvnih lezija, njihovo prisustvo u odnosu na frakturni nivo i procjenjene dobne skupine. Rezultati: najčešći etiološki faktor su bile radne mašine (67 slučajeva; 48,5%). Evaluacija izoliranih i kombiniranih fraktura šake u odnosu na anatomske nivoe lezije procijenjene anatomske nivoe potvrdila je statistički značajnu razliku (p=0.229; p= 0.007). Nisu pronađene statistički značajne razlike između anatomskog nivoa lezije i prisustva kombiniranih lezija drugih anatomskih struktura (p=0.312). Statistička značajnost nije utvrđena u procjenjenim dobnim skupinama prema etiološkim faktorima i izoliranim, odnosno kombiniranim frakturama (p=0.256). Zaključak: kombinirane koštano-mekotkivne lezije šake su sa rekonstruktivnog aspekta potencijalno složeniji problem. Klinička evaluacija se treba bazirati na procjeni opsega destrukcije tkiva vezanog za dejstvo etiološkog faktora, te definiranju kliničkih smjernica za selekciju rekonstruktivnog modaliteta. Dobna distribucija vezana za prevagu specifičnih etioloških faktora stvara veću mogućnost potencijalne ekspozicije određenim etiološkim faktorima kod radno sposobnog stanovništva.

Ključne riječi: povrede šake, etiološki faktori, kombinirana povreda, dobna skupina

INTRODUCTION

Acute hand trauma occupies a significant segment in traumatology and it is necessary to observe this segment of surgery from the point of a potentially complex lesion with a significant effect on the quality of life and disability (1). Maintaining hand functionality after the injury is the surgeon's ultimate priority from the perspective of the unique biomechanical characteristics of the hand (2). Although

potential etiological factors almost always cause a combined bone-soft tissue lesion, stable osteosynthesis at any level is important for satisfactory functional recovery, early mobilization is often a priority when it comes to sports injuries (3). Preoperative assessment of the extent of hand injury is very important, as well as proper indications for surgical treatment (4).

The main goal of each surgical procedure is to achieve the most optimal postoperative result and functionality (5). Isolated bone injuries, which typically involve a small percentage of soft tissue structures, are generally an indication for conservative treatment (6), with maintained functionality, as the ultimate criterion (5,7). Taking into account the fact that phalangeal and metacarpal fractures were often associated with injuries of the flexor and extensor tendons, which can lead to subsequent tendon rupture (8), surgical treatment has to provide the maintenance of the sliding mechanism as a prerequisite for functionality (9).

AIM

To assess the prevalence of complex hand injuries in terms of the existence of combined bone-soft tissue lesions, their correlation to etiological factors, fracture levels, and estimated age groups.

MATERIALS AND METHODS

We assessed 138 patients with complex hand trauma, who were diagnosed and treated with phalangeal, metacarpal, and carpal fractures assessed to etiological factors, potential associated soft tissue injuries, and the age distribution in the Clinic of Reconstructive and Plastic Surgery, Clinical Center University of Sarajevo, during the period from 2015 to 2020. All patients underwent clinical examination for functional failure and standard hand X-ray for assessment of bone injuries, as a diagnostic method of choice. The complex injury was assessed to etiological factors, the corresponding fracture level, and age distribution. Statistical data processing was done through IBM SPSS (SPSS-Statistical Package for Social Sciences) version 25.0 and Microsoft Excel computer program (Microsoft Office 2010). The results were shown in the graphs. The data were analyzed using X^2 and Fischer test. The $p<0.05$ was used as statistically significant.

Study inclusion criteria: patients of all ages and gender with acute hand trauma and combined bone-soft tissue lesions; patients with combined bone-soft tissue lesions of phalangeal, metacarpal, and carpal level.

Study exclusion criteria: patients primarily treated in other hospital facilities, cases without the possibility of adequate postoperative evaluation.

RESULTS

Various etiological factors are associated with the resulting fracture lesions of the hand (Figure 1). In our estimated group of 138 patients, we evaluated the predominance of hand injuries with working machines, 67 cases (48.55%), followed by sharp and heavy objects injuries, 58 cases (42%) and explosive injuries, 6 cases (4.35%). Contusion and traffic accidents injuries were represented in a significantly smaller number of cases, 5 (3.7%) and 2 (1.4%) cases, respectively.

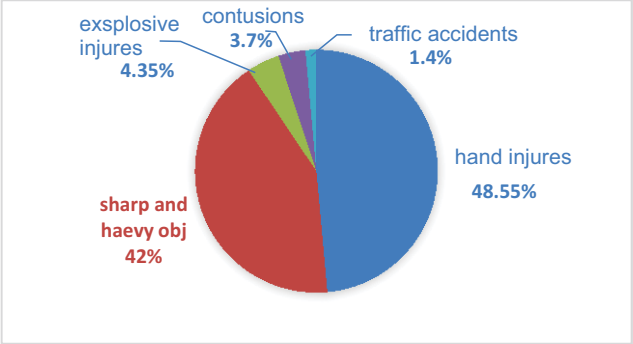

Figure 1 **Representation of etiological factors.**

Fracture lesions of the hand in most cases were combined with lesions of the adjacent soft tissue structures (Figure 2). In our estimated the combined fracture lesions were predominant, 129 cases (93.4%), compared to isolated fracture lesions, 9 cases (6.6%).

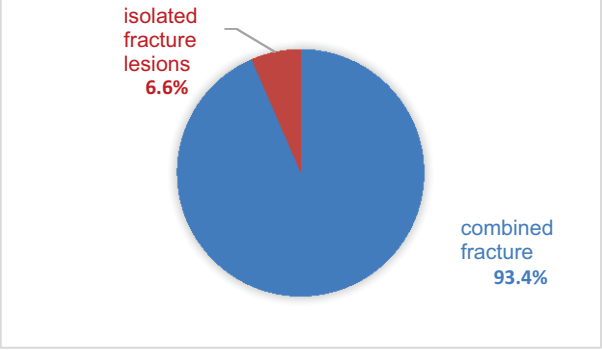

Figure 2 **Percentage distribution of combined and isolated hand fractures.**

The estimated age groups were with variable distribution of etiological factors, the largest number of injuries was represented in the age groups of 24-44 years and 45-64 years, which corresponded to the working population. Concerning the etiological factors, injuries to various facilities and working machines were the most common (Figure 3).

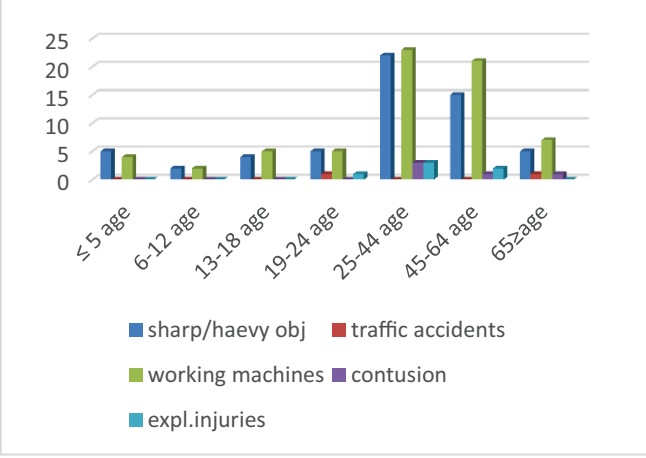

Figure 3 **Estimated age-group distribution of etiologic factors.**

The distribution of combined and isolated bone lesions was assessed according to the age groups of the patients (Figure 4) and it was found that most hand fractures have occurred in the adult population aged 25-44 years (37%), followed by the adult population aged 45-64 years (29%). In all age groups, the incidence of combined fractures was higher compared to the incidence of isolated fractures. The highest frequency of isolated fractures was observed in the population of children aged 6 -12 years (25%), followed by the population of young adults aged 19-24 years (17%), and in the adult population aged 25-44 years (8%). No isolated fractures were observed in the population of infants and preschool children less than 5 years of age, as well as in adolescents aged 13-18 years. Statistical significance was not confirmed concerning the age groups correlated with etiological factors and the presence of combined or isolated fracture lesions (p=0.256)

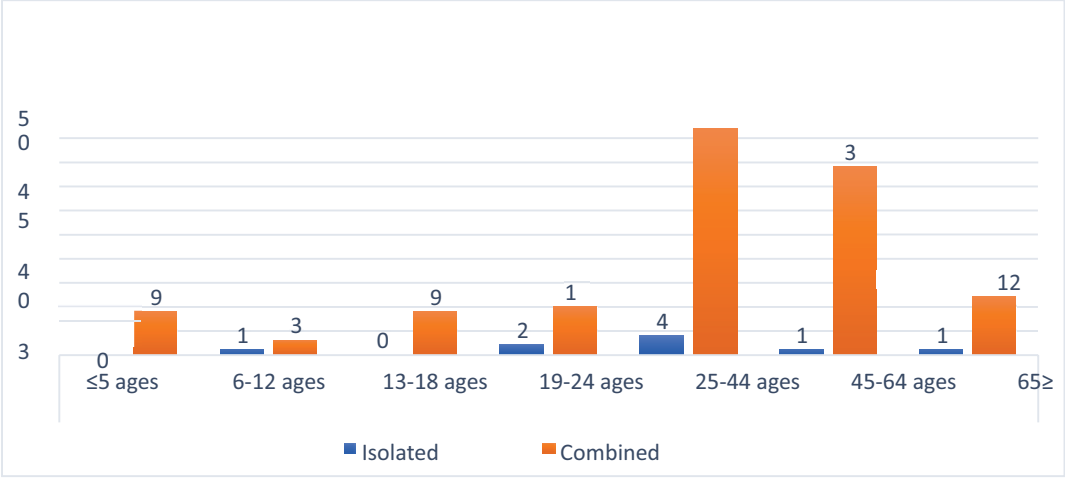

Figure 4 **Distribution of combined and isolated hand fractures estimated by age groups.**

Evaluation of the anatomical distribution of injury levels (Figure 5), we estimated that the phalangeal level has been the most common localization of fracture injuries, 112 cases (81%), followed by metacarpal level, 22 cases (16%). Carpal level and combined digital and metacarpal level were represented in 2 cases each (1%) in our estimated group of 138 patients.

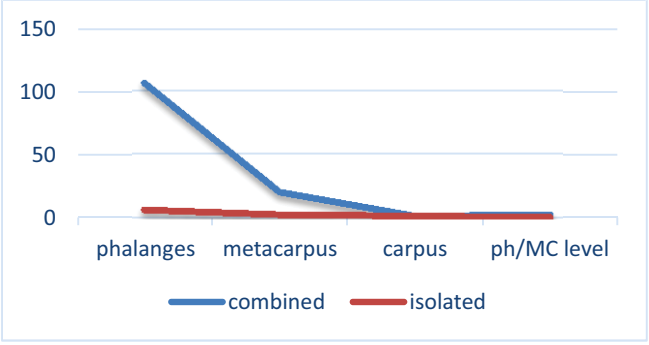

Figure 5 **Numerical distribution of combined and isolated fractures to an anatomical level estimated.**

Distribution of isolated fractures to the estimated anatomical level of injuries evaluated the highest percentage of isolated fracture lesions at phalangeal level (67%), followed by metacarpal level (22%), while the lowest percentage of isolated fracture injuries was at the carpal level. Combined phalangeal-metacarpal bone lesions were not confirmed in our study group due to the high probability of combined bone-soft tissue lesions (Figure 6). Distribution of isolated hand fractures to evaluated anatomical level confirmed statistically significant difference (p = 0.229).

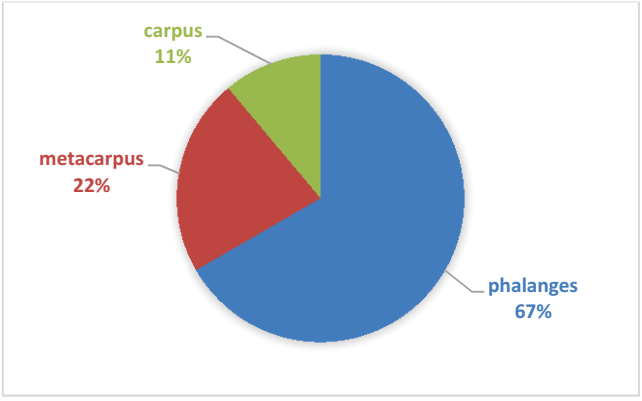

Figure 6 **Proportion of isolated fractures to an estimated anatomical level presented in percentages.**

The phalangeal level was the anatomical localization with the highest percentage of combined fractures (82%), followed by the metacarpal (15%) and combined phalangeal-metacarpal level (2%). Carpal lesions were present in a small number of cases (1%), although this level, due to its anatomical characteristics is associated with a high probability of combined lesions, which was not the case in our study group. Distribution of combined hand fractures to evaluated anatomical level confirmed statistically significant difference (p=0.007).

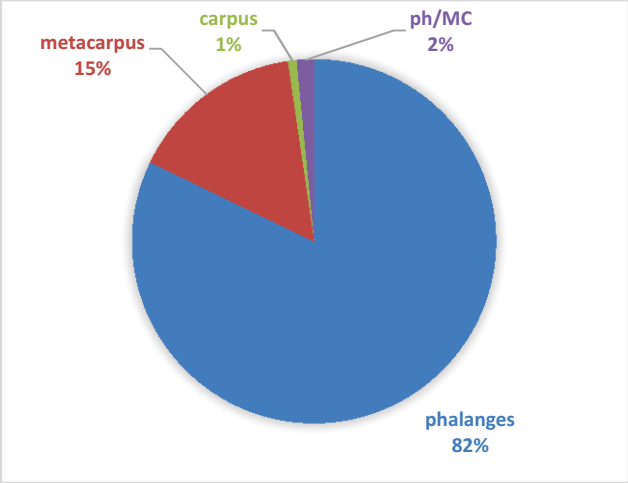

Figure 7 **Proportion of combined fractures to an estimated anatomical level presented in percentages.**

No statistically significant difference was found in terms of fracture levels and the presence of associated injuries (p=0.312).

DISCUSSION

Hand injuries represent an important segment in overall traumatology (10), with the necessity of timely reconstruction principles, taking into account the potential association of soft tissue and bone lesions and the reconstruction complex (11), whose choice must be adapted to each case (12). During the evaluation of the bone injury, it is important to consider the etiology as well, because various etiological factors, such as sports injuries, traffic accidents, fights injuries, participate in varying degrees of bone and soft tissue lesions (13).

Regardless of the type of etiological factor and potential destructiveness to tissues, the choice of an appropriate reconstructive procedure is mandatory for achieving the best postoperative results in terms of reduction of the potential scarring and reduced functionality due to postoperative contractures, regarding intraoperative soft tissue manipulation, despite existing soft tissue lesions, can affect scarring and reduction of ultimate functionality (14). Timely treatment and early mobilization protocols achieve rapid recovery and return to work, which is the result of appropriate preoperative assessment and treatment. (15). A meticulous surgical approach with experience in predicting all potential complications and how to deal with them is mandatory (16). The higher prevalence of combined to isolated bone injuries at all fracture levels requires special consideration (17). The level of injury does not correlate with the extent of combined soft tissue lesions, (18), but the mode of resolution depends on the surgeon's preferences, although no type of osteosynthesis is superior to others (19). Due to the need for additional surgical exploration, it is very important to apply less invasive surgical exploration with reduced tissue irritation, both in combined and isolated lesions (20). Considering to aspect of etiological factors, which rarely, except in the case of contusion injuries, result in isolated fractures, associated neurovascular and soft tissue lesions are regularly present in hand traumatology, with potential multiphase reconstructive procedures (21), so that it is almost impossible to separate bone injuries as an isolated entity (22).

The potential bone and soft tissue combined injuries require a serious approach to reconstruction, with the ultimate principle of a rapid return to functionality and social reintegration whenever possible (23). The distribution of etiology and combination of bone lesions of the hand is variable, but there is an evident higher prevalence of sports injuries and traffic accidents in young adult age groups, while older life groups are exposed to etiological factors of lower energy levels (24).

CONCLUSION

During the clinical evaluation of acute hand trauma, it is important to consider the potential complexity of the lesion, i.e., the combined lesion of bone and soft tissue elements, and its higher probability of isolated lesions. Complex hand lesions complicate reconstruction, carrying a greater possibility of complications and secondary operations. The choice of the most optimal reconstructive procedure is mandatory for the quality of postoperative recovery, disability reduction, and quality of life.

REFERENCES

1. Pulos N, Kakar S. Hand and wrist injuries: common problems and solutions. Clin Sports Med. 2018;37(2):217-43.
2. Omid R, Stone MA, Zalavras CG, Marecek GS. Gunshot wounds to the upper extremity. J Am Acad Orthop Surg. 2019;27(7):301-10.
3. Cotterell IH, Richard MJ. Metacarpal and phalangeal fractures in athletes. Clin Sports Med. 2015;34(1):69-98.
4. Wahl EP, Richard MJ. Management of metacarpal and phalangeal fractures in the athlete. Clin Sports Med. 2020;39(2):401- 22.
5. Oberreiter B, Kilgus M, Bodmer E, Platz A. Osteosynthesis of metacarpal fractures with 2.0 mm LCP system and functional postoperative treatment. Unfallchirurg. 2017;120(1):40-5.
6. Nellans KW, Chung KC. Pediatric hand fractures. Hand Clin. 2013;29(4):569-78.
7. Ahmed Z, Haider MI, Buzdar MI, Bakht Chugtai B, Rashid M, Hussain N. Comparison of miniplate and K-wire in the treatment of metacarpal and phalangeal fractures. Cureus. 2020;12(2):7039.
8. Jeong SH, Gu JH, Han SK, Kim WK. Two-staged tendon reconstruction in flexor tendon ruptures secondary to fracture of the hamate hook. Ann Plast Surg. 2012;69(2):157-60.
9. Lögters TT, Lee HH, Gehrmann S, Windolf J, Kaufmann RA. Proximal phalanx fracture management. Hand (N Y). 2018;13(4):376-83.
10. Ciclamini D, Panero B, Titolo P, Tos P, Battiston B. Particularities of Hand and Wrist Complex Injuries in Polytrauma Management. Injury. 2014;45(2):448-51.
11. Adani R, Tarallo L, Caccese AF, Delcroix L, Cardin -Langlois E, Innocenti M. Microsurgical soft tissue and bone transfers in complex hand trauma. Clin Plast Surg. 2014;41(3):361-83.
12. Vester H, Deiler S. Strategies for complex injuries of the hand. Unfallchirurg. 2017;120(3):237-51.
13. Lempesis V, Rosengren BE, Landin L, Tiderius CJ, Karlsson MK. Hand fracture epidemiology and etiology in children-time trends in Malmö, Sweden, during six decades. J Orthop Surg Res. 2019;14(1):213.
14. Taghinia AH, Talbot SG. Phalangeal and metacarpal fractures. Clin Plast Surg. 2019; 46(3):415-23.
15. Byrne B, Jacques A, Gurfinkel R. Non-surgical management of isolated proximal phalangeal fractures with immediate mobilization. J Hand Surg Eur Vol. 2020;45(2):126-30.
16. Henry MH. Fractures of the proximal phalanx and metacarpals in the hand: preferred methods of stabilization. J Am Acad Orthop Surg. 2008;16(10):586-95.
17. Gaston RG, Chaderdon C. Phalangeal fractures: displaced/nondisplaced. Hand Clin. 2012;28(3):395-401.
18. Köse A, Topal M, Engin MÇ, Şencan A, Dinçer R, Baran T. Comparison of low - profile plate-screw and Kirschner-wire osteosynthesis outcomes in extra-articular unstable proximal phalangeal fractures. Eur J Orthop Surg Traumatol. 2019;29(3):597-604.
19. Palibrk TD, Lesić AR, Anđelković SZ, Milošević I, Stefanović RB, Milutinović SM, et al. Operative treatment of metacarpal and phalangeal fractures with Kirschner wire fixation - a review. Acta Chir Iugosl. 2013;60(2):49-52.
20. Pandey R, Soni N, Bhayana H, Malhotra R, Pankaj A, Arora SS. Hand function outcome in closed small bone fractures treated by open reduction and internal fixation by miniplate or closed crossed pinning: a randomized controlled trial. Musculoskelet Surg. 2019;103(1):99-105.

21. Ferreira J, Fowler JR. Management of complications relating to complex traumatic hand injuries. Hand Clin. 2015;31(2):311-7.
22. Refromat DD, Nores GG, Lam G, Cuzzone D, Hill JB, Muresan H, Thanik V. Outcome analysis of metacarpal and phalangeal fixation techniques at Bellevue hospital. Ann Plast Surg. 2018;81(4):407-10.
23. Lahiri A. Managing mutilating hand injuries. Clin Plast Surg. 2019;46(3):351-57.
24. MacIntyre NJ, Dewan N. Epidemiology of distal radius fractures and factors predicting risk and prognosis. J Hand Ther. 2016;29(2):136-45.

