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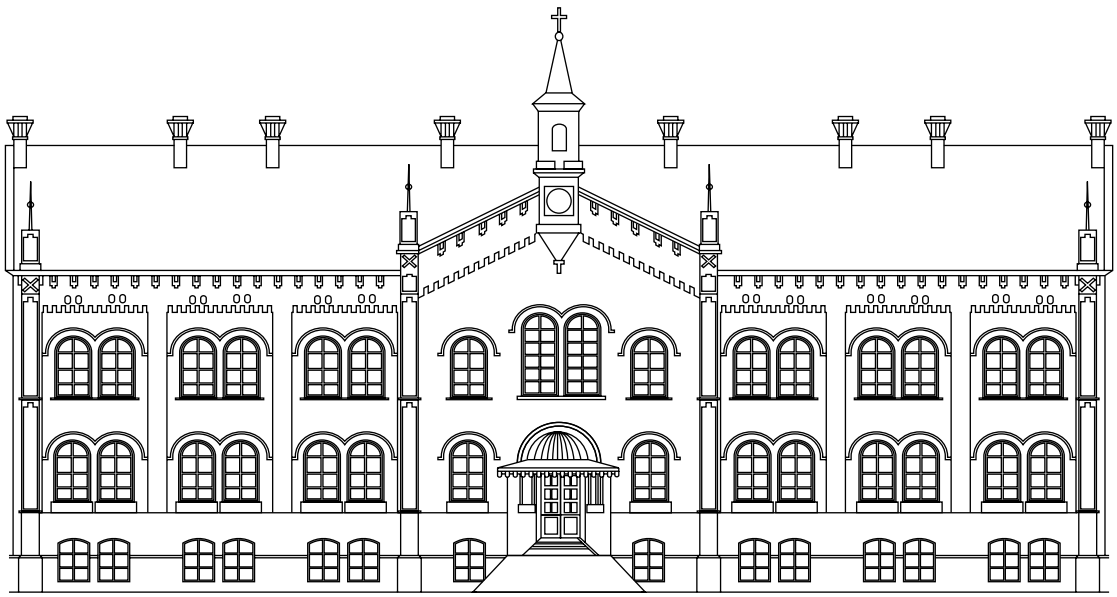

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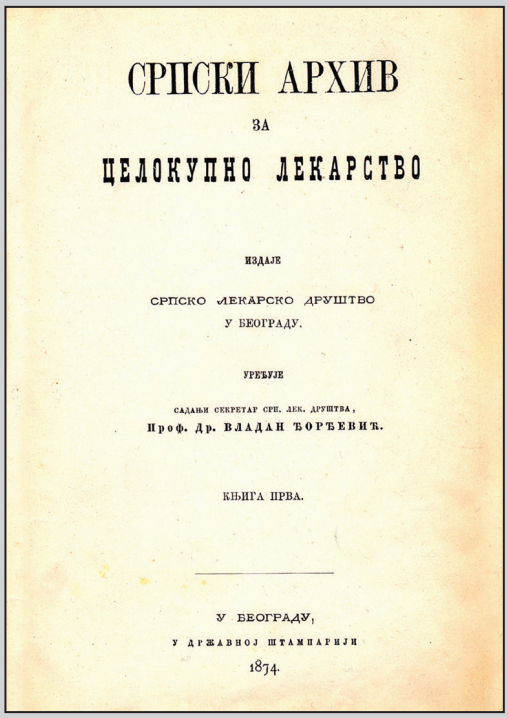

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

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У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

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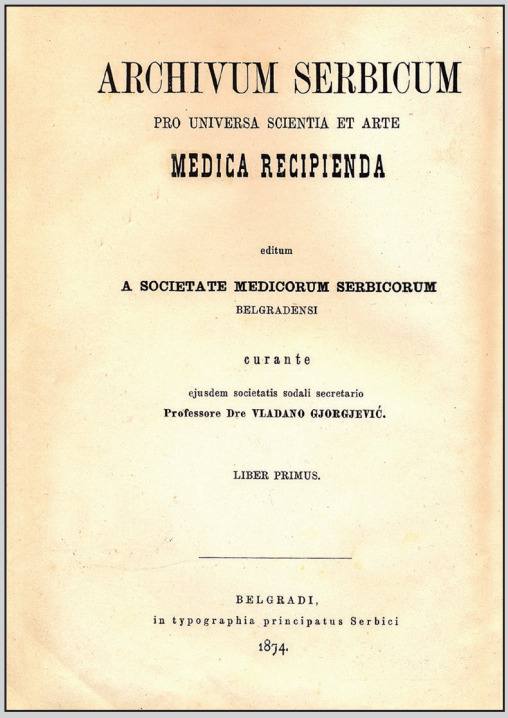

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1874.