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Correlation between preoperative serum C-reactive protein values and the Nottingham prognostic index score in early invasive breast cancer

Korelacija preoperativnih vrijednosti C-reaktivnog proteina u serumu i skora Notthingham prognostičkog indeksa kod ranog invazivnog karcinoma dojke

Sadat Pušina[1*], Emir Bičakčić[1], Mirhan Salibašić[1], Edin Hodžić[1], Lamija Zečević[2], Emsad Halilović[1], Salem Bajramagić[1]

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ABSTRACT

Introduction: breast cancer is a disease that continues to plague women throughout their lives. C-reactive protein (CRP) has been found to be elevated in a variety of inflammatory and malignancies and its level has been found to correlate with prognostic and predicted breast cancer variables. Aim: to determine the preoperative serum levels of CRP in breast carcinoma, and to correlate them with the Nottingham prognostic index. Materials and methods: a total of 71 patients were included in this retrospective, descriptive-analytical study. Serum CRP levels were assessed using an enzyme-linked immunosorbent assay (ELISA), and CRP was measured by immunoturbidimetry. Histological findings included tumor size, lymph node metastases (LN), and histological staging. Statistical analysis was made in the IBM SPSS Statistics v. 21.0 for Windows, and the most important results are presented in the form of tables and graphs. Results: CRP levels are not statistically significantly correlated with the Nottingham prognostic index. Also, CRP values didn't show a statistically significant association with ALN metastases, tumor size and histological stage. Conclusion: serum CRP levels don't correlate statistically significantly with tumor extent, ALN metastases, histological grade in non-invasive breast cancer. The relationship between the Nottingham prognostic index and CRP didn't prove to be statistically significant.

Keywords: breast cancer, C-reactive protein, Notthigham prognostic score

SAŽETAK

Uvod: rak dojke je bolest koja i dalje muči žene tijekom njihovog života. Utvrđeno je da je C-reaktivni protein (CRP) povišen kod raznih upalnih i zloćudnih bolesti, a također je utvrđeno da njegova razina korelira s prognostičkim i prediktivnim varijablama raka dojke. Cilj: primarni cilj ove studije bio je odrediti preoperativne razine CRP-a u serumu kod pacijentica oboljelih od raka dojke i povezati ih s Nottinghamovim prognostičkim indeksom. Materijali i metode: u ovu retrospektivnu, opisno-analitičku studiju uključena je ukupno 71 pacijentica. Razine CRP-a u serumu procjenjivale su se pomoću enzimski povezanog imunosorbentnog testa (ELISA), a CRP je mjeren imunoturbidimetrijom. Histološki nalazi uključuju veličinu tumora, metastaze u limfnim čvorovima (LN) i histološki gradus. Statistička analiza izvršena je pomoću IBM SPSS Statistics v. 21.0 za Windows, a najvažniji rezultati predstavljeni su u obliku tabela i grafikona. Rezultati: razine CRP-a nisu statistički značajno povezane s Nottinghamovim prognostičkim indeksom. Također, vrijednosti CRP -a nisu pokazale statistički značajnu povezanost sa metastazama u aksilarnim limfnim čvorovima, veličinom tumora i histološkim gradusom tumora. Zaključak: razine CRP u serumu statistički značajno ne koreliraju s veličinom tumora, metastazama u aksilarnim limfnim čvorovima, kao niti sa histološkim gradusom neinvazivnog karcinoma dojke. Povezanost Nottinghamovog prognostičkog indeksa i CRP-a nije se pokazala statistički značajnom.

Ključne riječi: karcinom dojke, C-reaktivni protein, Notthigham prognostički skor

INTRODUCTION

Malignant breast tumors are the most common type of malignancy in women, and the fifth most common cause of death associated with malignancy (1).

There is much evidence linking chronic inflammation and the process of human carcinogenesis (2). One of the biomarkers of inflammation is the C-reactive protein of the acute phase of inflammation, the value of which is also increased in conditions of chronic inflammation, infection and tissue damage (3). However, while some authors report an increase in preoperative CRP

concentration in advanced cancers, at the same time the data related to the relationship between preoperative serum CRP values and early invasive breast cancer are contradictory (4-6).

Combining the status of axillary lymph nodes, tumor size and histological grade, as the most important individual prognostic parameters of breast cancer, the Nottingham Prognostic Index (NPI) was established, which stratifies all patients with breast cancer into three prognostic groups: good, medium and poor (7,8,9) (Table 1).

Table 1 **Nottingham prognostic index (NPI).**

*NPI	
Values	Prognosis
≤ 3,44	Good
3,45 – 5,4	Medium
> 5,5	Poor

* NPI = tumor size in cm x 0.2 + histological grade (1-3) + number of positive axillary lymph nodes (1 = 0 positive axillary lymph nodes; 2 = 1 to 3 positive axillary lymph nodes; 3 => of 3 positive axillary lymph nodes)

AIM

The aim of the study was to correlate preoperative blood C-reactive protein values with the score of the Nottingham prognostic index in early invasive breast cancer.

MATERIALS AND METHODS

The research in the form of a retrospective, descriptive-analytical study was conducted at the Clinic of General and Abdominal Surgery, Clinical Center University of Sarajevo (CCUS), in the period from September 2017 to April 2018. The study included 71 patients, aged 18 to 75 years, in whom primary invasive breast cancer was preoperatively patho-histologically verified by needle biopsy.

Surgical treatment was performed at the Clinic of General and Abdominal Surgery of CCUS, in the form of radical or sparing surgery on the breast, with or without dissection (I and II floors) of axillary lymph nodes.

To determine the value of C-reactive protein (CRP), the nephelometric method was used, on the SIEMENS BN II device (Munich, Germany) and the finding was interpreted in the unit of measurement mg / L. Nephelometry is a technique that determines the presence of antibodies and antigens in a patient's serum based on sample turbidity. Polystyrene particles, coated with monoclonal antibodies, specific for human CRP aggregate when mixed with samples containing CRP. These aggregates scatter rays of light passing through the sample. The light intensity is measured and compared to a standard known concentration, and thus used to determine the CRP value in the sample. Normal CRP (cut-off) was taken as ≤ 3.36 mg/L. The measurement was performed at Clinical Biochemistry Clinic with Immunology of the CCUS.

Patho-histological analysis of surgical resection of breast with tumor and axillary lymph nodes (one or more) was performed at Clinic of Clinical Pathology, Cytology and Human Genetics of the CCUS according to appropriate protocols.

The stage of the tumor was determined on the basis of the "TNM" classification, and according to the pathological (p) "TNM" classification of the American Cancer Society (10).

Postoperatively, all patients were monitored on an outpatient basis according to current protocols.

Statistical analysis was made in the IBM SPSS Statistics v. 21.0 for Windows, and the most important results are presented in the form of tables and graphs. The data were processed using the descriptive and analytical statistics methods, and we used the Chi-square test to prove the correlation between the variables. Multinomial Logistic Regression was used to examine the predictive relationship between the variables. The statistical significance was $p<0.05$.

RESULTS

Table 2 **Descriptive data analysis.**

		Mean	Standard Deviation	Minimum	Maximum	Count	Column N %
Age		62	10	42	84		
NPI risk assessment	Low risk					30	42.3%
	Medium risk					21	29.6%
	High risk					20	28.2%
Histological grade of tumor	Histological grade 1					14	19.7%
	Histological grade 2					28	39.4%
	Histological grade 3					29	40.8%
CRP (mg/L)	≤ 3,36					54	76.1%
	>3,37					17	23.9%
Tumor size (cm)	<1.99					29	40.8%
	>2.0 – 5.0					42	59.2%
Infiltration of axillary lymph nodes	No positive axillary lymph nodes					32	45.1%
	1-3 positive axillary lymph nodes					25	35.2%
	4-9 positive axillary lymph nodes					10	14.1%
	≥10 positive axillary lymph nodes					4	5.6%

The mean age of the patients was 62 years (+/- 10 SD). The youngest patient was 42 and the oldest 84 years old. A total of 30 (42.3%) patients had a low risk estimated by the NPI score, 21 (29.6%) a medium risk, and 20 (28.2%) patients a high risk. Histological grade 1 of the tumor had 14 (19.7%), histological grade 2 had 28 (39.4%), and histological grade 3 had 29 (40.8%) patients. Most subjects in the study had a CRP value ≤ 3.36 mg / L, namely, 54 (76.1%) patients, and 17 (23.7%) patients had a CRP > 3.36 mg / L. In relation to tumor size, 29 (40.8%) patients had a tumor < 2 cm, and 42 (59.2%) patients > 2 cm. According to the numerical representation of infiltrated axillary lymph nodes, the patients were divided into four groups. Of the total number of subjects, 32 (45.1%) patients didn't have positive axillary lymph nodes; 25 (35.2%) patients had positive 1-3 axillary lymph nodes; 10 (14.1%) patients had 4-9 positive axillary lymph nodes, and more than 10 positive axillary lymph nodes had 4 (5.6%) patients.

Table 3 **Correlation between serum CRP values and NPI, histological grade, tumor size, and axillary lymph node infiltration.**

		CRP (mg/L)							
		≤ 3,36			>3,36				
		Count	Column N %	Row N %	Count	Column N %	Row N %	x^2	p
NPI risk assessment	Low risk	22	40.7%	73.3%	8	47.1%	26.7%		
	Medium risk	17	31.5%	81.0%	4	23.5%	19.0%	0.41	0.814
	High risk	15	27.8%	75.0%	5	29.4%	25.0%		
Histological grade of tumor	Histological grade 1	10	18.5%	71.4%	4	23.5%	28.6%		
	Histological grade 2	24	44.4%	85.7%	4	23.5%	14.3%	2.40	0.301
	Histological grade 3	20	37.0%	69.0%	9	52.9%	31.0%		
Tumor size (cm)	< 1.99	22	40.7%	75.9%	7	41.2%	24.1%		
	≥ 2 – 5	32	59.3%	76.2%	10	58.8%	23.8%	0.01	1.00
Infiltration of axillary lymph nodes	No positive axillary lymph nodes	25	46.3%	78.1%	7	41.2%	21.9%		
	1-3 positive axillary lymph nodes	19	35.2%	76.0%	6	35.3%	24.0%		
	3-10 positive axillary lymph nodes	7	13.0%	70.0%	3	17.6%	30.0%		
	>10 positive axillary lymph nodes	3	5.6%	75.0%	1	5.9%	25.0%	0.279	0.964

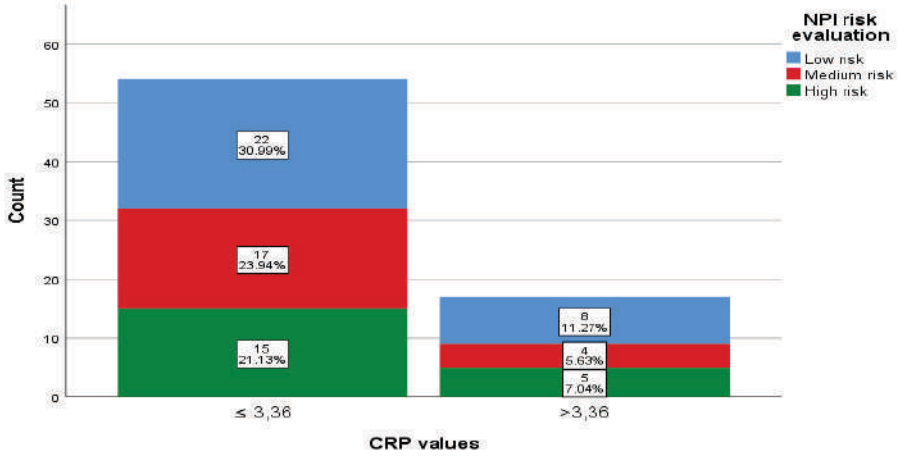

Figure 1 **Prevalence of NPI scores relative to serum CRP values.**

A higher prevalence of patients with low NPI risk was in the group with serum CRP values ≤ 3.36 mg / l (73.3%) compared to the group of patients with serum CRP values > 3.36 mg /l. However, patients with medium and high NPI risk were more represented in the group of patients with serum CRP values ≤ 3.36 mg /l compared to patients with serum CRP values > 3.36 mg /l. The hi-square test didn't show a statistically significant correlation between serum CRP values and NPI risk assessment (p>0.05), (Table 1, Figure 1).

Within the group of patients with serum CRP values ≤ 3.36 mg /l, 10 (18.5%) patients had a histological grade of 1.24 (44.4%) patients had a histological grade of 2, while 20 (37%) patients had histological grade 3. Within the group of patients with serum CRP values >3.36 mg/l, histological grade 1 and histological grade 2 had 2 (23.5%) patients and histological grade 3 had 9 (52.9%) patients. A statistically significant association between serum CRP values and tumor histological grade (p>0.05) was not demonstrated by the Hi-square test (Table 1).

Serum CRP values are equally represented in patients relative to tumor size. Within the group of patients with serum CRP values ≤ 3.36 mg/l, 22 (40.7%) patients had tumor size < 1.99 cm, while 32 (59.3%) patients had tumor size above ≥ 2 cm. Within the group of patients with serum CRP values > 3.36 mg/l, 7 (41.2%) patients had tumor size < 1.99 cm, and 10 (58.8%) patients had tumor size ≥ 2cm. Fisher's exact test didn't prove a statistically significant association between serum CRP values and tumor size (p>0.05), (Table 1).

Within the group of patients without infiltrated axillary lymph nodes, 25 (78.1%) patients had serum CRP values ≤ 3.36 mg/l, and 7 (21.9%) patients had serum CRP values > 3.36 mg/l. Within the group of patients with positive axillary lymph nodes, (1-3, 4-9 and > 10) it is evident that a larger number of study patients in all three cases were in the group with serum CRP values ≤ 3.36 mg/l. The hi-square independence test didn't show a statistically significant association between axillary lymph node infiltration status and serum CRP values (p>0.05), (Table 1).

Table 4 **Multinomial logistic regression of NPI score prediction based on serum CRP values.**

								95% Confidence Interval for Exp(B)	
NPI score[a]		B	Error	Wald	df	Sig.	Exp(B)	Lower Bound	Upper Bound
0	Intercept	.470	.570	.680	1	.410			
	[CRP =0]	-.087	.661	.017	1	.895	.917	.251	3.350
	[CRP =1]	0[b]	.	.	0	.	.	.	.
1	Intercept	-.223	.671	.111	1	.739			
	[CRP =0]	.348	.759	.211	1	.646	1.417	.320	6.266
	[CRP =1]	0[b]	.	.	0	.	.	.	.

a. The reference category is: 2.

b. This parameter is set to zero because it is redundant.

Patients with serum CRP values > 3.36 mg/l had 9.1% or 1.1 times higher statistically insignificant chance (p=0.895) of being in low than in high NPI risk group compared to patients with serum CRP values ≤ 3.36 mg/l. Patients with serum CRP values less than 3.36 mg/l were 41.7% or 1.42 times more likely to be in the group of patients with medium than high NPI risk compared to patients with serum CRP>3, 36 mg/l, but without statistical significance (p= 0.646). Patients with serum CRP values ≤ 3.36 mg/l were 54.5% or 1.54 times more likely to be in the group of patients with medium than low NPI risk compared to patients with serum CRP > 3,36 mg/l, but also without statistical significance (p=0.529).

DISCUSSION

In our study, we analyzed the correlation of serum C-reactive protein values and the Nottingham prognostic index score in 71 patients with primary early invasive breast cancer. By analyzing the study data, we didn't prove a statistically significant association between serum CRP values and NPI prognostic index scores, nor between serum CRP values and individual NPI score prognostic parameters.

Clinical studies that analyzed the relationship between standard clinical-pathological parameters and serum CRP values interpreted a variety of results.

C-reactive protein is a nonspecific prognostic parameter, whose elevated values are not only specific for breast cancer, but can be a sign of various physiological and pathological conditions (aging, smoking, lack of physical activity, myocardial infarction, stroke, peripheral vascular disease, increased physical severity, hypertension, type 2 diabetes mellitus, venous thrombosis, obstructive pulmonary disease, mortality of any cause, etc.) (11-25).

Some authors did not find statistically significant correlation between preoperative serum CRP values and individual prognostic parameters of NPI score in breast cancer or with the occurrence of distant metastases and overall survival of patients of the mentioned studies (26). Also, baseline plasma CRP values are not associated with an increased risk of developing breast cancer in clinically healthy women (27).

Contrary to the above, there are results of studies that indicate a significant association between serum CRP values, but high sensitivity CRP, and the degree and size of the tumor and the formation of metastases. Macrophage infiltration, as one of the consequences of the inflammatory process, results in elevated CRP values and increased vascularization, which leads to increased tumor aggressiveness in the form of earlier recurrence of the disease and shortened survival time. It also leads to disruption of the DNA repair process (deoxyribonucleic acid) resulting in disease progression due to more pronounced tumor invasion and metastasis process (28-30).

A study of the relationship between preoperative CRP values in patients with breast cancer and negative axillary lymph nodes has similar results. The results of the study confirmed that preoperative serum CRP values correlated with shortening of disease-free survival (DFS) or overall survival (OS) as an independent parameter. However, in a subgroup of 72 patients in whom CRP values and genetic tests were correlated, no association was found between these parameters (31).

A clinical study of 2910 patients with invasive breast cancer showed that elevated CRP values at the time of breast cancer diagnosis were associated with larger tumor diameters, the presence

of distant metastases, and a lower histological grade of the tumor, and these factors were associated with a poorer prognosis (32).

In our study, all patients, in relation to serum CRP values were divided into two groups: the first with ≤3.36 mmol / l and the second with> 3.36 mmol / l. We failed to show a linear relationship between tumor size, histological grade and axillary lymph node involvement predicted by an increase in serum CRP, nor did we obtain a statistically significant difference in serum CRP and individual variants of the above standard clinical-pathological prognostic parameters.

By additional statistical analysis of our study data, we found that patients with lower CRP values were slightly more likely to be in the group of patients with medium than high NPI risk. Paradoxically, patients with higher serum CRP values are slightly more likely to be in the group of patients with low than high NPI risk.

Also, the results obtained may be due to study limitations. Namely, no agreement has yet been reached on benchmarks for CRP or high sensitivity CRP. CRP measurement was performed once as a baseline measurement. In addition, although we correlated serum CRP values with NPI score and its individual clinical-pathological parameters, we are not sure whether we could avoid all pathological and physiological conditions that interfere with elevated preoperative CRP values in the blood of patients with early invasive breast cancer. Another limitation is the number of patients and the duration of the study, which are insufficient compared to other studies.

Therefore, additional studies need to be undertaken to clarify whether preoperative blood CRP values, as a cheap and affordable parameter, can be used in the prognosis and prediction of breast cancer patients, alone or in combination with standard clinical-pathological parameters of breast cancer.

CONCLUSION

The value of CRP in the serum of patients with primary early invasive breast cancer in our study cannot be taken as a separate prognostic-predictive factor that correlates with standard prognostic clinical-pathological variables of Nottingham's prognostic index of early invasive breast cancer. The search for a serum biomarker (alone or in combination with other indicators) that would meet the specificity criteria for assessing the prognosis of breast cancer continues.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-49.
2. Chan DSM, Bandera EV, Greenwood DC, Norat T. Circulating C -Reactive Protein and Breast Cancer Risk-Systematic Literature Review and Metaanalysis of Prospective Cohort Studies. Cancer Epidemiol Biomarkers Prev. 2015;24(10);1439-49.
3. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140(6):883-99.
4. De Nardo DG, Coussens LM. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Res. 2007;9(4):212.
5. Han Y, Mao F, Wu Y, Fu X, Zhu X, Zhou S, et al. Prognostic role of C-reactive protein in breast cancer: a systematic review and meta-analysis. Int J Biol Markers. 2011;26(4):209-15.
6. Guo YZ, Pan L, Du CJ, Ren DQ, Xie XM. Association between C-reactive protein and risk of cancer: a meta-analysis of prospective cohort studies. Asian Pac J Cancer Prev. 2013;14(1):243-8.
7. Todd JH, Dowle C, Williams MR, Elston CW, Ellis IO, Hinton CP, et al. Confirmation of a prognostic index in primary breast cancer. Br J Cancer 1987;56(4):489-92.
8. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham Prognostic Index in primary breast cancer. Breast Cancer Res Treat. 1992;22(3):207-19.
9. Rakha EA, Soria D, Green AR, Lemetre C, Powe DG, Nolan CC, et al. Nottingham Prognostic Index Plus (NPI+): a modern clinical decision making tool in breast cancer. Br J Cancer 2014;110(7):1688-97.
10. Sobin LH, Gospodarowicz Mk, Wittekind Ce. TNM Claassification of malignat tumors, 7th ed. Oxford: Wiley-Blackwell; 2009.310.
11. Zacho J, Tybjærg-Hansen A, Nordestgaard BG. Creactive protein and all-cause mortality-the Copenhagen City Heart Study. Eur Heart J. 2010;31(13):1624-32.
12. 19Timpson NJ, Nordestgaard BG, Harbord RM, Zacho J, Frayling TM, Tybjærg-Hansen A, et al. C-reactive protein levels and body mass index: Elucidating direction of causation through reciprocal Mendelian randomization. Int J Obes. 2011;35(2):300-8.
13. Hutchinson WL, Koenig W, Fröhlich M, Sund M, Lowe GD, Pepys MB. Immunoradiometric assay of circulating C-reactive protein: Age-related values in the adult general population. Clin Chem. 2000;46(7):934-8.
14. Woloshin S, Schwartz LM. Distribution of C-reactive protein values in the United States. N Engl J Med. 2005;352(15):1611-3.
15. Nordestgaard BG, Adourian AS, Freiberg JJ, Guo Y, Muntendam P, Falk E. Risk factors for near-term myocardial infarction in apparently healthy men and women. Clin Chem. 2010;56(4):559-67.
16. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med. 2002;347(20):1557-65.
17. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. N Engl J Med. 2008;359(18):1897-908.
18. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: A comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA. 2001;285(19):2481-5.
19. Pradhan AD, Cook NR, Buring JE, Manson JE, Ridker PM. C-reactive protein is independently associated with fasting insulin in nondiabetic women. Arterioscler Thromb Vasc Biol. 2003;23(4):650-5.
20. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD, et al. West of Scotland Coronary Prevention Study. C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. Diabetes. 2002;51(5):1596-600.
21. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Jama. 2001;286(3):327-34.
22. Marott SC, Nordestgaard BG, Zacho J, Friberg J, Jensen GB, Tybjaerg-Hansen A, et al. Does elevated C-reactive protein increase atrial fibrillation risk? A Mendelian randomization of 47,000 individuals from the general population. J Am Coll Cardiol. 2010;56(10):789-95.
23. Zacho J, Tybjaerg-Hansen A, Nordestgaard BG. C-reactive protein and risk of venous thromboembolism in the general population. Arterioscler Thromb Vasc Biol. 2010;30(8):1672-8.
24. Sesso HD, Buring JE, Rifai N, Blake GJ, Gaziano JM, Ridker PM. C-reactive protein and the risk of developing hypertension. Jama. 2003;290(22):2945-51.
25. Dahl M, Vestbo J, Zacho J, Lange P, Tybjærg-Hansen A, Nordestgaard BG. C reactive protein and chronic obstructive pulmonary disease: A Mendelian randomisation approach. Thorax. 2011;66(3):197-204.
26. Al Murri AM, Wilson C, Lannigan A, Doughty JC, Angerson WJ, McArdle CS, et al. Evaluation of the relationship between the systemic inflammatory response and cancer-specific survival in patients with primary operable breast cancer. Br J Cancer. 2007;96(6):891-5.
27. Ravishankaran P, Karunanithi R. Clinical significance of preoperative serum interleukin-6 and C-reactive protein level in breast cancer patients. World J Surg Oncol. 2011;9:18.
28. Zhang SM, Lin J, Cook NR, i sar. C-Reactive Protein and Risk of Breast Cancer. J Natl Cancer Inst. 2007;99(1):890-4.
29. Asegaonkar S, Takalkar U, Kodlikeri P, Pagdhune A, Bonduliya V, Thorat A. Serum high sensitivity C-reactive protein in breast cancer patients. IJRMS. 2014;2(4):1408-11.
30. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour progression. Nat Rev Immunol. 2004;4(8):641-8.
31. Sicking I, Edlund K, Wesbuer E, Weyer V, Battista MJ, Lebrecht A, et al. Prognostic Influence of Pre-Operative C-Reactive Protein in Node-Negative Breast Cancer Patients. PLoS One 2014;9(10) e111306.
32. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. Crit Rev Clin Lab Sci. 2011;48(4):155-70.

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Influence of microsatellite instability as a predictive biomarker on the length of overall survival in patients with metastatic colorectal cancer

Utjecaj mikrosatelitne nestabilnosti kao prediktivnog biomarkera na dužinu ukupnog preživljenja kod pacijenata sa metastatskim kolorektalnim karcinomom

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ABSTRACT

Introduction: colon cancer is one of the most common forms of cancers. The incidence of colorectal cancer is 19.7 per 100.000 population according to Global Cancer Observatory (GLOBOCAN) worldwide in 2018. Microsatellite instability (MSI) is characterized by inactivation of the DNA repair system (deoxyribonucleic acid) and occurs in 5-15% of colorectal cancers. The presence of MSI is phenotypic evidence that mission repair systems (MMR) are not normally working. Aim: To determine the impact of microsatellite instability on the overall survival of patients with metastatic colorectal cancer. Materials and methods: The research is a retrospective, descriptive-analytical study was conducted at the Clinic of General and Abdominal Surgery, Clinic of Oncology and Clinical Pathology, Cytology and Human Genetic of the Clinical Center University of Sarajevo (CCUS) in the period from 2014 to 2020. A total of 101 patients were included in the study. The study included patients diagnosed with colorectal cancer (stages II and III according to AJCC - The American Joint Commission on Cancer) who underwent surgery and then received adjuvant chemotherapy. After adjuvant chemotherapy, patients were followed up to five years. Those patients whose distant metastasis was verified during the follow-up period were included in the study. The biomarkers of microsatellite instability (MSI) were determined in the patients included in the study, and for those who lacked MSI findings, additional/subsequent ones were performed in order to complete the findings. Overall survival (OS) calculation was done from the moment of metastasis until the end of the study, until the last written finding or death of the patient. Results: the average age was 59.69 (± 11,42). A comparison of the estimated OS (in months) by the Log Rank Mantel Cox test showed that there was no statistically significantly longer estimate of OS in the group of patients with MSS-marker compared to MSI-L cpf" OUKH marker with 4? 0.527 and p=0.768. Conclusion: predictive biomarker MSI has no direct effect on the overall survival of patients with metastatic CRC. MSI influences the choice of oncology therapy and the overall survival accordingly.

Keywords: microsatellite instability (MSI), colorectal cancer (CCR), biomarkers

SAŽETAK

Uvod: rak debelog crijeva jedan je od najčešćih oblika karcinoma. Incidenca kolorektalnog karcinoma je 19,7 na 100.000 stanovnika prema GLOBOCAN-u (Global Cancer Observatory) na svjetskom nivou u 2018. godini. Mikrosatelitnu nestabilnost (MSI-Microsatellite instability) karakterizira inaktivacija sistema za popravljanje neusklađenosti DNK (deoxyribonucleic acid) i primjećuje se kod 5-15% kolorektalnih karcinoma. Prisustvo MSI predstavlja fenotipski dokaz da MMR (mismatch repair systems) ne funkcionira normalno. Cilj rada: utvrditi utjecaj MSI na ukupno preživljenje pacijenata oboljelih od metastatskog kolorektalnog karcinoma. Materijali i metode: provedeno istraživanje je retrospektivna, deskriptivno-analitička studija provedena na Klinici za opštu i abdominalnu hirurgiju, Klinici za onkologiju te odjelu Kliničkoj patlogiji, citologiji i humanoj genetici Kliničkog centra Univerziteta u Sarajevu u periodu od 2014. do 2020. godine. U istraživanje je bio uključen ukupno 101 pacijent. Istraživanje je obuhvatilo pacijente dijagnosticirane s kolorektalnim karcinomom (stadija II i III prema AJCC-The American Joint Commission on Cancer) koji su operirasi a potom primili adjuvantnu hemoterapiju. Nakon adjuvatne hemoterapije, pacijenti su praćeni u periodu od pet godina. Oni pacijenti kojima je verificirana udaljena metastaza u periodu praćenja uključeni su u istraživanje. Pacijentima koji su uključeni u istraživanje rađeno je određivanje biomarkera MSI, a za one pacijente kojima su nedostajali nalazi MSI, izvršene su dodatne/naknadne pretrage u cilju kompletiranja nalaza. Ukupno preživljavanje (OS-overall survival) kalkulacija, rađeno je od momenta pojave metastaze pa do kraja istraživanja, tj. do zadnjeg pisanog nalaza ili do smrti pacijenta. Rezultati: prosječna starosna dob iznosi 59,69±11,42 SD. Komparacija procijenjenog vremena OS (u mjesecima) putem Log Rank Mantel Cox testa pokazuje da ne postoji statistički signifikantno duža procjena OS u grupi pacijenata s MSS-markerom u odnosu na MSI-L i MSI-H marker uz $\chi 2=0,527$ i p=0,768. Zaključak: provedenim naučno-istraživačkim radom pokazali smo da prediktivni biomarker MSI nema direktan utjecaj na ukupno preživljenje pacijenata oboljelih od metastatskog kolorektalnog karcinoma. MSI ima utjecaj pri određivanju onkološke terapije, a time i na ukupno preživljenje.

Ključne riječi: mikrosatelitska nestabilnost (MSI), kolorektalni karcinom, biomarker

INTRODUCTION

Colon cancer is one of the most common forms of cancer, affecting both sexes equally with a peak incidence in the seventh decade of life (1). Colorectal cancers (CRCs) are relatively rare in young people; only about 9% of these tumors are diagnosed before the age of 50, and 5-8% in those under the age of 40 (1). Majority of colon cancers are caused by malignant changes in colon polyps (more than 95% of colon cancers are polyps and they most commonly relate to adenocarcinomas) (2). The incidence of CRC is 19.7 per 100.000 population according to Global Cancer Observatory (World Health Organization/International Agency for Research on Cancer)- GLOBOCAN worldwide in 2018 for both sexes and for all age groups (3).

Microsatellite instability (MSI) is characterized by inactivation of the deoxyribonucleic acid (DNA) repair system and occurs in 5-15% of CRC. The presence of MSI is phenotypic evidence that mission repair systems (MMR) are not functioning normally (4). MSI-high tumors are less likely to metastasize compared to other phenotypes and have favorable survival outcomes (4).

According to present, modern divisions, CRCs are divided based on the presence or absence of changes in the microsatellite sequences of DNA of tumor cells into two large groups: microsatellite stable and microsatellite unstable CRC. In the development of CRC, 5-10% of cases have a clear hereditary background, of which 3-4% are hereditary non-polyposis colorectal cancers (HNPCC), the so-called Lynch syndrome, which is associated with MSI (5). About 15% of sporadic CRCs show microsatellite instability, caused in about 95% of cases by methylation of the MLH1 gene promoter region. Tumors of high microsatellite instability (MSI-H) are more commonly seen in the earlier stages of cancer and tend to have a better overall prognosis (5). Only about 3 to 6% of advanced CRC patients have MSI-H or dMMR. MMR specifically recognizes and corrects insertions, deletions, and incorrect incorporations of DNA bases during DNA replication. Lack of MMR system leads to accumulation of mutations.

These types of DNA errors, which the MMR recognizes and corrects, preferentially occur in areas of DNA replication, called microsatellites. Due to this association, patients with dMMR will show variations in the length of different microsatellites when comparing normal and tumor sequences, which is called MSI-H. Deficiency or dysfunction of MMR proteins such as MLH1, MSH2, MSH6, PMS2 and TACSTD1 / EPCAM will result in dMMR (6). Classical testing for MSI-H is based on guidelines recommending testing of five specific microsatellites (BAT25, BAT26, D2S123, D5S346 and D17S250) by polymerase chain reaction with determination of MSI-H based on instability on more than 30% of tested microsatellites.

Approximately 15% of CRCs have dMMR, however, this rate decreases in stages, with approximately 4% of patients with stage IV showing dMMR. Among patients with dMMR, Lynch syndrome or hereditary nepolyposis CRC will be present in about one-third of patients. It is recommended that all patients with metastatic CRC undergo dMMR testing to identify not only patients with Lynch syndrome but also patients who could be treated with anti-PD-1 therapy (5,6). Consensus determined that the microsatellite status of tumor tissue was divided into three groups, by determining a panel of 5 microsatellite markers from the National Cancer Institute (NCI), also known as Bethesda markers, two of which were mononucleotide (BAT25 and BAT26), and three dinucle otide (02S123, 05S346 and OJ7S250).

Tumors are classified as tumors with a high degree of MSI if they show instability in at least two examined microsatellite markers of tumor tissue. If MSI is registered in only one marker, it is defined as a tumor with a low degree of MSI (7). If no microsatellite locus shows a change in length, the tumor is characterized as microsatellite stable. Data from the literature indicate different clinical and pathological characteristics of microsatellite stable and microsatellite unstable colorectal cancers, significant differences in the prognosis and response to adjuvant chemotherapy (7).

AIM

The aim of the study was to determine the impact of microsatellite instability (MSI) on the overall survival of patients with metastatic colorectal cancer.

MATERIALS AND METHODS

The research is a retrospective, descriptive-analytical study conducted at the Clinic of General and Abdominal Surgery and Clinical Pathology, Cytology and Human Genetic of the Clinical Center University of Sarajevo (CCUS) in the period from 2014 to 2020. The study included a total of 101 patients diagnosed with colorectal cancer (stages II and III according to AJCC - The American Joint Commission on Cancer) who underwent surgery at the Clinical Center University of Sarajevo, and who subsequently received adjuvant chemotherapy. Following the adjuvant chemotherapy the patients were monitored for five years. The patients whose distant metastasis was verified during the follow-up period were included in the study. The biomarkers of MSI were determined in the patients included in the study, and for those who lacked MSI findings, additional/subsequent ones were performed in order to complete the findings.

At the Clinical Pathology, Cytology and Human Genetic, the "models" of patients included in the research were identified and sorted. Some patients included in the study started their oncological treatment before 2014, but their biomarkers were measured in the given period (2014-2020). Statistical processing was done in the Statistical Package for the Social Sciences (SPSS), program for Windows application. All data are presented in tables and figures. Overall survival (OS) calculation was done from the moment of metastasis until the end of the study, specifically until the last written finding or death of the patient.

FFPE sections of 10 micrometer-thick tissue samples cut into sterile tubes or glasses were used to isolate DNA (3,6). The following protocols were used to isolate DNK from FFPE tissue samples: QIAamp DNA Blood Mini Kit and QIAamp DNA FFPE Tissue Kit. AB StepOne PCR was used for Real-Time PCR. Fragmentary sequencing is done on 3130 Genetic Analyzer ABI Prism, and analysis of results on Thermo Fisher Scientific7GeneMapper v.4.0 software.

RESULTS

Table 1 shows demographic characteristics (gender, age, localization of tumor, tumor grade, pathohistological finding, MSI (microsatellite instability) and overall survival (OS) values of colorectal cancer (CRC) in 101 patients included in the study.

Table 1 **Demographic characteristics, OS values, and clinical and pathological features of CRC c in 101 patients included in the study.**

		Count	Percentage
Gender	Male	49	48.5%
	Female	52	51.5%
Age distribution	<20	1	1.0%
	21-40	4	4.0%
	41-60	40	39.6%
	>60	56	55.4%
Tumor localization	Right colon	22	22.4%
	Left colon	75	76.5%
	Synchronous	1	1.0%
Tumor grade	Grade I	2	2.2%
	Grade II	67	72.0%
	Grade III	24	25.8%
Pathohistological finding of the tumor	Adenocarcinoma	79	79.8%
	Adenocarcinoma mucinous	19	19.2%
	Adenocarcinoma signet ring cells	1	1.0%
T	T1	1	1.3%
	T2	10	12.8%
	T3	47	60.3%
	T4	20	25.6%
N	N0	21	27.3%
	N1	28	36.4%
	N2	25	32.5%
	Nx	3	3.9%
M	Mx	40	65.6%
	M1	17	27.9%
	M2	1	1.6%
	M0	3	4.9%
Resection margine	R0	61	89.7%
	R1	7	10.3%
OS range	<3 years	41	40.6%
	3-5 years	15	14.9%
	>5 years	45	44.6%
MSI	MSS	53	93.0%
	MSI-H	3	5.3%
	MSI-L	1	1.8%

Table 2 **Analysis of patients' age.**

	N	Min.	Max.	Mean	Std. Deviation
Age	101	18	80	59.69	11.417
Valid N (list wise)	101				

The youngest patient was 18 years old and the oldest was 80. The average age was 59.69±11.42.

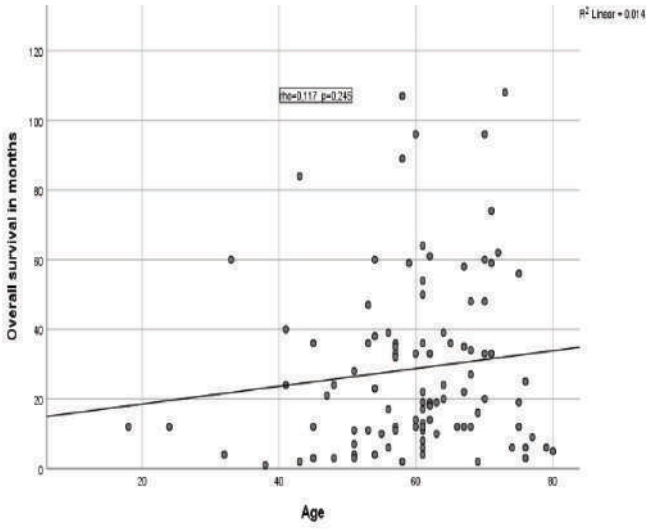

Figure 1 **Relation between the age of the respondents and overall survival (OS).**

Correlation analysis of age and OS in the total sample shows that there is a slightly positive correlation, but without statistically significant correlation (rho=0.117; p=0.245) (Figure 1).

Table 3 **Relationship between the type of pathohistological finding and the MSI marker.**

		Pathohistological finding of the tumor								
		Adenocarcinoma			Adenocarcinoma mucinous			Adenocarcinoma signet ring cells		
		Count	Row N %	Column N %	Count	Row N %	Column N %	Count	Row N %	Column N %
MSI	MSS	42	80.8%	95.5%	9	17.3%	81.8%	1	1.9%	100.0%
	MSI-H	2	66.7%	4.5%	1	33.3%	9.1%	0	0.0%	0.0%
	MSI-L	0	0.0%	0.0%	1	100.0%	9.1%	0	0.0%	0.0%

X^2=4,667 p=0,323

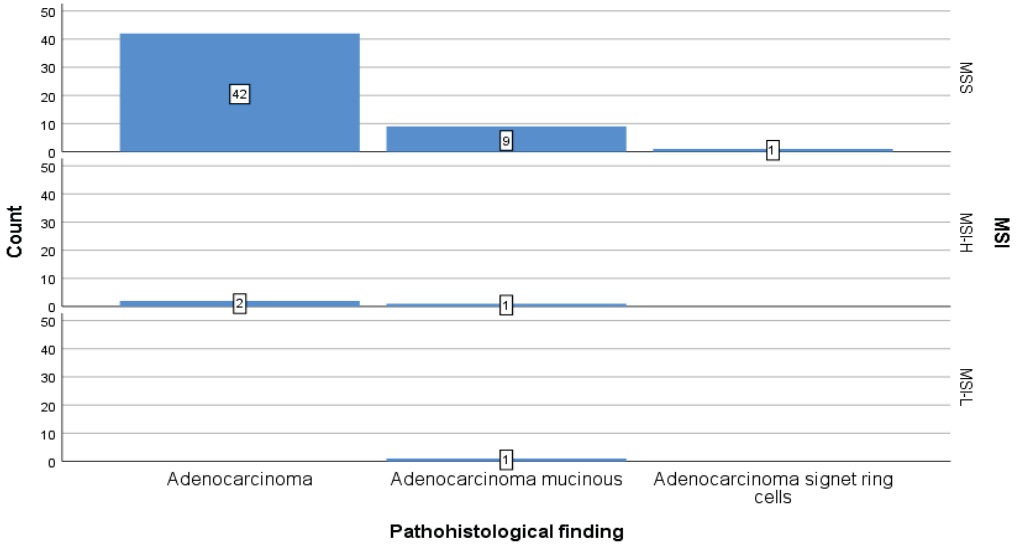

Figure 2 **Prevalence of microsatellite instability in relation to pathohistological findings.**

A total of 42 patients (80.8%) with elevated MSS values had pathohistologically verified adenocarcinoma, 9 patients (1.7%) had mucinous adenocarcinoma, and only 1 patient (1.9%) had signet ring cells adenocarcinoma. MSI-H and MSI-L had a lower percentage in different tumor types (Table 3 and Figure 2). Chi-square test of independence did not show statistically significant association between the type of pathohistological finding and the type of MSS marker (X^2=4,667 p=0,323).

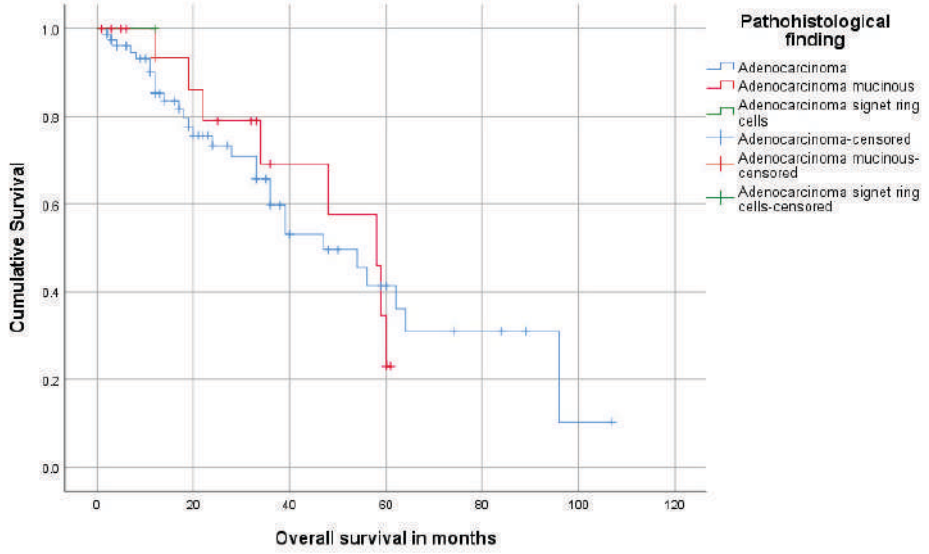

Figure 3 **Length of overall survival (OS) in a group of 101 colon cancer patient, in relation to pathohistological finding.**

Comparison of OS time (in months) by Log Rank Mantel Cox test shows that there is no statistically significant difference in overall survival in the group of patients with adenocarcinoma, mucinous adenocarcinoma and ring cell carcinoma with $\chi 2=0,146$ i $p=0,930$ (Figure 3).