The title page of the first journal volume in Latin

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Morphometric and morphological study of mandibular foramen and lingula in dry human mandibles

Braca Kundalić[1], Slađana Ugrenović[1], Ivana Graovac[1], Vladimir Petrović[2], Jovana Čukuranović Kokoris[1], Vesna Stojanović[1], Branka Marković Ilić[3]

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SUMMARY

Introduction/Objective The mandibular foramen (MF) and lingula (ML) are key landmarks on the medial surface of the mandibular ramus. Their position relative to the ramus borders, dentition, and lingula morphology is clinically important for anesthesia and surgical procedures. The study aimed to assess the morphological and morphometric characteristics of MF and ML in relation to ramus borders, alveolar presence, and lingula shape in a Serbian population sample.

Methods The material comprised 50 human dry hemimandibles from the bone collection of the Department of Anatomy, Faculty of Medicine, University of Niš. Distances from MF and ML to the superior, anterior, and posterior ramus borders, temporal crest, and gonion were measured, along with MF diameter and ML height. Hemimandibles were classified by the number of alveoli present (0, 1–4, or 5–8). Lingulae were classified by shape. Measurements were obtained using a digital vernier caliper, and mean values with standard deviations were analyzed by ANOVA. Statistical significance was defined as $p < 0.05$.

Results The MF–posterior border and ML–posterior border distances (14.49 and 16.03, respectively) were significantly larger in partially edentulous hemimandibles, compared with the dentulous subjects (11.97 and 13.95 mm, respectively). The triangular lingula was most prevalent (48%), followed by truncated (32%), nodular (16%), and assimilated (4%) forms. The ML–gonion distance was significantly greater in mandibles with a truncated *vs.* triangular lingula (33.37 *vs.* 27.99 mm).

Conclusion This study suggests considering the posterior ramus distance in partially edentulous patients and the shape of the ML to optimize MF localization for anesthesia and surgery.

Keywords: anatomic variation; mandible; morphometry; anesthesia; mandibular osteotomy

INTRODUCTION

The mandibular ramus features landmarks including the four borders, the mandibular foramen, and the mandibular lingula. Knowledge of their spatial relationship may have clinical implications in oral and maxillofacial surgery.

The mandibular foramen (MF), located on the medial surface of the mandibular ramus, is the entry point for the inferior alveolar neurovascular bundle into the mandibular canal and represents a critical landmark in dental anesthesia and ramus surgery [1, 2]. Even minor positional variations significantly affect the trajectory of inferior alveolar nerve (IAN) blocks and the safety of medial osteotomies, underscoring the importance of population- and modality-specific morphometric mapping [3, 4]. Studies on dry skulls and imaging reveal systematic differences in MF location influenced by age, sex, dentition, and ethnicity, explaining variable anesthetic success and complication rates [1, 4].

The mandibular lingula (ML) is a variable bony projection adjacent to the MF, serving as the sphenomandibular ligament attachment [4–6]. Its morphology is commonly classified into triangular, truncated, nodular, and assimilated types [5], with distribution varying between populations and between dry bone and CBCT studies [4, 6, 7]. The ML position (vertical height above and anteroposterior relation to the MF) affects its reliability as a surgical landmark [7]. The ML shape may be assessed differently in dry bone *vs.* CBCT studies, where the latter report a nodular shape more frequently than a triangular one, due to the rounder appearance on imaging caused by soft tissue, periosteum, and volume averaging [8].