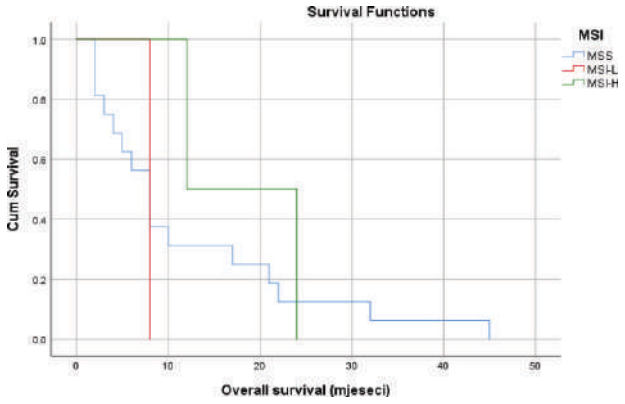

Figure 4 **Overall survival (OS) in a group of 101 patients with colon cancer in relation to MSI marker type.**

A comparison of the estimated overall OS (in months) by the Log Rank Mantel Cox test shows that there is no statistically significantly longer assessment of OS in the group of patients with MSS-marker compared to MSI-L and MSI-H marker, with $\chi 2=0,527$ and $p=0,768$.

DISCUSSION

The study included 101 patients, of which 53 (52%) were males and 48 patients (48%) were females (8,9). The obtained data on the sexual structure correspond to the data from the literature (8). Examination of the age structure shows that the largest number of patients (n = 86) or 85.15% of them were over 50 years of age. While younger patients (up to 50 years of age) made only 14.85% of patients (n=15) (10), there were only 2 patients (1.98%) under the age of 30. The American Cancer Society (ACS) study from 2020 showed that there was only 12% of sick patients under the age of 50 (10). The youngest patient included in the study was 18 years old, and the oldest patient was 80. The obtained data correspond to the data of the consulted literature (White A, et al) which shows that the largest percentage related to third age patients (8,10).

Adenocarcinoma is the most common pathohistological verification of colon cancer found in 78 patients (77.23%), followed by mucinous adenocarcinoma found in 19 patients (18.81%) (11,12). The consulted literature also shows that the most common colon cancers are adenocarcinomas with a share of 98% (8). With regard to tumor grade, the most common were grade II tumors (n=67) 66.34%, followed by grade III tumors (n=24) 23.76%. The research conducted at the Clinical Center of Vojvodina also speaks in favor of the most common grade II colon tumors. In a study conducted in 2010 (one-year study) on 100 patients, 60.3% related to grade II tumors (13). Out of the total number of patients (n=101) 63 patients (62.37%) had an MSI done by the end of the study. For some patients it was not technically possible to perform additional cytogenetic analyzes (due to the age of the available molds or due to some missing molds - patients took them away).

Microsatellite instability (MSI-H) was detected in only three patients (4.7%). An MSI-L mutation (1.5%) was found in one patient. Microsatellite stability (MSS) was verified in 59 patients (93%). In 38 patients (37.6%) there were no MSI findings (due to inhibition towards certain preparations, mold obsolescence, and due to the fact that some patients (~ 10) removed molds from Clinical Pathology of the Clinical Center University of Sarajevo.

In their study, Ooki A, et al. stated that about 5% of patients had positive MSI-H test, which corresponds to the data obtained from our study (4.7%) (14). MSI is a specific genetic marker in colorectal cancer which may be useful in diagnosing, anticipating and predicting the effectiveness of chemotherapy (12). In their study, Battaglin F, et al stated that MMR had an important predicting role, especially in the early stage of colon cancer (15). From the prognostic point of view, patients with MSI-H, have a good prognosis, but it is very important to determine the existence of a BRAF mutation at an early stage, which is a bad sign. Some authors also state that MSI depends on the location of the tumor and that it is more common in right-sided colon cancers (16,17). Our research, although conducted on a small sample of patients with MSI-H, can confirm those allegations.

CONCLUSION

The predictive biomarker (MSI-microsatellite intstability) has no direct effect on the overall survival of patients with metastatic colorectal cancer. On the other hand, it has indirect effect, determination of oncology therapy, and thus on overall survival.

REFERENCES

1. Fenjveši A. Prognostički značaj mikrosatelitne nestabilnosti kod pacijenata mlađih od 50 godina, oboljelih od kolorektalnog karcinoma. Med Pregl. 2009;LXII (5-6): 217-223.
2. Europacolon.com (homepage on the Internet). Helping you understand the importance od predictive RAS (KRAS, NRAS) biomarkers in colorectal cancer treatment. Updated 2018 February 09; cited 2018 Mart 15. Available from: http://www.europacolon.com/Documents/Uploaded/. 20.10.2020. 06:20PM
3. gco.iarc.fr (home page on the Inernet). International Agency for Research on Cancer. World Health Organization. Global Cancer Observatory. Available from: https://gco.iarc.fr/today/online-analysis-multi-bars. 20.10.2020. 05:54PM.
4. ghr.nlm.nih.gov (homepage on the Internet) U.S Department of Health & Human Services. National Library of Medicine. Genetics Home Reference. 8600 Rockville Picke. Bethesda MD, 20894 USA. Updated 2018 Mart 15; cited 2018 Mart 25. Available from: https://ghr.nlm.nih.gov/gene/BRAF
5. Yurgelun MB, Hampel H. Recent Advances in Lynch Syndrome: Diagnosis, Treatment, and Cancer Prevention. Am Soc Clin Oncol Educ Book. 2018;38:101-9.
6. https://ascopubs.org/doi/full/10.1200/EDBK_200821. 24.05.2021, at 06:30AM
7. Richard BC, Ajay G. Microsatellite Instability in Colorectal Cancer. Gastroenterology. 2010;138(6): 2073-87.
8. White A, Ironmonger L, Steele RJC, Ormiston-Smith N, Crawford C, Seims A. A review of sex-related differences in colorectal cancer incidence, screening uptake, routes to diagnosis, cancer stage and survival in the UK. BMC Cancer. 2018;18(1):906.
9. nccn.org (home page on the Internet) Clinical Practice Guidelines of Oncology (NCCN Guidelines). Colon Cancer. Version 4.2020. - Jun.15, 2020. https://www.nccn.org/professionals/physician_gls/pdf/colon_blocks.pdf. 09.10.2020, at 00:43AM
10. Siegel R, Miller DK, Sauer GS, Fedewa SA, Butterly LF, Anderson JC. Colorectal Cancer Statistics, 2020. CA Cancer J Clin. 2020;70(3):145-64.
11. Luo C, Cen S, Ding G, Wu W. Mucinous colorectal adenocarcinoma: clinical pathology and treatment options. Cancer Commun (Lond). 2019;39(1):13.
12. Nojadeh JN, Behrouz Sharif S, Sakhinia E. Microsatellite instability in colorectal cancer. *EXCLI J.* 2018;17:159-68.
13. Sečen S, Moljević N, Vuković M, Somer Lj. Histopathological finding as a prognostic factor of the surgical treatment outcome in colorectal cancer. Vojnosanit Pregl. 2010; 67(8):638-43.

14. Ooki A, Shinozaki E, Yamaguchi K. Immunotherapy in Colorectal Cancer: Current and Future Strategies. J Anus Rectum Colon. 2021;5(1):11-24.
15. Battaglin F, Naseem M, Lenz HJ, Salem ME. Microsatellite Instability in Colorectal Cancer: Overview of Its Clinical Significance and Novel Perspectives. Clin Adv Hematol Oncol. 2018;16(11):735-45.
16. Li K, Luo H, Huang L, Luo H, Zhu X. Microsatellite instability: A review of what the oncologist should know. Cancer Cell Int. 2020;20:16.
17. Arakawa K, Hata K, Kawai K, Tanaka T, Nishikawa T, Sasaki K, et al Predictors for high microsatellite instability in patients with colorectal cancer fulfilling the revised bethesda guidelines. Anticancer Res. 2018;38(8):4871-6.

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Analysis of the severity of influenza epidemics: severe acute respiratory infection (SARS) sentinel surveillance data in the Federation of Bosnia and Herzegovina during the 2016/17, 2017/18 and 2018/19 seasons

Analiza ozbiljnosti epidemije gripe: podatci sentinel nadzora nad teškim akutnim respiratornim infekcijama (SARI) u Federaciji Bosne i Hercegovine tokom sezona 2016/17, 2017/18 i 2018/19

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ABSTRACT

Introduction: influenza is a major public health burden. In the Federation of Bosnia and Herzegovina (FBiH, one of two entities which compose the state of Bosnia and Herzegovina), a severe acute respiratory infection (SARI) sentinel surveillance system was established in the 2014/15 season. Aim: to analyse the clinical, epidemiological and virological characteristics of laboratory confirmed influenza cases at the SARI sentinel site at the Clinical Center University of Sarajevo (CCUS), during the 2016/17, 2017/18 and 2018/19 seasons. Material and methods: sentinel surveillance included clinics for pulmonary diseases, infectious diseases, intensive care and pediatrics. From each patient who met the SARI case definition, a nasopharyngeal swab, BD universal viral transport (UVT) system, was collected and real-time RT-PCR was performed for influenza virus detection. Results: throughout the 2016/17 season 31 laboratory confirmed influenza cases were reported, representing 49.2% (31/63) of all reported SARI cases, 60 cases in the 2017/18 season (48.0% or 60/125), and 83 cases in the 2018/19 season (37.2% or 83/223). Influenza virus types/subtypes and chronic health conditions were significantly different between influenza patients who were admitted to ICU and required mechanical ventilation and other hospitalized influenza cases. Conclusion: this study emphasizes the importance of SARI sentinel surveillance data, and the need for system improvement for assessment of the burden of seasonal influenza and mobilization of resources for prevention and control.

Keywords: sentinel surveillance, SARI, RT-PCR method

SAŽETAK

Uvod: gripa je veliki javnozdravstveni teret. U Federaciji Bosne i Hercegovine (FBiH, jedan od dva entiteta koja čine državu Bosnu i Hercegovinu), sentinel nadzor nad teškim akutnim respiratornim infekcijama (SARI) je uspostavljen u sezoni 2014/15 godina. Cilj: analizirati kliničke, epidemiološke i virusološke karakteristike laboratorijski potvrđenih slučajeva gripe kod SARI slučajeva, na sentinel mjestu za SARI u Kliničkom centru Univerziteta u Sarajevu (KCUS), tokom sezona 2016/17, 2017/18 i 2018/19. Materijal i metode: klinike uključene u nadzor su Klinika za plućne bolesti, Infektivna klinika, Klinika za anesteziju i reanimaciju i Pedijatrijska klinika. Od pacijenata koji odgovaraju definiciji SARI slučaja uzet je bris nazofarinksa u virusnoj transportnoj podlozi, BD Universal Viral Transport (UVT) System, i proveden RT-PCR u stvarnom vremenu radi otkrivanja virusa influence. Rezultati: tokom sezone 2016/17 prijavljen je 31 laboratorijski potvrđen slučaj gripe, što predstavlja 49,2% (31/63) svih prijavljenih SARI slučajeva; 60 slučajeva tokom sezone 2017/18 60 (48,0% ili 60/125); i 83 slučaja tokom sezone 2018/19 (37,2% ili 83/223). Tipovi i podtipovi virusa gripe i kronična zdravstvena stanja su značajno različiti između pacijenta koji su primljeni na odjel intenzivne njege i koji su zahtijevali mehaničku ventilaciju, i drugih hospitaliziranih slučajeva oboljelih od gripe. Zaključak: ova studija naglašava važnost SARI sentinel nadzora i potrebu za njegovim unapređenjem radi procjene tereta sezonske gripe i mobilizacije resursa za prevenciju i kontrolu.

Ključne riječi: sentinel nadzor, SARI, RT-PCR metoda

INTRODUCTION

Influenza is a major public health burden with an estimated 3 to 5 million cases of severe illness, and about 290 000 to 650 000 respiratory deaths each year (1). In the EU/EEA, seasonal influenza causes 4-50 million symptomatic cases each year, and 15 000 – 70 000 deaths (2).

In response to pandemic influenza in 2009, the Federation of Bosnia and Herzegovina (FBiH, one of two entities which composed the State of Bosnia and Herzegovina), redesigned the existing passive surveillance system. Universal syndromic surveillance for influenza-like illness (ILI), acute respiratory infections (ARI) and severe acute respiratory infections (SARI) were introduced, as well as influenza mortality. Influenza is reported in aggregate weekly on Mondays for the previous week. Health care providers' data on influenza are further aggregated within two levels: county level by the Cantonal public health institutes (PHI), and Federation level by the PHI of the FB&H.

In FB&H, sentinel surveillance of ILI and SARI was introduced in the 2014/15 season, with support of the Centers for Disease Control and Prevention (CDC) and the South East European Center of Infectious Diseases (SECID), as part of the project, "Surveillance and Response to Avian and Pandemic Influenza by National Health Authorities of Countries of South East Europe", and from the season 2016/17 further developed as part of the project, "Strengthening the Surveillance and Response Capacity of Avian and Pandemic Influenza in Bosnia and Herzegovina".

The ILI sentinel site was established in the Primary Health Center Novi Grad in Sarajevo and the SARI sentinel site at the CCUS. Focal points for both sites were selected, and guidelines for influenza surveillance were created (i.e., guidelines for ILI sentinel surveillance; guidelines for SARI sentinel surveillance; and guidelines for collection, transport and storage of samples). Feasibility and representativeness were the most important factors considered when choosing the sentinel sites. Analysis of surveillance data and writing of reports were conducted by the Department of Epidemiology at the PHI of the FB&H.

Surveillance data are sent to the Federal Ministry of Health which further sent them to the Ministry of Civil Affairs of Bosnia and Herzegovina to aggregates data from both entities of Bosnia and Herzegovina and the Brčko District, before submitting it to The European Surveillance System (TESSy)

During the 2017/18 influenza season, three additional SARI sentinel sites were piloted in FB&H at the University Clinical Hospital Mostar, the Cantonal Hospital Zenica and the University Clinical Centre Tuzla.

In this study we analyzed the clinical, epidemiological and virological characteristics of laboratory confirmed influenza cases at the SARI sentinel site at the CCUS, during the 2016/17, 2017/18 and 2018/19 seasons.

MATERIALS AND METHODS

SARI sentinel surveillance system

SARI sentinel surveillance lasted from week 40 to week 20 of the following year. Severe cases of influenza are monitored through a surveillance system that includes laboratory and clinical data.

The CCUS is a leading professional, scientific and educational health institution in Bosnia and Herzegovina. It has 43 clinics and 1524 beds in 33 separate buildings, and 3365 employees. It covers about 415000 residents of Sarajevo Canton.

Clinics of Pulmonary Diseases, Infectious Diseases, Intensive Care Unit and Pediatrics participate in the sentinel SARI surveillance system. Data and samples were collected from all cases meeting the SARI case definition.

SARI sentinel staff (physicians or other trained medical staff) complete the forms and collect samples in accordance with the guidelines. Completed forms and samples are sent to the laboratory. Laboratory results are sent to the sentinel doctor and to the cantonal/federal PHIs on weekly basis. Laboratory and epidemiological data are entered into the SARI surveillance database.

Clinical Microbiology located in the CCUS contains 12 laboratories and 3 departments (Department for Bacteriology, Department for Mycology and Parasitology and Department for Serology and Molecular Diagnostics). The laboratories perform about 120 000 analyses per year. Since 2009, the Clinical Microbiology has been included in the external quality control program for the detection and typing of influenza viruses by molecular methods and has acquired a certificate of quality from the World Health Organization (WHO) and Global Influenza Surveillance and Response System (GISRS). In addition to sentinel surveillance, the laboratories also participate in the influenza surveillance program from non-sentinel sites in FB&H. The registration process for the National Referral Center for Influenza is ongoing.

This study included only laboratory confirmed influenza SARI cases admitted at four sentinel clinics of the CCUS and reported during three seasons (2016/17, 2017/18 and 2018/19). For those SARI cases who were omitted from the standardized reporting form, but were tested positive and entered into the laboratory database, clinical data were retrospectively reviewed from medical files.

Case definition

A SARI case is defined as an acute respiratory infection with: history of fever or measured fever of ≥38°C and cough, with onset within the last 10 days and requires hospitalization (3).

Laboratory investigation

From each patient that met the SARI case definition, a nasopharyngeal swab was collected, labeled with the patient's ID number, immediately sent to the laboratory and evaluated for the presence of influenza virus (Clinical Microbiology at CCUS) via the BD universal viral transport (UVT) system. Upon arrival of the sample in the laboratory, real-time RT-PCR (rRT-PCR) was used for detection. The CDC test was chosen as the reference method given its proven performance (4).

Viral RNA was extracted with QIAamp Viral RNA Mini Kits (Qiagen, Germany) according to the manufacturer's instructions. (QIAamp Viral RNA Mini Handbook 03/2018). The primer and probe sequences from previously reported rRT-PCR assays developed at the CDC were used for the detection of influenza A and B viral RNA. The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel consists of nucleic acid amplification assays that detect influenza A and B viruses and further characterize influenza A subtypes A/H1, A/H1pdm09, A/H3, and influenza B lineages B/Victoria and B/Yamagata (4). Amplification was performed by one-step RT-PCR using the SuperScript III One-Step RT-PCR System with Platinum™ (Invitrogen). Real-time RT-PCR was performed for influenza virus detection, first for detection of Influenza A or

Influenza B. After that all positive samples were tested on influenza A subtyping (influenza A H3, influenza A pdmH1) and influenza B lineage determination (Yamagata and Victoria).

Analyses were performed on the Applied Biosystem platform (ABI 7500). All specimens with rRT-PCR cycle threshold (CT) values <37 were recorded as positive, while CT values ≥37 were recorded as negative.

Data analysis

Patient characteristics were summarized using descriptive statistics. Differences between categorical and continuous variables were calculated using Pearson's Chi-square test, Fisher's exact test and Student's t-test, as appropriate. After univariable analysis, variables that had a p-value <0.08 were introduced into a multivariable analysis. Logistic regression was used to identify risk factors for ICU admission related to influenza infection. Analyses were performed using Stata 15.

RESULTS

Throughout the 2016/17 season, 31 laboratory confirmed influenza cases from SARI sentinel sites were reported which represent 49.2% (31/63) of all reported SARI cases; 60 cases in the 2017/18 season (48.0% or 60/125), and 83 cases in the 2018/19 season (37.2% or 83/223).

Most influenza positive SARI cases were aged 30-64 years (2017/18 48.3% of cases and 42.0% of cases in 2018/19), except during the 2016/17 season when cases among the older age group (65+ years) were most prevalent (51.6% of cases), followed by persons aged 30-64 years (49.2%) (Table 1).

The most frequently reported comorbidities among influenza cases during the examined seasons were chronic respiratory disease (43.8% in 2018/19), cardiac disease (27.6% in 2016/17) and immunodeficiency (19.2% in 2017/18), though percentages varied across seasons (Table 1).

The highest percentage of influenza cases admitted to ICU was during the 2016/17 season with 51.6%. In the other two seasons almost 40% of SARI cases required admission to ICU. The majority of cases required non-invasive ventilation. During the 2018/19 season seven people died; one death was reported in the 2017/18 season and no death cases in the 2016/17 season. For most of the influenza cases, vaccination status was registered, and majority stated that they had not received the seasonal flu vaccine (Table 1).

Table 1 **Characteristics influenza-positive SARI cases, SARI sentinel site FB&H, influenza seasons 2016-2018.**

Influenza season	2016/17 (n= 31)	2017/18 (n= 60)	2018/19 (n=81)
Age and sex			
Male	14 (45.2)	42 (70.0)	42 (51.9)
Female	17 (54.8)	18 (30.0)	39 (48.2)
0-4	0 (0)	3 (5.0)	12 (14.8)
5-14	1 (3.2)	17 (28.3)	8 (9.9)
15-29	0 (0)	3 (5.0)	3 (3.7)
30-64	14 (45.2)	29 (48.3)	28 (34.6)
65+	16 (51.6)	8 (13.3)	30 (37.0)
Risk factors	(n=29)	(n=52)	(n=73)
Chronic respiratory disease	1 (3.5)	8 (15.4)	32 (43.8)
Diabetes	0	3 (5.8)	5 (6.9)
Cardiac disease	8 (27.6)	7 (13.5)	19 (26.0)
Neuromuscular disease	2 (6.9)	4 (7.7)	3 (4.1)
Obesity (Body mass index > 30	2 (6.9)	0	2 (7.4)
Pregnancy	0	1 (1.9)	1 (1.4)
Hepatic disease	0	0	0
Immunodeficiency	0	10 (19.2)	1 (1.4)
Haemodylisis	1 (3.5)	0	1 (1.4)
Cancer	0	0	6 (8.2)
Not reported	2	5	4
Influenza severity	(n=31)	(n=58)	(n=81)
SARI non ICU	15 (48.4)	35 (60.3)	50 (61.7)
SARI ICU	16 (51.6)	23 (39.7)	31 (38.3)
Mechanical ventilation	15 (50.0)	16 (27.6)	23 (28.4)
Death	0	1 (1.8)	7 (8.9)
Influenza virus types/ subtypes	(n=31)	(n=60)	(n=80)
Influenza A	1 (3.2)	4 (6.7)	0
Influenza A(H3)	30 (96.8)	4 (6.7)	47 (58.0)
Influenza A(H1N1)pdm09	0	28 (46.7)	34 (41.98)
Influenza B	0	24 (40.0)	0
Seasonal vaccination	(n=31)	(n=60)	(n=81)
Unvaccinated	29 (93.6)	55 (91.7)	79 (97.5)
Not reported	2 (6.5)	5 (8.3)	2 (2.5)

Virus A was predominant among hospitalized influenza cases across the three seasons studied, varying from 100% during the 2016/17 and 2018/19 seasons to 60% during the 2017/18 season. Virus B was detected only during the 2017/18 season (40% of cases). When compared with subtypes, the virus A(H3N2) was overrepresented during the 2016/17 season and co-circulated with A(H1N1)pdm09 during the 2018/19 season (Table 1, Figure 1). Clinical and epidemiological data were missing for seven cases.

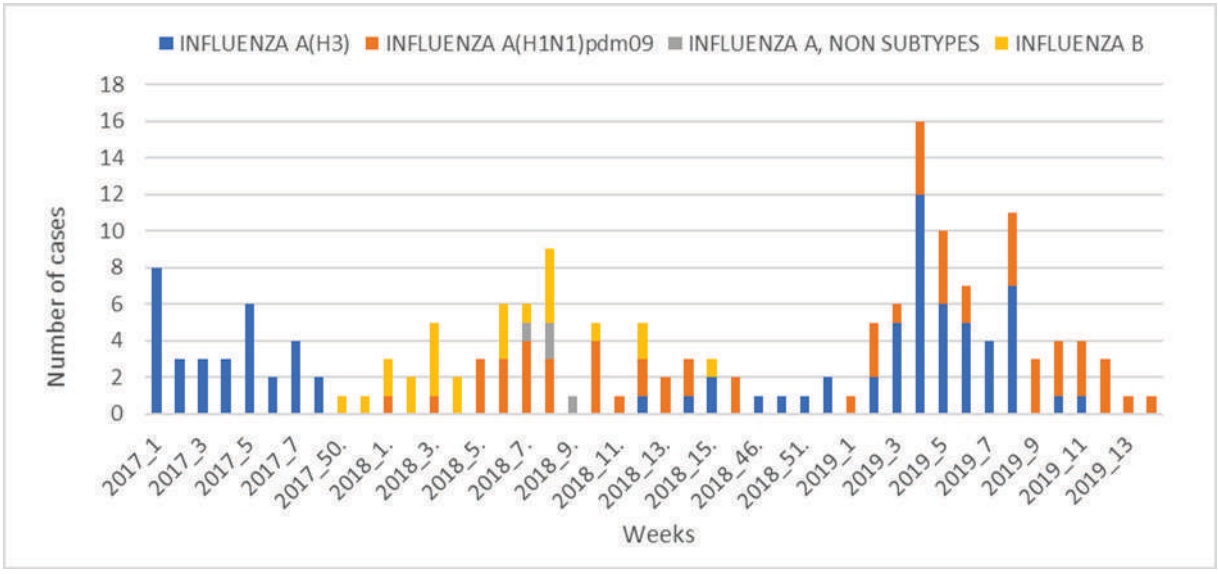

Figure 1 **Influenza-positive SARI cases by influenza virus type and subtype during 2016-2018 seasons.**

Only influenza virus types/subtypes and chronic health conditions were significantly different between influenza patients who were admitted to ICU and required mechanical ventilation and other hospitalized influenza cases (Table 2).

Table 2 **Univariable analysis of factors related to patient admisson to ICU requiring machanical ventilation due to influenza infection during the 2016-2018 seasons.**

	ICU (n=54)	Non-ICU (n=115)	p-value
Sex (male)	28 (51.9)	68 (59.1)	0.373
Age, years (range)	46.9 (1-91)	46.0 (1-91)	0.8554
Influenza A(H3) Influenza A(H1N1)pdm09 Influenza A* Influenza B	28 (51.9) 23 (42.6) 0 3 (5.6)	53 (46.1) 38 (33.0) 4 (3.5) 20 (17.4)	0.08
Chronic respiratory disease	15 (27.8)	26 (22.6)	0.465
Diabetes	1 (1.9)	7 (6.1)	0.437
Cardiac disease	14 (25.9)	20 (17.4)	0,195
Neuromuscular disease	1 (1.9)	8 (6.96)	0.273
Obesity (Body mass index > 30	3 (5.6)	1 (0.9)	0.097
Pregnancy	0	2 (1.7)	1.000
Immunodeficiency	5 (9.3)	6 (5.2)	0.322
Chronic renal diseases	0	2 (1.7)	1.000
Carcinoma	1 (1.9)	5 (4.3)	0.665
0 risk factors	11 (20.4)	42 (36.5)	0.005**
1 risk factor	36 (66.7)	48 (41.7)	0.030***
2 or more risk factors	2 (3.7)	14 (12.2)	

*excluded from analysis
**when comparing all 3 groups
***when comparing two groups

In the multivariable model, influenza virus types/subtypes were found to be associated with increased risk for admi ssion to ICU requiring mechanical ventilation: influenza A(H3) with OR=8.8 and influenza A(H1N1)pdm09 with OR=10.4 (reference group: influenza B) (Table 3).

Table 3 **Multivariable analysis of variables related to patient admisson to ICU requiring mechanical ventilation due to influenza infection during the 2016-2018 seasons.**

Variable	OR	p	(95% CI)
Influenza B Influenza A(H3) Influenza A(H1N1)pdm09	Ref. 8.8 10.4	 0.041 0.029	 1.1 – 70.0 1.3 – 84.1
Risk factors yes/no	2.1	0.077	0.9 – 4.6

DISCUSSION

Severe acute respiratory infection (SARI) is an important cause of morbidity and mortality worldwide. Influenza, in particular, is a very common and sometimes serious infectious disease, with seasonal peaks in winter months and with antigenic drift and shift threat for global pandemics (5).

We present an analysis of data obtained from SARI sentinel surveillance site and virus characterization to provide an overview of three consecutive influenza seasons in FB&H. The majority of laboratory-confirmed influenza SARI cases were detected between December and March, but seasonal peaks occurred in different weeks.

In other European countries influenza activity started to increase in early December (week 49/2018) and continued into early February (week 5/2019). The 2018/19 season started one week later than the B-dominated 2017/18 season, and three weeks later than the A(H3N2)-dominated 2016/17 season (6).

A SARI sentinel surveillance system was established in FB&H in the 2014/15 season and was strengthened in the following years. Sentinel surveillance is the most efficient and useful way for monitoring and assessing the severity of seasonal outbreaks and the impact on high-risk populations (3). ICU is included in sentinel clinics. Several countries set up an ICU surveillance system during the influenza A(H1N1) pandemic in 2009/10 (7). Studies found a greater under-detection of influenza in the adult ICU (8).

In our study we analyzed characteristics and risk factors of severe influenza cases in intensive care units compared with SARI non-ICU cases. The majority of cases reported they had not received a seasonal influenza vaccine, despite more than 90% of this case population showing indications for vaccination. Host characteristics influenced severity and the need for ICU or intermediate care beds and/or ventilatory assistance. Low coverage of the influenza vaccine decreased the protective effect of seasonal vaccination, which increased risk of hospitalizations among elderly patients and those with diabetes mellitus, cardiac disease and chronic respiratory disease.

The proportion of patients needing ventilatory support in this study ranged between 28% and 50%. Data obtained from surveillance systems enables healthcare authorities to better understand the timing of peak influenza activity and to mobilize resources accordingly, such as vaccines and antivirals (9).

In this study, the percentage of laboratory-confirmed influenza cases among SARI cases ranged between 37-49%. The lowest percentage of positivity was recorded in the 2018/19 season, when the most samples were taken. The percent of specimens testing positive for influenza varies by year and country ranging from 4.3-9.0% positive among children aged <5 years, 10.0-23.1% among those aged 5-64 years, and 6.8-17.6% among those aged 65+ years (10).

Applying the virologic surveillance data to attribute respiratory hospitalizations to influenza, the observed crude rates of influenza-associated respiratory hospitalizations varied from 3 per 100,000 in people aged 5-64 years in Cuba to 487 per 100,000 in among people 65+ years in the United States of America (10).

During the peak winter months (December and January) positive cases, with any of the three viruses, represented about 15% of respiratory virus infection-related hospitalizations (11). Studies suggest there is a difference between age groups affected by different influenza viruses. The higher burden of influenza A(H3N2) infection is associated with the greater susceptibility to this virus subtype among elderly populations, as older age groups represent the largest population at risk for severe and complicated influenza in industrialized countries (12). A number of studies have found that influenza-associated hospitalizations and deaths are highest in seasons dominated by A(H3N2), suggesting that the clinical presentation and severity of influenza may be worse for this subtype (13,14). However, there was some evidence that the clinical outcomes of influenza illness could be worse for the A(H1N1)pdm09 strain (15). In our study we found that patients with influenza A(H1N1)pdm09 have increased risk for admission to ICU requiring mechanical ventilation.

The surveillance system has several limitations, particularly as a result of underreporting, lack of representativeness, and lack of timeliness, and there is a need for periodic evaluations.

Surveillance data collected over three seasons underscore the importance of prevention by vaccination in order to avoid serious complications among the most vulnerable persons, such as ICU admission and mechanical ventilation.

CONCLUSION

This study emphasizes the importance of a SARI sentinel surveillance system that includes ILI/ARI syndromic and virological surveillance data, and the need for system improvement for assessment of the burden of seasonal influenza and mobilization of resources for prevention and control. This is especially relevant in light of an overall low vaccination uptake and antiviral use in our country, since information on risk factors will help in targeting and prioritising vulnerable populations.

REFERENCES

1. World Health Organization. Fact sheets: influenza (seasonal). Available at: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal) [Accessed Oct 2019].
2. ECDC. Factsheet about seasonl influenz. Available at: https://ecdc.europa.eu/en/seasonal-influenza/facts/factsheet [Accessed Oct 2019].
3. World Health Organization. Global Epidemiological Surveillance Standards for Influenza. Available at : http://www.who.int/influenza/resources/documents/WHO_Epidemiological_Influenza_Surveillance_Standards_2014.pdf. [Accessed Oct 2019].
4. World Health Organization. CDC Protocol of real time RTPCR for influenza A(H1N1). Available at: http://www.who.int/csr/resources/publications/swineflu/CDCRealtimeRTPCR_SwineH1Assay-2009_20090430.pdf?ua=1) [Accessed Oct 2019].
5. Hayward AC, Fragaszy EB, Bermingham A, Wang L, Copas A, Edmunds WJ, et al. Comparative community burden and severity of seasonal and pandemic influenza: Results of the Flu Watch cohort study. Lancet Respir Med. 2014;2:445-54.
6. Flu News Europe. Joint ECDC-WHO/Europe Weekly influenza update, season 2016-2017, Week 20, 2017. Available from: https://flunewseurope.org/Archives.
7. Bonmarin I, Belchior E, Bergounioux J, Brun-Buisson C, Mégarbane B, Chappert JL, et al. Intensive care unit surveillance of influenza infection in France: the 2009/10 pandemic and the three subsequent seasons. Eurosurveillance. 2015;20(46).
8. Ortiz JR, Neuzil KM, Shay DK, Rue TC, Neradilek MB, Zhou H, et al. The burden of influenza-associated critical illness hospitalizations. Crit Care Med. 2014;42(11):2325-32.
9. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data–from vision to reality. Euro Surveill. 2017;22(13):30494.
10. Palekar RS, Rolfes MA, Arriola CS, Acosta BO, Guidos PA, Vargas XB et al. Burden of influenza-associated respiratory hospitalizations in the Americas, 2010-2015. PLoS One. 2019;14(9):e0221479.
11. Cohen R, Babushkin F, Geller K, Finn T. Characteristics of hospitalized adult patients with laboratory documented Influenza A, B and Respiratory Syncytial Virus - A single center retrospective observational study. PLoS One. 2019;14(3):e0214517.
12. Mertz D, Kim TH, Johnstone J, Lam PP, Science M, Kuster SP, et al. Populations at risk for severe or complicated influenza illness: systematic review and meta-analysis. BMJ. 2013;347:f5061.
13. Kaji M, Watanabe A, Aizawa H. Differences in clinical features between influenza A H1N1, A H3N2, and B in adult patients. Respirology. 2003;8(2):231-3.
14. Irving SA, Patel DC, Kieke BA, Donahue JG, Vandermause MF, Shay DK, et al. Comparison of clinical features and outcomes of medically attended influenza A and influenza B in a defined population over four seasons: 2004-2005 through 2007-2008. Influenza Other Respir Viruses. 2012;6(1):37-43.
15. Chaves SS, Aragon D, Bennett N, Cooper T, D'Mello T, Farley M, et al. Patients hospitalized with laboratory-confirmed influenza during the 2010-2011 influenza season: exploring disease severity by virus type and subtype. J Infect Dis. 2013;208(8):1305-14.

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Arrival time of emergency medical teams on out-of-hospital cardiac arrest and percentage of successful reanimations in the 2015-2019 period

Vrijeme dolaska ekipe hitne medicinske pomoći na vanbolnički srčani arest i procenat uspješnih reanimacija u periodu od 2015-2019. godine

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ABSTRACT

Introduction: sudden cardiac arrest causes nearly 20% of all cardiovascular (CVD) deaths in Europe. It is lethal within minutes if left untreated, with a survival rate of 5-20%. Urgent medical treatment includes cardiopulmonary resuscitation (CPR) and early defibrillation. Aim: to explore how fast Emergency Medical Teams (EMTs) arrive on site of an incident in Canton Sarajevo and to explore the rate of achieved return of spontaneous circulation (ROSC). Materials and methods: this is a retrospective descriptive study which included all out-of-hospital cardiac arrest (OHCA) events occurred between 1 January 2015 and 31 December 2019 in the Canton Sarajevo. All patients from above mentioned period were consecutively included in the study, without exclusion criteria related to their age, gender or any other personal factors. Data was extracted from data registry of the EMTs of Canton Sarajevo. Results: during the study period a total of 1274 OHCA were recorded. An average arrival time of EMTs for cases of successful ROSC was 5.37±0.38 minutes, and 7.27±0.42 minutes for unsuccessful ones. The successful ROSC was achieved in 292 cases (22.54%). Conclusion: the time of EMTs arrival in Sarajevo Canton on OHCA cases is similar to the world standards. There is a low number of bystanders who start CPR and these results suggests the urgent need for mass education of citizens and installation of Automatic External Defibrillators (AEDs) on public places. Good survival rates are a result of good organization, high quality and good equipment and education of the emergency medical staff in Canton Sarajevo.

Keywords: out-of-hospital cardiac arrest, emergency medical services, reaction time, survival rate

SAŽETAK

Uvod: iznenadni srčani zastoj uzrokuje blizu 20% svih smrtnih slučajeva u Evropi. Smrtonosan je unutar nekoliko minuta ako se ne tretira, astopa preživljavanjase kreće od 5 do 20%. Urgentni medicinski tretman uključuje kardiopulmonalnu reanimaciju (CPR) i ranu defibrilaciju. Cilj: pokazati koliko brzo ekipe hitne pomoći dolaze na lice mjesta u Kantonu Sarajevo (KS), te pokazati procenat povratka spontane cirkulacije (ROSC). Materijali i metode: članak je deskriptivnog retrospektivnog karaktera. Korišteni su podaci iz registra Centra za edukaciju Hitne medicinske pomoći Kantona Sarajevo. Uključeni su svi vanbolnički srčani zastoji u period od 1. januara 2015. do 31. decembra 2019. godine u Kantonu Sarajevo (KS). Pacijenti su uključeni neovisno o dobi, spolu i ličnoj historiji. Također, uključeni su pacijenti koji su reanimirani (sa kompresijama grudnog koša i/ili defibrilacijom) od strane očevidaca i medicinskih službi. Rezultati: ukupan broj vanbolničkih srčanih zastoja u definisanom period bio je 1274. Prosječno vrijeme dolaska ekipa hitne medicinske pomoći na slučajeve uspješne reanimacije bilo je 5,37±0,38 mnuta, dok je prosječno vrijeme dolaska za neuspješne reanimacije iznosilo 7,27±0,42 minuta. Uspješan ROSC ostvaren je u 292 slučaja (22,54%) Zaključak: brzina dolaska ekipa Hitne medicinske pomoći KS na vanbolnički srčani zastoj odgovara svjetskim standardima. Veoma visok procenat uspješne reanimacije rezultat je dobre organizacije Hitne medicinske pomoći KS, dobre opreme i edukacije osoblja.

Ključne riječi: vanbolnički srčani zastoj, hitna medicinska pomoć, vrijeme započinjanja intervencije, procenat preživljavanja

INTRODUCTION

Cardiac arrest presents unexpected circulatory arrest, occurring within one hour of onset of acute symptoms (1). It causes loss of heart function, with an onset in the point when the heart stops beating or when the heart beats are not adequate to ensure perfusion that is essential for life. It can start suddenly and is often fatal if adequate steps are not made immediately. The chances for survival are higher if termination of mechanical activity of the heart happens in a hospital environment, but most of the cases account for Out-of-hospital cardiac arrest (OHCA). OHCA presents loss of cardiac function and lack of pulse, and it is the main cause of

mortality, with survival rates 4.0-39.3% (2, 3). Cardiopulmonary resuscitation (CPR) should be done as soon as possible, and hospitalization is of utmost importance (3).

Sudden cardiac arrest causes almost 20% of all deaths in Europe and it is lethal if left untreated in the first couple of minutes, with the survival rate of 5-20% (4). On the contrary to the popular beliefs, sudden cardiac arrest is not just a heart attack. Heart attack happens when one or more coronary arteries are clothed, preventing the heart muscle to receive enough oxygenated blood. If the oxygenated blood cannot get to the heart muscle, the heart gets damaged. Opposite to this, cardiac arrest happens when the heart electrical system is deranged due to heart attack and other heart conditions, but also due to hypovolemic shock, hypoxia, drug overdose, electrocution etc. The heart beat becomes irregular and dangerously fast. The heart chambers flutter or fibrillate and the blood cannot be transported to the rest of the body. In the first few minutes the biggest problem is that the blood flow to the brain is decreased drastically and the victim loses consciousness. Death follows if urgent medical treatment is not started.

Coronary artery disease is the most common cause of cardiac arrest, accounting for up to 80% of all cases (5). The most common cause of non-ischemic cardiac arrest is hypertrophic cardiomyopathy that develops in correlation with obesity, alcoholism and fibrosis (6).

There are over 400.000 victims of OHCA per year in Europe, with survival rate lower than 10% (1). In United States of America, there are around 350.000 victims of OHCA yearly, while in Russian Federation 200.000-250.000 patients yearly dies from cardiac diseases (6).

Urgent treatment of sudden cardiac arrest implies cardiopulmonary reanimation (CPR) and early defibrillation. (1) Compressions and defibrillation within 3-5 minutes from the cardiac arrest results in survival rate of 49-75%. Every minute of defibrillation delay reduces survival rate for 10-15%. International studies that were published from 1990 to 2005 showed that starting CPR 5 to 8 minutes after the onset of cardiac arrest correlates with the average survival rate of 6,4%. In most of the countries, the average time from the call to the emergency medical service to the arrival of Emergency Medical Teams EMTs is 5-8 minutes (7).

Considering that very few laic bystanders start basic life support in our country, the survival of victims of OHCA mostly depends on the prompt reaction of the EMTs i.e., and their training, equipment and speed of arrival. Therefore, it is very important that the EMTs are well-organized, so that teams can reach the victim of a cardiac arrest as soon as possible. The Emergency Medical Centre of Canton Sarajevo is organized in a way that there are 24/7 emergency medical stations in nine municipalities in Canton Sarajevo. Each station has an EMT that consists of a medical doctor (emergency medicine specialist) and two emergency medical technicians with mandatory additional training in basic life support (BLS), advanced life support (ALS), pre-hospital trauma life support (PTLS). The ambulance cars on each station contain full equipment needed for reanimation.

All medical doctors and emergency medical technicians are obliged to learn and follow European Research Council (ERC) and American Heart Association (AHA) algorithms in reanimation. Continuous education and training for medical staff are regularly provided in the Emergency Medical Centre of Canton Sarajevo.

AIM

The aim of this study is to show successfulness and work quality of the emergency medical teams in Sarajevo Canton. Show on which segments we need to work more and better to increase the number of successful reanimations.

MATERIALS AND METHODS

This is a retrospective descriptive study which included all out-of-hospital cardiac arrest (OHCA) events occurred from 1 January 2015 to 31 December 2019 in Canton Sarajevo. All patients from above mentioned period were consecutively included in the study, without exclusion criteria related to their age, gender or any other personal factors.

Data was extracted from data registry of the Educational Center of Emergency Medical Center of Sarajevo, Sarajevo, Bosnia and Herzegovina.

RESULTS

EMTs had a total of 1274 CPRs in the period 2015-2019 on the area of Canton Sarajevo. Out of the total number, 292 patients (22.92%) account for successful reanimations that reached ROSC.

Furthermore, we analyzed long-term outcomes of successful reanimations and found that 142 patients (11.15%) had no neurological deficits, reaching both cardiopulmonary and mental status recovery (Table 1).

Table 1 **Reanimation success rates of Emergency medical center of Canton Sarajevo in the 2015-2019 period.**

Outcome	Number of patients (n)	Rate (%)
Unsuccessful	982	77.08
Relatively successful	150	11.77
Completely successful	142	11.15
TOTAL	1274	100

General average arrival time of emergency medical center teams was 6.15±0.34 minutes for all cases. Furthermore, average arrival time was 5.37±0.38 minutes in cases of successful reanimations and 7.27±0.42 minutes in unsuccessful cases.

Total number of witnessed OHCA is 183 or 14.36% from the total number of reanimations. Ratio of successful to unsuccessful outcomes of OHCA is 50.82% to 49.18%. For the purpose of comparison, our Emergency medical Centre staff had 46 cases of cardiac arrest in the emergency room, of which 50% had successful outcome.

Out of all cases of OHCA that took place in 4 years period in Sarajevo Canton, 66.80% occurred in patients' homes, while 29.43% happened in public places (Table 2).

Table 2 **Outcome of reanimation according to place of reanimation.**

	Successful		Unsuccessful		Total	
Place	N	%	n	%	n	%
Home	144	16.86	707	83.14	851	66.79
Public places	146	38.94	229	61.16	375	29.43

From the total number of OHCA (n=1274), medical professionals started with basic life support (CPR) in only 32 cases (3.45%) and laic bystanders started CPR in only 12 cases (0.94%). In all the other cases (96.54%), CPR was provided only by EMTs of the Emergency Medical Center of Canton Sarajevo.

DISCUSSION

Sudden cardiac death caused by cardiac arrest is the most important global public health problem, for which is estimated to be the cause of 15-20% of all deaths (8). Primary cardiac etiology is widespread in patients with OHCA. Because of that there are urgent medical needs for improvement in prevention and treatment of OHCA. Although the number of reanimations globally is much higher, the biggest number of individuals that have an OHCA still does not survive. The incidence of OHCA is 0.04% to 0.1% in Europe and about 0.1% in the USA (5). According to the available data (Ruzman, et al.) (9), about 6000 people a year develop a cardiac arrest per year. It is estimated that in Bosnia and Herzegovina there is about 3000 to 4000 out of hospital cardiac arrests per year or 0.11% (10). Total number of interventions of our emergency medical teams for OHCA in the period of five years was 1274. That is 254.8 cases per year, or 0.06% of 420.000 citizens living in Canton Sarajevo.

Sudden cardiac death usually develops in older people that have previously developed a heart condition, and it is rare to find it among young people, in which it usually occurs due to hereditary diseases (11). Our findings do not differ a lot from other published studies, as average age of patients in our sample was 63.49 years.

When cardiac arrest occurs, it is necessary to begin cardiopulmonary reanimation in the first 4-5 minutes. Those 4-5 minutes are also called "golden minutes", in which the EMTs hurry to get to the patient that had an OHCA, so that the patient has a chance to survive. Unfortunately, not a single emergency medical service in the world, no matter how well-organized it is, cannot always reach the victim of a sudden cardiac arrest within 3 to 5 minutes in order to start with resuscitation and avoid permanent brain damage. Even in highly developed countries, EMTs cannot respond to a cardiac arrest in ideal time - global average time from the call to the EMS to the arrival of the EMTs is 5-8 minutes (7). It implies that the survival rate of patients with OHCA depends on the arrival time of EMTs on site. Our findings support those claims. Speed of arrival of the EMTs of Sarajevo Canton on OHCA cases is similar to world standards. Very high percentages of successful resuscitation are a result of good emergency medical organizations in Canton Sarajevo, good equipment and educated medical staff.

Another indicator of EMTs short arrival time importance is witness cardiac arrests. The analysis showed that reanimations in witnessed cardiac arrest have a little bit higher success rate (50.82%) than reanimations done in medical facilities (50.0%). A recent study on 20453 victims of OHCA, with data from Copenhagen, Stockholm, west Scotland and Amsterdam (7), showed that 13% of patients survived at least 30 days after reanimation. In the other hand, our study showed that the rate of successful reanimations was 22.29%. These rates show percentage of OHCA survivors who reached return of spontaneous circulation (ROSC), without more details about neurological deficits and mental status of the survivors. It is important to bear in mind that there is a significant number of comatose OHCA victims, who never experienced return of brain functions. These patients and their families have very poor quality of life. That is the reason that we decided to examine the number of reanimated patients that have no neurological deficit, i.e., patients that experienced full return of quality of life as it was before the incident. Our study showed that there were 142 OHCA survivors in the sample (11.15%) that remained without any neurological deficits.

Considering that our medical workers are very often witnesses of cardiac arrests, it was important to determine successfulness of these cases. In our sample, the survival rates of witnessed OHCA were the same regardless of location of the incident (medical facility or the field). However, when we further analyze field reanimations, the successfulness rates are around two times higher in cases of OHCA that took place in public places, comparing to those that happened at patients' homes. It is also important to note that around 1/3 of field reanimations happened at public places.

The European resuscitation council (ERC) project called "EuReCa two" (12) analyzed the role of general population in resuscitation across 28 European countries in the period of three months (01/10/2017-31/12/2017). According to their results, the bystander cardiopulmonary resuscitation (CPR) rate ranged from 13% to 82% between countries, with an average of 58%. Our data shows that Canton Sarajevo had bystander CPR rate of only 3.45%. Furthermore, Scandinavian data from the same study showed that a total of 2289 OHCA victims (81% of the total sample) were defibrillated, 59% of them were defibrillated by the EMTs and the rest of them (41%) were defibrillated by first responders or witnesses, i.e., medical laics. In our study, all defibrillations were done by EMTs, without a single one performed by anybody else.

CONCLUSION

Our results showed that EMTs arrival time in Canton Sarajevo was in line with international standards. The survival rate of OHCA was even higher than average reported in the literature, with a very good rate of survivors without any neurological deficits. However, it is noticeable that there is a very low number of bystanders who start CPR in Canton Sarajevo. Also, considering that there were no bystanders who started defibrillation, good survival rates speak in favor of good organization, high quality and good equipment and education of the emergency medical staff in Canton Sarajevo. Results from our study imply the urgent need for mass education of citizens and installation of Automatic External Defibrillators (AED) in public places, as recommended by ERC.

REFERENCES

1. Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J; ESC Scientific Document Group. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC). Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Eur Heart J. 2015;36(41):2793-2867.
2. Le Guen M, Nicolas-Robin A, Carreira S, Raux M, Leprince P, Riou B, et al. Extracorporeal life support following out-of-hospital refractory cardiac arrest. Crit Care. 2011;15(1):R29.
3. Jea JL, Sang JH, Hyoung SK, Kyung SH, Hyun HC, Kye TP, et al. Out-of-hospital cardiac arrest patients treated with cardiopulmonary resuscitation using extracorporeal membrane oxygenation: focus on survival rate and neurologic outcome. Scand J Trauma Resusc Emerg Med. 2016;24:74.
4. Hanno LT, Dagres N, Böttiger B, Schwartz PJ; ESCAPE-NET Investigators. European Sudden Cardiac Arrest network: towards Prevention, Education and New Effective Treatments (ESCAPE-NET): A major European Horizon 2020 project focused on cardiac arrest. European Heart Journal. 2018;39(2):86-8.
5. Yow AG, Rajasurya V, Sharma S. Sudden Cardiac Death. StatPearls Publishing LLC.; 2020.
6. Linchak R, Boytsov S, Ardashev A, Kuzovlev A. Public-Access Defibrillation in Sudden Cardiac Arrest. In: Cardiac Diseases and Interventions in 21st Century. 2018.
7. Zijlstra JA, Koster RW, Blom MT, Lippert FK, Svensson L, Herlitz J, et al. Different defibrillation strategies in survivors after out-of-hospital cardiac arrest. Heart. 2018; 104(23):1929-36.
8. Hayashi M, Shimizu W, Albert CM. The spectrum of epidemiology underlying sudden cardiac death. Circ Res. 2015;116(12):1887-906.
9. Ružman T, Tot O K, Ivić D, Gulam D, Ružman N, Burazin J. In-hospital cardiac arrest: can we change something? Wiener klinische Wochenschrift. 2013;125(17-18):516-23.

10. Zavod za javno zdravstvo FBiH. Zdravstveno stanje stanovništva i zdravstvena zaštita u Federaciji Bosne i Hercegovine. 2015.
11. Katritsis DG, Gersh BJ, Camm AJ. A Clinical Perspective on Sudden Cardiac Death. Arrhythm Electrophysiol Rev. 2016;5(3):177-82.
12. Gräsner JT, Wnent J, Herlitz J, Perkins GD, Lefering R , Tjelmeland I, et al. Survival after out-of-hospital cardiac arrest in Europe - Results of the EuReCa TWO study. Resuscitation. 2020;148:218-26.

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Clamshell incision as a method of choice in the surgical treatment of large tumors of the anterior mediastinum: two case reports

Clamshell incizija kao metoda izbora kod operativnog liječenja gigantskih tumora prednjeg medijastinuma: dva prikaza slučaja

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ABSTRACT

Introduction: Clamshell incision is often used in extensive bilateral tumors, with bilateral lymphadenopathy, and anterior mediastinal tumors that spread to both hemithorax. This approach has proven excellent access to both hilar regions. Aim: to present the significance of bilateral thoracotomy with transverse sternotomy, a clamshell incision in the treatment of large mediastinal tumors. Case report: two patients with large tumors of anterior mediastinum were surgically treated at our clinic. Bilateral thoracotomy with transverse sternotomy - Clamshell incision was used as the method of choice in the surgical treatment of these patients. The definitive histopathological finding in the first patient was mature cystic teratoma, while our second patient had a large colloid nodular goiter. In both patients, the tumor of the anterior mediastinum was completely resected. Their postoperative course was uneventful. Conclusion: Clamshell incision is a significant approach in the treatment of massive mediastinal, lung, and pleural tumors or extensive bilateral lung metastases as well as large tumors of the anterior mediastinum.

Keywords: bilateral tumors, clamshell incision, mediastinum

SAŽETAK

Uvod: Clamshell incizija je čest operativni pristup kod opsežnih obostranih tumora, s bilateralnom limfadenopatijom i tumora prednjeg medijastinuma koji se šire na oba hemitoraksa. Ovim pristupom se odlično prikazuju obje hilarne regije. Cilj: prikazati značaj bilateralne torakotomije s poprečnom sternotomijom - clamshell incizije u tretmanu velikih tumora medijastinuma. Prikaz slučaja: dva pacijenta s velikim tumorima prednjeg medijastinuma hirurški su liječena na našoj klinici. Bilateralna torakotomija s poprečnom sternotomijom - Clamshell incizija korištena je kao metoda izbora u hirurškom liječenju ovih bolesnika. Definitivni histopatološki nalaz kod prvog bolesnika je bio zreli cistični teratom, dok je naš drugi pacijent imao veliku koloidnu nodularnu strumu. U oba bolesnika tumor prednjeg medijastinuma je u potpunosti reseciran. Njihov postoperativni tok bio je bez komplikacija. Zaključak: Clamshell incizija značajan je pristup u tretmanu velikih tumora medijastinuma, pluća i pleure ili opsežnih obostranih metastaza na plućima, kao i velikih tumora prednjeg medijastinuma.

Ključne riječi: bilateralni tumori, clamshell incizija, medijastinu

INTRODUCTION

In the early days of cardiac surgery, operative access to the heart was through a clamshell incision. With the increased popularity and use of median sternotomy, the clamshell incision became an almost forgotten surgical approach due to significantly less pain in patients operated with median sternotomy. Recently, it has found its place again, primarily due to lung transplantation, large mediastinal tumors that spread to both hemithorax, and in oncological thoracic surgery in the removal of bilateral lung metastases. A full clamshell incision combined with a partial upper median sternotomy, the so-called "inverse T-incision", has been described recently (1). This incision allows excellent access to the upper mediastinum and both the pulmonary hili, therefore an effective dissection of the entire

upper third of the thorax. Despite the extensity of the incision, satisfying results in terms of chest wall stability and preservation of the sternocostal arch functionality have been reported. In cases of extremely large mediastinal tumors, when the size of the tumor disables safe resection due to the limited visualization, combined sternotomy/thoracotomy or clamshell thoracotomy, with or without partial upper hemisternotomy remains a viable option (2).

AIM

To present two cases of large mediastinal tumors that were successfully treated using bilateral thoracotomy with transverse sternotomy - clamshell incision.

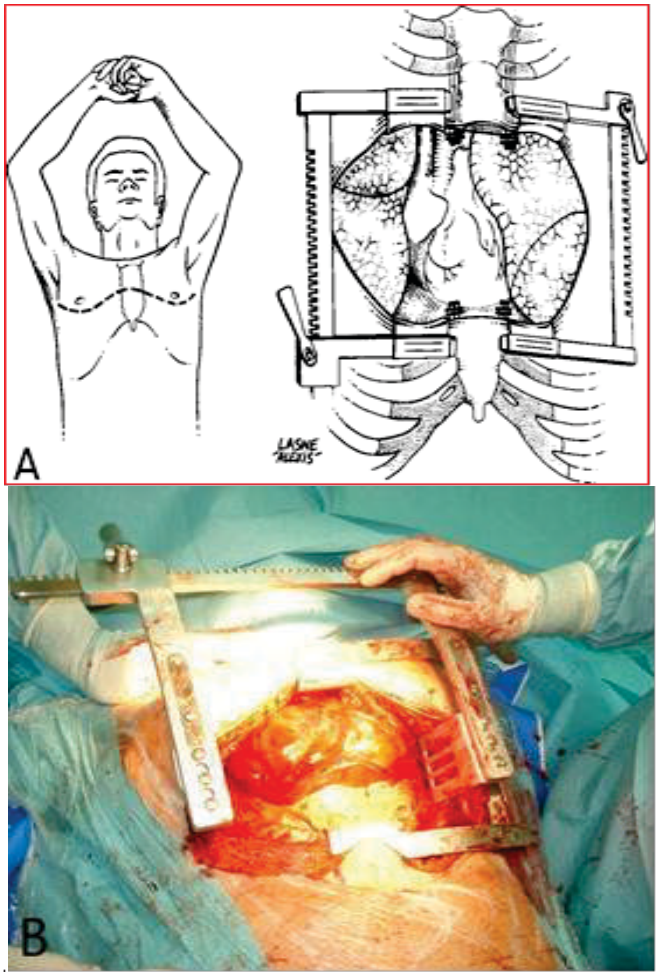

Figure 1A **Schematic representation of the clamshell incision.**
Figure 1B **- Intraoperative view.**

CASE REPORTS

CASE 1

A previously healthy sixteen-year-old male patient was admitted to Clinic of Thoracic Surgery of the Clinical Center University of Sarajevo for further evaluation and surgical treatment of a tumor located in the anterior mediastinum. The patient was previously treated at the Clinic of Hematology for one month. During preoperative preparation, chest X-ray (CXR) (Figure 2A) and magnetic resonance imaging (MRI) (Figure 2B) of the thorax were performed.

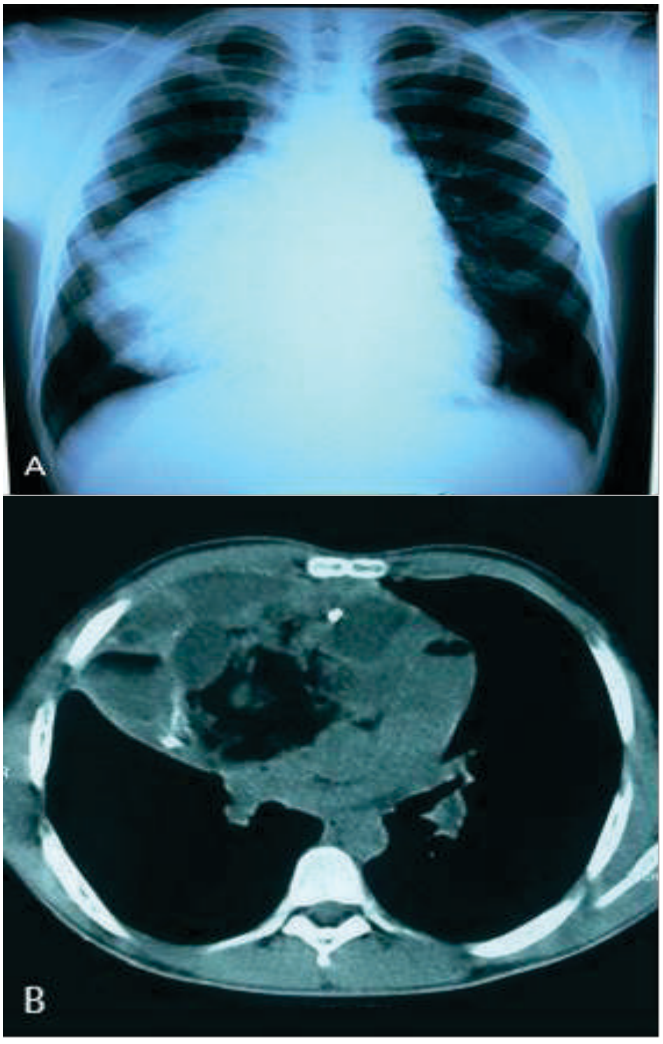

Figure 2A - **Chest X-ray on the day of admission.** B - **Magnetic resonance imaging (MRI) of the thorax in the preoperative preparation of the patient**.

A curvilinear bilateral submammary incision was performed, extending from one midaxillary line to the other side across the anterior aspect of the chest wall. Both internal mammary arteries were ligated at the beginning of the procedure. An irregular mass was identified in the anterior and middle mediastinum, the largest dimension being 16 cm x 12.5 cm x 10 cm (Figure 3A). The tumor was completely resected with careful dissection and separation of both phrenic nerves and recurrent laryngeal nerves from the tumor.

The postoperative course was uneventful. Chest drains were removed successively on the fifth and sixth postoperative day, respectively. The patient was discharged from our Clinic on the seventh postoperative day. Definitive histopathological finding - *mature cystic teratoma.*

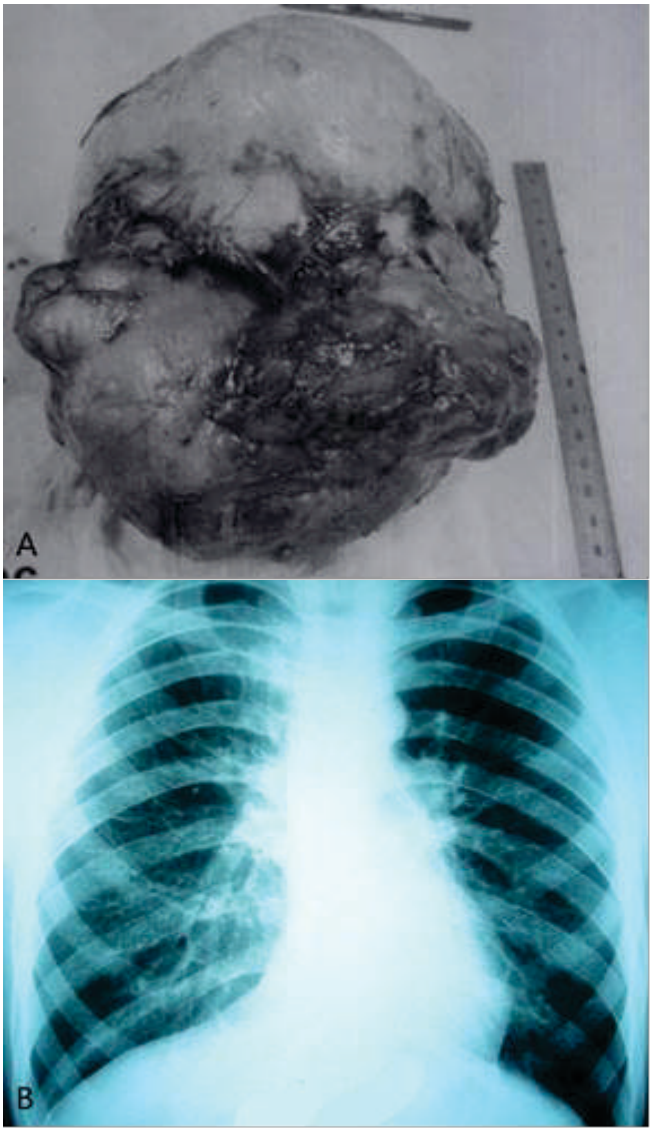

Figure 3A - **Specimen of completely resected mediastinal mature cystic teratoma.** B - **Chest X-ray before discharge from the Clinic**.

CASE 2

A thirty-four-year-old female patient was admitted to the Clinic of Hematology. For the past two years, the patient was complaining of malaise, intermittent pain along the spine, and occasional shortness of breath as well as palpitations. She was also sweating excessively and experienced migraine-type headaches, denied having difficulties swallowing. She underwent thyroid surgery twice and was not taking any medications. During preoperative preparation, CXR (Figure 4A) and MRI (Figure 4B of the thorax were performed.

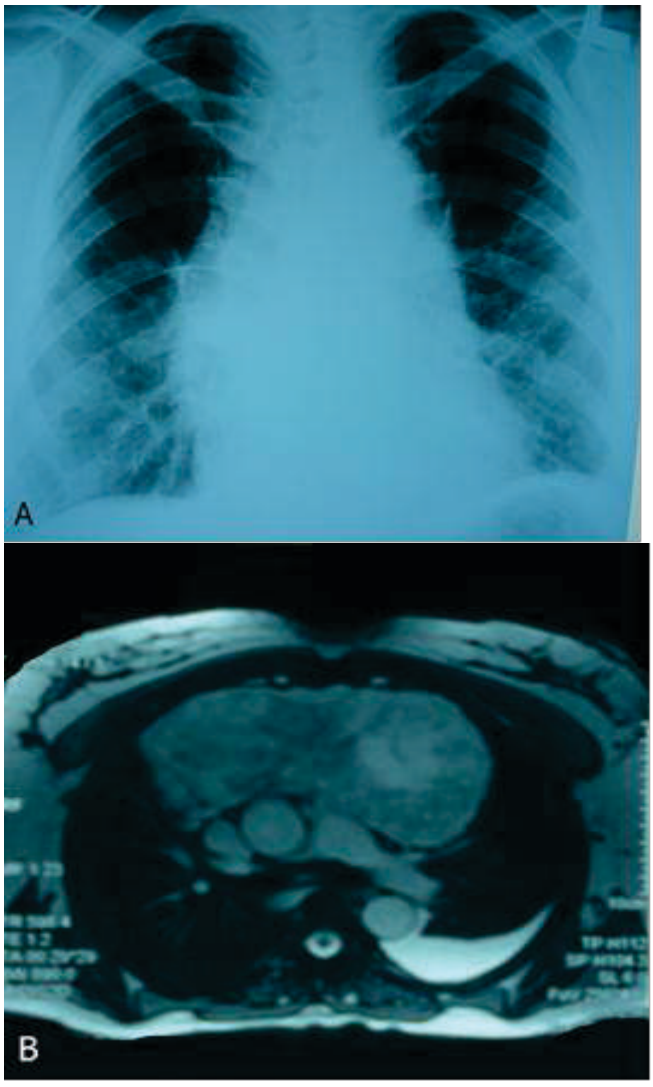

Figure 4A - **CXR on the day of admission.** B - **MRI of the thorax in the preoperative preparation of the patient**.

The MRI of the thorax demonstrated an expansive, sharply limited cystic formation that compresses the horizontal part of the aortic arch and partially superior vena cava and descends to the level of the upper parts of the heart, more to the right, in the area of the superior and middle mediastinum.

The clamshell incision was the surgical approach of choice. After a submammary skin incision, the breasts were separated from the fascia of the pectoral muscle, which enabled access to the fourth intercostal space on both sides. The sternum was cut transversely, and both internal mammary arteries were ligated. A large tumor (dimensions 15 cm × 15 cm × 5 cm) was identified in the anterior mediastinum (Figure 5A). The tumor was then completely resected (Figure 5B). The patient was intubated and transferred to the Intensive Care Unit (ICU) for continuous patient monitoring.

The postoperative course was uneventful. Chest drains were removed successively on the eighth and ninth postoperative day, respectively. The patient was discharged from our Clinic on the tenth postoperative day (Figure 5C). Before discharge, the level of thyroid gland hormones was examined, and the values were in reference

ranges. Definitive histopathological finding - a large colloid nodular goiter.

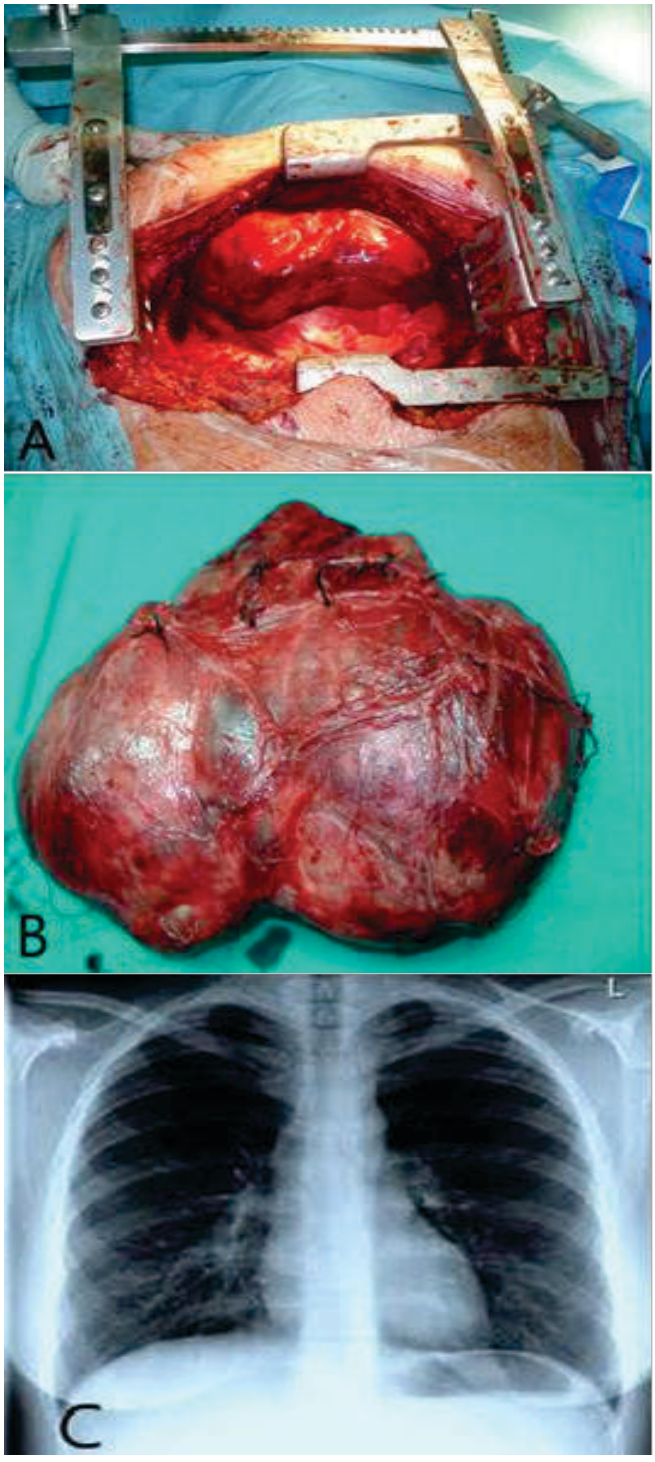

Figure 5A - **Intraoperative finding of the tumor.** B - **Specimen of completely resected colloid nodular goiter.** C - **Chest X-ray before discharge from the Clinic.**

DISCUSSION

Clamshell incision was the surgical approach of choice in the early days of heart surgery. With the increased popularity of minimally invasive surgical procedures, the use of clamshell incision became mostly limited to large tumors of the mediastinum (3).

Recently, it has found its place again, primarily due to bilat eral lung transplantation. Bains, et al. have investigated and confirmed its usefulness in the treatment of bilateral mediastinal masses (4). The choice of this incision requires good preoperative diagnostics, a good understanding of the anatomy of the mediastinum, as well as sufficient knowledge and experience of the surgeon regarding the treatment of large mediastinal masses (5).

This operative technique is often used in the treatment of extensive bilateral lung tumors and large mediastinal tumors that spread to both hemithorax. Median sternotomy is a widely used approach, but it may not afford adequate exposure of mediastinal tumors that extend into the thoracic cavity. Because a component of clamshell incisions is a large transverse incision, it allows good access to the intrapleural space bilaterally (6). In addition to the clamshell incision, there is a modification of the procedure called "hemiclamshell" where unilateral thoracotomy is combined with median sternotomy with the possibility of access to the apex of the lung and the base of the neck - superior sulcus tumor surgery, carotid space and superior vena cava pathologies (7). Several authors have proposed the use of these combined surgical approaches. Kao, et al. described a successful simultaneous clamshell thoracotomy with median sternotomy in a 17-year-old man who developed mediastinal growing teratoma syndrome following two cycles of chemotherapy, after an initial diagnosis of primary mediastinal nonseminomatous germ cell tumor (8). Minegishi et al. have reported a case of a 41-year-old man with multiple endocrine neoplasia type I, who had previously undergone extended thymothymectomy. They described the successful use of a mini-clamshell approach through the third intercostal space for complete resection of recurrent thymic carcinoid tumors (9).

Complications of this surgical procedure may include increased postoperative pain and surgical wound infections. If the latter occurs, it is necessary to administer antibiotics intravenously for 5-7 days, at least based on the findings of the wound swab and laboratory findings of the causative agent(s) (10). However, due to the excellent exposure of both the pleural cavities and pulmonary hili, this approach remains of great importance for the treatment of large tumors of the mediastinum and in the modern surgical settings provides excellent access for bilateral lung and heart-lung transplantation.

CONCLUSION

Median sternotomy is the surgical approach of choice in the treatment of most of the mediastinal tumors and bilateral pulmonary diseases. Clamshell and hemiclamshell operative techniques are still considered widely useful in the treatment of massive mediastinal, lung, and pleural tumors or extensive bilateral lung metastases as well as large tumors of the anterior mediastinum.

REFERENCES

1. Marta GM, Aigner C, Klepetko W. Inverse T incision provides improved accessibility to the upper mediastinum. J Thorac Cardiovasc Surg. 2005;129:221-223.

2. Kesler KA, Patel JB, Kruter LE, Birdas TJ, Rieger KM, Okereke IC, Einhorn LH. The "growing teratoma syndrome" in primary mediastinal nonseminomatous germ cell tumors: Criteria based on current practice. J Thorac Cardiovasc Surg. 2012;144(2):438-43.
3. Shameem M, Qaseem SM, Siddiqui MA, Shah NN. Mature mediastinal teratoma in adult. Respir Med CME. 2010;3:116-7.
4. Bains MS, Ginsberg RJ, Jones WG, McCormack PM, Rusch VW, Burt ME, et al. The clamshell incision: An improved approach to bilateral pulmonary and mediastinal tumor. The Annals of Thoracic Surgery. Elsevier. 1994;58(1):30- 33.
5. Kim D, Kim S-W, Hong J-M. Rapid growing huge teratoma: complete surgical resection. Journal of thoracic disease. 2014;6(10):E217-9.
6. Toda M, Izumi N, Tsukioka T, Komatsu H, Okada S, Hara K, et al. Huge mediastinal liposarcoma resected by clamshell thoracotomy: a case report. Surg Case Rep. 2017;3(1):16.
7. Drevelegas A, Palladas P, Scordalaki A. Mediastinal germ cell tumors: a radiologic–pathologic review. European Radiology. 2001;11(10):1925 -1932.
8. Kao CN, Chou SH, Huang CL, Lin CH, Chiang HH, Lee JY, et al. Salvage surgery using simultaneous clamshell thoracotomy with median sternotomy for mediastinal growing teratoma syndrome. Thorac Cancer. 2020;11(3):785 -788.
9. Minegishi K, Tsubochi H, Taki T, Endo S. Mini-clamshell sternotomy for resection of recurrent thymic carcinoid tumors after extended thymothymectomy: report of a case and surgical procedure. J Thorac Dis. 2019;11(3):969-971.
10. Hayati F, Ali NM, Kesu Belani L, Azizan N, Zakaria AD, Rahm an MRA. Giant Mediastinal Germ Cell Tumour: An Enigma of Surgical Consideration. Case Reports in Surgery. 2016;2016:1-5.

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Leukodystrophies - diagnostic approach and diagnostic possibilities in Bosnia and Herzegovina

Leukodistrofije - dijagnostički pristup i dijagnostičke mogućnosti u Bosni i Hercegovini

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ABSTRACT

Introduction: leukodystrophies are hereditary disorders affecting the white matter of the central nervous system with or without peripheral nervous system involvement. The symptoms depend on a specific disorder and the age of onset. Aim: to present a clinical course, diagnostic procedures and results for patients with different type od leucoystrophies. Case reports: this paper presents five cases of children with leukodystrophy treated at Department of Neuropediatrics of the Pediatric Clinic, Clinical Center University of Sarajevo (CCUS). Presented pateints were admitted in the period from October 2015 to July 2019. Magnetic resonance imaging (MRI) of the brain represents the current imaging standard for diagnosis of leukodystrophies. Diagnostic imaging is followed by biochemical and single-gene testing. Diagnostic procedures are limited in many cases of leukodytrophies due to unavailability of genetic testing. Clinical course of the disease and MRI of the brain findings may help in the diagnostic evaluation process of leukodystrophies. For reliable diagnosis, specific MRI findings should be completed with biochemical and gene testing.

Keywords: leukodystrophies, magnetic resonance, gene testing

SAŽETAK

Uvod: leukodistrofije predstavljaju nasljedna oboljenja bijele mase centralnog nervnog sistema sa ili bez oštećenja perifernog nervnog sistema. Simptomi zavise od specifičnog poremećaja i dobi u kojoj se pojave. Cilj: prikazivanje dijagnostičih procedura, kliničkog toka i ishoda pacijenata sa različitim vrstama leukodistrofija. Prikazi slučajeva: u radu je prezentirano petero djece sa leukodistrofijom, koja su se liječila na Odjelu za neuropedijatriju Pedijatrijske klinike Kliničkog centra Univerziteta u Sarajevu. Prezentirani pacijenti su primljeni u vremenskom periodu od oktobra 2015. do jula 2019. godine. Magnetska rezonancija (MR) mozga predstavlja trenutni standard za dijagnosticiranje leukodistrofije, nakon čega slijede biohemijska i genetska testiranja Dijagnostički postupci su ograničeni u mnogim slučajevima leukodistrofija zbog nedostupnosti genetskog testiranja. Klinički tok bolesti i nalazi MR mozga mogu pomoći u dijagnostičkoj evaluaciji leukodistrofija. Za pouzdanu dijagnozu, specifične MR karakteristike treba dopuniti sa biohemijskim i genskim testovima.

Ključne riječi: leukodistrofije, magnetna rezonanca, genetska testiranja

INTRODUCTION

Leukodystrophies are hereditary disorders affecting the white matter of the central nervous system with or without peripheral nervous system involvement, sharing glial cell or myelin sheath abnormalities. The neuropathology is primarily characterized by involvement of oligodendrocytes, astrocytes and other non-neuronal cell types, although in many disorders pathophysiology remains unknown and in other cases is suspected to include significant axonal pathology. They are mostly progressive and degenerative, but some are found to be static or even improving, only impairing white matter function (1,2,3). Reported incidence of a broad group of these disorders varies in different studies, from 1.2/100000 in Washington, 2/100 000 in Germany, 3.1/100000 in United Kingdom (4). According to the latest adopted classification of inherited white matter disorders, leukodystrophies must be distinguished from a large group of disorders called genetic leukoencephalopathies, diseases of white matter, where strong evidence for primary neuronal involvement and prominent systemic manifestations overshadow the white matter abnormalities. Genetic leukoencephalopathies are considered inborn errors of metabolism and mitochondrial disorders that don't meet criteria for leukodystrophies. A specific diagnosis is established in less than half of all patients with leukodystrophies. The diagnosis relies on clinical presentation, neuroimaging, particularly magnetic resonance imaging of the brain, as well as biochemical and genetic tests. After a diagnosis is made, the physician can give prognostic information, monitor for known complications, and may ultimately allow use of disease-specific therapeutics Leukodystrophies can be classified according to the main pathogenic process into the following categories: myelin disorders

(hypomyelinating, demyelinating or myelin vacuolization), astrocytopathies, leuko-axonopathies, microgliopathies and leuko-vasculopathies. Another classification of leukodystrophies is in relation to MRI findings as hypomyelinating and demyelinating leukodistrophies. There is also another molecular and metabolic based classification: lysosomal, ribosomal, peroxisomal and mitochondrial leukodistrophies, leukodistrophies caused with amino-acid, organic acid and myelin proteins metabolic disorders (1,2,3). Many diseases in this broad group of conditions have a similar clinical presentation, so diagnostic process can often be challenging.

Clinical presentation

The symptoms depend on a specific disorder and the age of onset. Motor manifestations (spasticity, weakness, pyramidal tract signs and cerebellar signs) are clinically observable. In some disorders, involuntary movements, macrocephaly, peripheral neuropathy, cognitive and behavioral deterioration may be present. Clinical presentation depends on the age of onset. Axial hypotonia, which evolves over time into spastic tetraparesis, nystagmus or seizures are present in patients with neonatal or infantile presentation. Motor delay and regression, with progressive upper motor neuron signs, ataxia and dysarthria are typical for childhood-onset leukodytrophies. Diagnostic procedures are limited in many cases of leukodytrophies due to unavailability of genetic testing.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) represents the current imaging standard for diagnosis of leukodystrophies. Different types of leukodystrophies have different MRI signal characteristics that can provide specific diagnostic information. Demyelinating leukodystrophies are characterized by prominent white matter hyperintensity in T2-weighted and prominent hypointensity in T1-weighted images compared with gray matter structures. In hypomyelinating leukodystrophies, the white matter abnormalities appear mildly hyperintense in T2-weighted images and have a variable signal (hyper-, iso- or hypo-intense) in T1-weighted images. Involvement or sparing of subcortical U-fibers is one of common findings. Each leukodystrophy has some specific MRI features which are listed below. After classifying the disease in this manner, precise location of the white matter signal abnormalities must be identified in order to narrow down the differential diagnosis to the exact one. MRI lesions in leukodystrophies may predominantly affect central white matter (i.e. metachromatic leukodystrophy), gray and white matter-pandystrophy (i.e. Canavan disease, Krabbe disease), may show postcontrast enhancement (i.e.X-linked adrenoleukodystrophy) or restricted diffusion (i.e. Canavan disease, metachromatic leukodystrophy-MLD). In MLD, in T2-weighted images, affected areas may show a "tigroid pattern" on axial plane or "leopard pattern" on sagittal plane (5,6).

Biochemical and molecular genetic testing

Diagnostic imaging is followed by biochemical and single-gene testing, which are essential for a reliable diagnosis. Leukodytrophies characterized by lysosomal storage disorder are metachromatic leukodystrophy (MLD), multiple sulfatase deficiency (MSD) disorder, Krabbe disease (globoid cell leukodystrophy) and gangliosidosis GM1/GM2. X-linked adrenoleukodystrophy is characterized by peroxismal disorder and Canavan disease by organic acid disorder. Alexander disease is associated with mutations of the glial fibrillary acidic protein (GFAP) gene. Vanishing white matter (VWM) disease is characterized by elevation of glycine in cerebrospinal fluid and urine caused by a gene defect. Pelizaeus Merzbacher disease (PMD) is caused by mutation in the proteolipid protein (PLP) gene on chromosome Xq22.

In cases with no specific MRI patterns, genetic testing using either gene panels, whole exome sequencing (WES) or whole genome sequencing (WGS) is recommended (6).

MATERIALS AND METHODS

Five cases with five different types of the leukodystrophies were presented in this paper. The patients were admitted at Neuropediatric Department of the Pediatric Clinic of CCUS in the period from October 2015 to July 2019.

First case

A boy, 2.5 years old, was admitted due to additional diagnostic process. He was the first child from the first pregnancy, born at 38 weeks of gestation. Intrauterine growth retardation was noticed during pregnancy. He started to walk independently at the age of 14 months. No obvious signs of neurological dysfunction were found on admission. Motor development was adequate for his age, but the ability to speak was undeveloped. The anterior fontanel was widely opened. A hearing test revealed the hearing loss of 60% on both ears. MRI scans showed multifocal confluent lesions in periventricular white matter of the frontal and parietal lobes, indicative of leukodystrophy (Figure 1,2).

Routine laboratory tests, visual acuity and electroencephalography were normal. Further examinations were performed at another center abroad. The genetic analysis confirmed heterozygous mutation of SNORD 118 gene (SNORD-small nuclear RNA) and heterozygous mutation of NOTCH 3 gene.

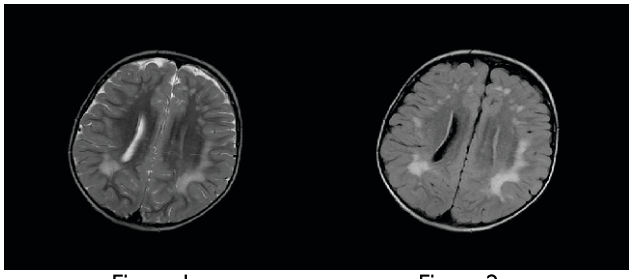

Figure 1 Figure 2

Second case

The patient was admitted to the Neuropediatric Department for the first time at the age of 2, for neurological evaluation due to deviation in motor development. Pregnancy, labor, neonatal period and infancy were uneventful. The p
independently at the age of 19 months. In neurological findings, paraparesis with pronounced tendon reflexes was present. The patient walked with a wide-based and instable gait.

Ophthalmological examination, electroencephalography and electroneuromyography showed normal results.

MRI of the brain (Figure 3,4) revealed diffuse zones of high-signal intensity in T2-weighted sequences in the deep white matter of both cerebral hemispheres, predominantly affecting periventricular regions of parietal lobes. Internal capsules and subcortical U-fibers were

spared. T2-w high-signal intensity lesions of the white matter were indicative of possibly metachromatic leukodystrophy. The routine laboratory tests were normal. Further laboratory tests for enzyme activity were performed at the center abroad, and they showed markedly reduced activity of arylsulfatase A in the blood. This finding indicated the diagnosis of metachromatic leukodystrophy for this patient. Current treatment options for this type of leukodystrophy are limited. There are some ongoing clinical studies. For this child, prevention and treatment of respiratory infections, adequate nutrition via nasogastric tube or percutaneous gastrostomy was recommended.

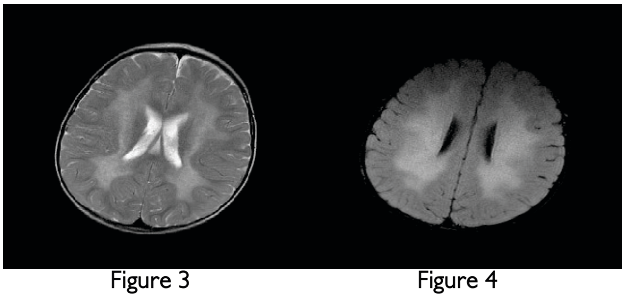

Figure 3 Figure 4

Third case

A 2 year old boy was transferred to the Clinical Center University of Sarajevo from another hospital in Bosnia and Herzegovina for further diagnostic evaluation. Pregnancy, perinatal and early infancy period were uneventful. First signs of axial hypotonia were evident at the age of 7 months. At the admission profound central generalized hypotonia and minimal spontaneous movements were evident. Patient could open his eyes spontaneously, but he did not follow objects. He did not speak, but occasionally cried, with disarticulated voices. Subtle focal seizures were noticed.

MRI of the brain (Figure 5,6) showed existence of bilateral confluent regions of T2-w high signal in periventricular white matter, cerebellum and thalami, with sparing of the corona radiata region. The described changes corresponded primarily to leukodystrophy.

Electroencephalography revealed epileptic activity. Levetiracetam was introduced due to convulsions. Fundoscopy revealed pale optic nerve discs, consistent with optic nerve atrophy.

A blood sample was sent to a clinical center abroad. The activity of galactocerebrosidase in the sample was significantly reduced, indicating a globoid cell leukodystrophy (Krabbe disease). The gene analysis was suggested and the blood sample has been sent abroad. Due to feeding difficulties, placement of percutaneous gastrostomy was indicated.

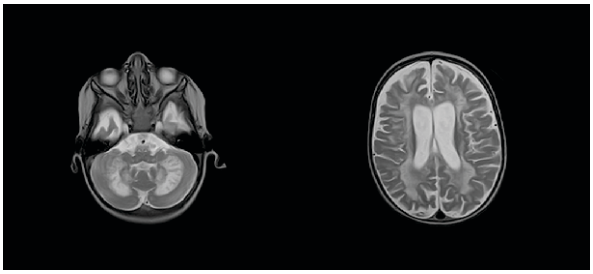

Figure 5 Figure 6

Fourth case

A four-month-old female infant was admitted to the Neuropediatric Department for additional diagnostic work up. Macrocephaly was evident. Spontaneous motor activity was very poor, with severe axial hypotonia. Patelar reflexes were exaggerated. Due to subtle seizures and epileptic activity on electroencephalography, antiepileptic treatment with levetiracetam was initiated. The first MRI of the brain showed alteration of signal intensity with swelling of the medulla oblongata, pons and mesencephalon. Additional zones of T2-w high-signal intensity affecting white matter in the corticospinal tracts and optical radiations, as well as periventricular zones along temporal white matter were also present. Lesions on brain MRI were most likely indicative of leukodystrophy. A follow-up MRI of the brain (Figure 7,8,9) revealed progression in the extensity of lesions. A urine analysis performed at another center showed elevated excretion of N-acetyl aspartic acid, which is a feature of Canavan leukodistrophy. The genetic analysis homozygous, most likely pathogenic mutations of ASPA gene has been detected. Genetic analysis of parents detected heterogeneous mutation of ASPA gene.

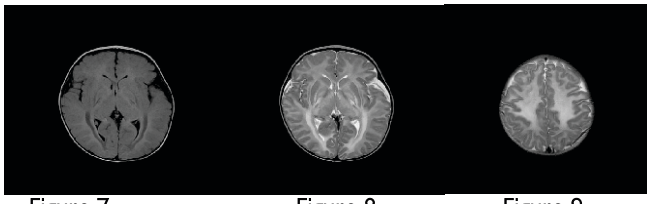

Figure 7 Figure 8 Figure 9

Fifth case

At nine months old female infant was admitted for the first time to the Neuropediatric Department due to deviation in psychomotor development and convulsions.

It was the fourth term born child of the same parents. Since birth, spontaneous motor skills were scarce. The first child of the same parents was a healthy 18 years old girl. Their second child died at the age of 1 year. The child had rough facial features, organomegaly and severe hypotonia. Neurometabolic disease was suspected. However, laboratory diagnostic tests for metabolic disorders were not available at that time at the Clinical Center of the University of Sarajevo. Their third child died at the age of 22 months, with diagnosis of Gangliosidosis GM 1. The diagnosis was confirmed based on laboratory tests carried out in another medical center abroad. The leukocyte enzyme activity of beta galactosidase lysosomal enzyme was significantly reduced. MRI revealed low signal in ventral parts of thalami (Figure 10,11). Due to progression of underlying disease and feeding and swallowing difficulties, percutaneous gastrostomy was performed.

The fourth child had similar clinical signs as previous siblings. After the initial evaluation, the fourth child was hospitalized abroad where the diagnosis of Gangliozidosis GM1 was established.

The child died at the age of 3 years and 6 months at the Pediatric Intensive Care unit due to complications of the underlying disease. The genetic pedigree for the child was presented in Figure 12.

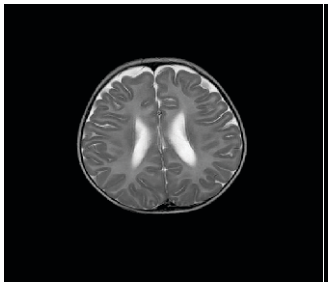

Figure 10

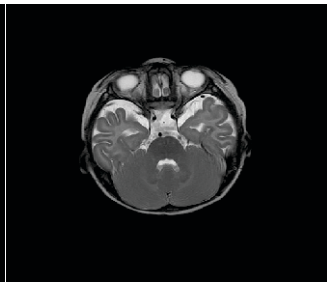

Figure 11

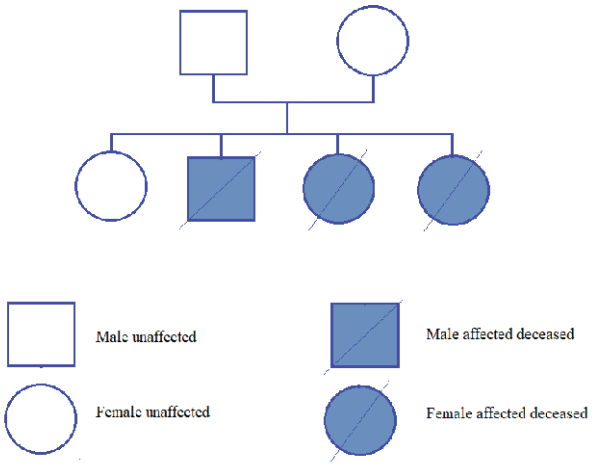

Figure 12 **Genetic pedigree chart for patient with gangliosidosis GM1.**

DISCUSSION

A patient with leukodystrophy and SNORD 118 gene heterozigous mutation was presented in the first case. This variant in SNORD 118 gene (chr 17:8,076,770G>A,hg 19) was previously reported.

Ribosomes are ubiquitously expressed and essential for life. The ribosome consists of ribosomal RNA (rRNA) and ribosomal proteins (RPs), with snoRNAs being an evolutionarily conserved group of non-protein encoding RNAs involved in the modification and processing of rRNAs. Alterations in ribosomal components, structure or function can cause a heterogeneous group of human disoders that are known as ribosomopathies, with this diversity in clinical phenotype.

Biallelic mutations in the gene SNORD118, encoding the box C/D snoRNA U8, cause the cerebral microangiopathy leukoencephalopathy with calcifications and cysts (LCC), presenting at any age from early childhood to late adulthood. LCC is the first example of a human disease to be associated with point mutations in a C/D box snoRNA (8).

The heterozigous mutation of SNORD 118 gene which was detected in our patient has been previously published in literature, but only biallelic variant may cause phenotype changes. Another heterozigous variant detected in our patient NOTCH3 (c.1775G>T;p.Arg.592Leu) was not reported till now. The phenotype of our patients with leukodystrophy could not be explained with detected mutations and its etiology has been looking for yet.

The second case related to a patient with metachromatic leukodystrophy. Metachromatic leukodystrophy (MLD) is a rare autosomal recessive lysosomal storage disease that causes progressive demyelination of the central and peripheral nervous system. It is caused by deficient activity of arylsulfatase A and sphingolipid activator B due to mutations in the ARSA and PSAP genes respectively. Decreased ARSA activity leads to accumulation of sulfatides and sphingolipids in oligodendrocytes, microglia, the central nervous system neurons, Schwann cells, peripheral nerve macrophages, gall bladder, kidneys, and other visceral organs. The accumulation causes central and peripheral demyelination. It is a progressive disease characterized by gradual regression of motor skills, spastic tetraparesis, optic atrophy, intellectual deterioration and pyramidal and cerebellar dysfunction. Three major subtypes considering the age of onset of the disease are late infantile, juvenile, and adult form. The diagnosis is confirmed by demonstrating deficient arylsulfatase A enzyme (ARSA enzyme) activity in leukocytes or cultured skin fibroblasts or urine. Currently, there is no curative treatment available for metachromatic leukodystrophy , although there are promising results in ongoing trials using gene-cell therapy particularly transplantation of genetically modified CD34+ HSCs overexpressing the ARSA gene (9).

The third case presented a patient with confirmed globoid cell leukodystrophy (Krabbe disease). Krabbe disease is a rare autosomal recessive lysosomal storage disorder caused by a deficiency of galactocerebrosidase (GALC enzyme) due to mutations in GALC gene. The substrate of the GALC enzyme is galactosylsphingosine, also known as psychosine, which is responsible for the damage of glial and neuronal cells. Pathological changes in the peripheral and central nervous system are globoid cell formation and demyelination. Considering the age of onset, there are three types of this disease: infantile, juvenile and adult. Symptoms start to present from infancy, between second and fifth month, and include irritability, developmental delay or regression, limb spasticity, axial hypotonia, absent reflexes, optic atrophy and microcephaly. Seizures eventually appear. Affected children have rapid motor regression and life expectnca usually till 2 years. The diagnosis is based on clinical course of the disease, neuroimaging, cerebrospinal fluid examinations (elevated proteins). Diagnosis is confirmed by measurement of galactocerebrosidase (GALC) activity in leukocytes or cultured skin fibroblasts or by genetic testing. Typical values of GALC activity in patients with Krabbe disease are <5 percent of the normal value. Still, no curative treatment is available. In USA phosphodiesterase inhibitor (ibudilast) was granted designation for treatment, while in EU only for amyotrophic lateral sclerosis. Also, there are no promising results from hematopoietic stem cell transplantation (2/5 survived with moderate to severe developmental delay) (10).

The fourth case presented a patient with confirmed Canavan disease, autosomal recessive leukodystrophy, caused by mutations in the aspartoacylase (*ASPA*) gene. The ASPA gene provides instructions for producing an enzyme aspartoacylase. In the brain, this enzyme breaks down a compound called N-acetyl-L-aspartic acid (NAA). The production and breakdown of NAA appears to be critical for maintenance of the brain's white matter. A defective gene causes elevated levels of NAA in urine, which characterizes this condition. In most cases, children have no specific clinical features in the first few months of their life. But at the age of six months, head circumference is above the 90th percentile in most patients. Motor development is delayed. Poor head control and macrocephaly are constant features. Patients have feeding difficulties, seizures, optic nerve atrophy and sleep disorders. There is no specific treatment for this condition (5,11).

The fifth case presents a patient with gangliosidosis GM 1. Gangliosidosis GM1 is a lysosomal storage disorder caused by ß galaktosidase enzyme (ßgal enzyme) deficiency due to biallelic mutations in the *GLB1* gene. It has an autosomal recessive inheritance pattern. ßgal enzyme is responsible for the degradation of GM1 ganglioside, oligosaccharides, and keratan sulfate. Toxic accumulation of these substrates in lysosomes leads to cellular apoptosis. GM1 ganglioside production occurs primarily in the central nervous system (CNS). Its toxic accumulation cause severe progressive neurological deficits. Systemic disease manifestations are also common due to keratan sulfate and oligosaccharide buildup in peripheral organs, such as the heart, bone, liver, and spleen. Diagnostic criteria include no ability to stand at all, hypotonia, cognitive/motor delay, macular cherry-red spot, hepatosplenomegaly, skeletal abnormalities, cardiomyopathy and coarse facies. Confirmation of diagnosis is obtained from biochemical and gene testing (enzyme deficiency in leukocytes and cultured fibroblasts). The disease is lethal with life expectancy of maximum 3 years (12).

All of the presented patients were younger than two years at the time of symptoms onset. Three of them were infants. Two of the patients were transferred from another hospital to the Pediatric Clinic for further examinations. One of the patients had two siblings who suffered from the same disease, with lethal outcome. The disease was suspected in one sibling, and was confirmed in the other. The older brother died at 12 months of age (Neurometabolic disease was suspected).

All of the patients underwent brain MRI examination. MRI features may help in establishing a diagnosis. For further confirmation of a presumed diagnosis, additional biochemical and gene tests were needed to be performed at other clinical centers. Biochemical confirmation was received for five patients.

One of the patients in present case series died, while other four patients are alive.

The relevant clinical data for these five patients were summarized in Table 1.

Table 1 **Summary of relevant clinical data for the patients with leukodystrophies.**

	Gender	Time of first symptoms	MRI	Laboratory tests	Genetic analysis	Diagnosis	Inheritance	Incidence	Outcome	Involved organelle
1	Male	2 years-hearing impairment	multifocal confluent lesions PVWM	peroxysome enzymes elevated	homozygous mutation of SNORD 118 gene ---------- heterozygous mutation of NOTCH 3 gene	Ribosomopathy	AR	unknown	hearing impairment	ribosome
2	Male	22 months paraparesis	diffuse zones of high signal intensity (T2w) of deep WM of both hemispheres	arylsulfatase A activity in leukocytes severely reduced	not done	Late infantile metachromatic leukodystrophy	AR	1:40 000 - 1:170 000	tetraparesis PEG	lysosome
3	Male	7 months-axial hypotonia	bilateral confluent region of T2w high signal in PVWM , cerebellum and thalami	Galacto - cerebrosidase activity in leukocytes reduced	not done	Leukodystrophy Krabbe	AR	1:100 000	tetraparesis PEG	lysosome
4	Female	4 months-generalized hypotonia	T2-w high signal intensity afecting PVWM	N-acetyl-L-aspartic acid(NAA) elevated in urine	homozygous mutation of ASPA gene	Leukodystrophy Canavan	AR	1: 100 000	tetraparesis epilepsy	mitochondria?
5	Female	2 months-generalized hypotonia	low signal in ventral part of thalami	β galactosidase in leucocytes reduced	not done	Gangliosidosis GM1	AR	1:100 000	lethal at the age of 3 1/2 y	lysosome

CONCLUSION

Clinical course of the disease and MRI findings may help in the diagnostic evaluation process of leukodystrophies. For the final diagnosis, further laboratory tests and a genetic analysis should be performed. Since the diseases are rare, establishment of neurometabolic laboratories at the Clinical Center University of Sarajevo is economically questionable. This is why the official cooperation with appropriate neurometabolic laboratories in neighbouring countries is a reasonable solution. A proper diagnosis and genetic tests are essential for genetic counseling. One of our patients had two older siblings with the same diagnosis, and all three children died before reaching the age of two.

REFERENCES

1. Ashrofi MR, Amant M, Garshasbi M, Kameli R, Nilipor Y, Heidori M, et al. An update on clinical, pathological diagnostic and therapeutic perspective on childhood lecodiystephies. Expert Rev Nurother. 2020;20(1):65-84.
2. Van der Knaap MS, Bugiani M. Leukodystrophies: a proposed classification system based on pathological changes and pathogenetic mechanisms. Acta Neuropathol. 2017;134:351-82.
3. Vanderver A, Prust M, Tonduti D, Mochel F, Hussey HM, Helman G, et al. Case definition and classification of leukodystrophies and leukoencephalopathies. Mol Genet Metab. 2015;114(4):494-500.
4. Ashrafi MR, Rezaei Z, Heidari M, Nikbakht S, Malamiri RA, Mohammadi M, et al. The First Report of Relative Incidence of Inherited White Matter Disorders in an Asian Country Based on an Iranian Bioregistry System. J Child Neurol. 2018;33(4):255-59.
5. Choudhri AF. Pediatric Neuroradiology: Clinical Practice Essentials. New York: Thieme Medical Publishers Inc. 2017:, pp 288; p115
6. Saber M. Leukodystrophies. Last revision made on May 5th, 2021 Available at: https://radiopaedia.org/articles/leukodystrophies. Accessed: May 14th, 2021
7. Jenkinson EM, Rodero MP, Kasher PR, Uggenti C, Oojageer A, Goosey LC, et al. Mutations in SNORD118 cause the cerebral microangiopathy leukoencephalopathy with calcifications and cysts [published correction appears in Nat Genet. 2017;49(2):317]. Nat Genet. 2016;48(10):1185-92.
8. Venturi G, Montanaro L. How Altered Ribosome Production Can Cause or Contribute to Human Disease: The Spectrum of Ribosomopathies. Cells. 2020;9(10):2300.
9. Shaimardanova AA, Chulpanova DS, Solovyeva VV, Mullagulova AI, Kitaeva KV, Allegrucci C, Rizvanov AA. Metachromatic leukodystrophy: diagnosis, modeling, and treatment approaches. Front. Med. 2020:7:576221
10. Komatsuzaki S, Zielonka M, Mountford WK, Kölker S, Hoffmann GF, Garbade SF, Ries M. Clinical characteristics of 248 patients with Krabbe disease: quantitative natural history modeling based on published cases. Genet Med. 2019;21(10):2208-15.
11. Pleasure D, Guo F, Chechneva O, Bannerman P, McDono ugh J, Burns T, Wang Y, Hull V. Pathophysiology and Treatment of Canavan Disease. Neurochem Res. 2020;45(3):561-65.
12. Lang FM, Korner P, Harnett M, Karunakara A, Tifft CJ. The natural history of Type 1 infantile GM1 gangliosidosis: A literature-based meta-analysis. Mol Genet Metab. 2020;129(3):228-35.

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SADRŽAJ

Sadržaj rada mora biti sistematično i strukturno pripremljen i podijeljen u poglavlja i to:

- **UVOD**
- **MATERIJAL I METODE**
- **REZULTATI**
- **DISKUSIJA**
- **ZAKLJUČAK**
- **LITERATURA**

UVOD

Uvod je kratak, koncizan dio rada i u njemu se navodi svrha rada u odnosu na druge objavljene radove sa istom tematikom. Potrebno je navesti glavni problem, cilj istraživanja i/ili glavnu hipotezu koja se provjerava.

MATERIJAL I METODE

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Navode se glavni rezultati istraživanja i nivo njihove statističke značajnosti. Rezultati se prikazuju tabelarno, grafički, slikom i direktno se unose u tekst gdje im je mjesto, s rednim brojem i konciznim naslovom. Tabela treba imati najmanje dva stupca s obrazloženjem što prikazuje; slika čista i kontrastna, a grafikon jasan, s vidljivim tekstom i obrazloženjem.

DISKUSIJA

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Treba da bude kratak, da sadrži najbitnije činjenice do kojih se došlo u radu tokom istraživanja i njihovu eventualnu kliničku primjenu, kao i potrebne dodatne studije za potpuniju aplikaciju. Obavezno navesti i afirmativne i negirajuće zaključke.

LITERATURA - Upute za citiranje - pisanje literature

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