Incorrect estimation of the MF and/or ML positions contributes to IAN block failures and increases risks of vascular or neural injury [3]. A clinically significant asymmetry of the mandible was reported in 10–25% of individuals [8]. The data reported on sex-specific or ethnic differences in the distance from the lingula to the posterior border of ramus suggest that measurements on one side should not be extrapolated to the contralateral one, but assessed preoperatively [8]. In orthognathic surgery, such as sagittal split ramus osteotomy (SSRO) and intraoral vertical ramus osteotomy (IVRO),

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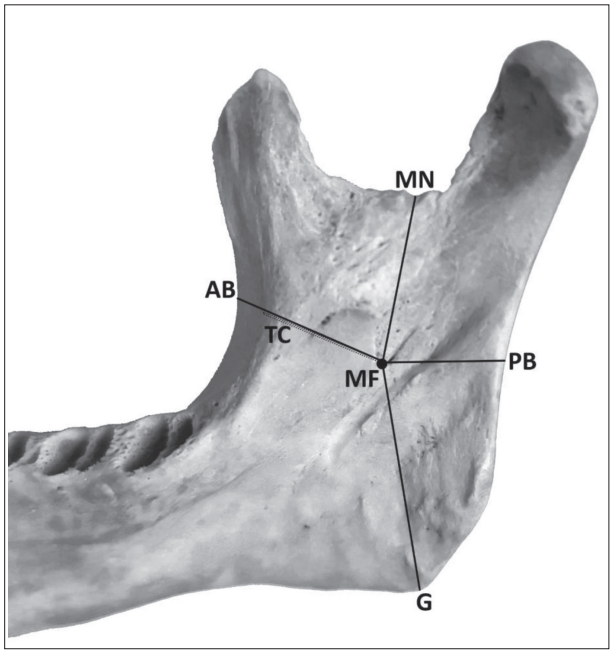

Figure 1. Distance measurements (full black line; MF–TC distance with the dotted line) and landmarks on the right hemimandible;
MF – mandibular foramen; TC – temporal crest; AB – anterior border of ramus; PB – posterior border of ramus; MN – mandibular notch; G – gonion

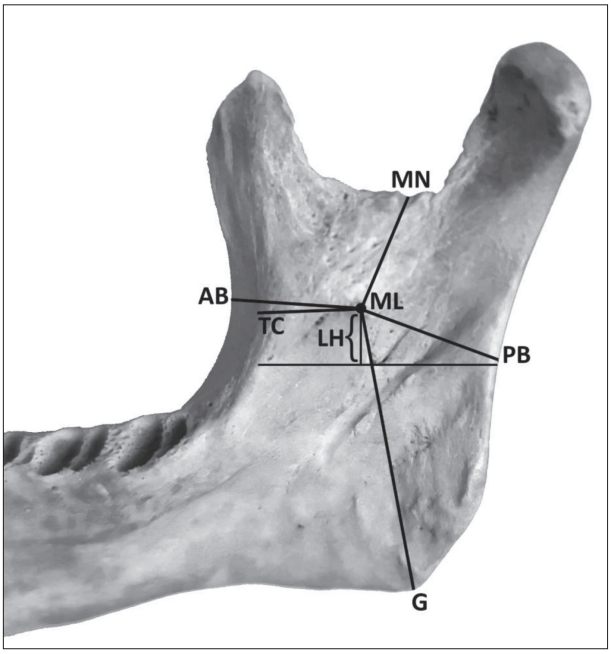

Figure 2. Distance measurements (full black line) and landmarks on the right hemimandible;
ML – lingula; TC – temporal crest; AB – anterior border of ramus; PB – posterior border of ramus; MN – mandibular notch; G – gonion; LH – height of the lingula;
LH is depicted as a vertical line drawn perpendicular to the horizontal plane passing through the inferior border of the mandibular foramen, and oriented parallel to the inferior border of the ramus

osteotomy placement must respect the MF–ML complex to avoid nerve injury or unfavorable fractures. Alveoli number may influence MF position [9], but no data were reported regarding the ML. The relationship between alveoli number and mandibular lingula morphology/position is underexplored but may be of clinical importance. Variations in diameters in partially or totally edentulous patients may influence anesthesia success and surgical safety, yet no available studies have addressed this relationship. Morphometric data thus provide essential guidance for anesthesia accuracy and surgical safety [7, 10].

The aim of this study was to investigate the morphological and morphometric characteristics of the mandibular foramen and lingula in relation to the borders of the ramus, the number of present alveoli, and the shape of the lingula.

METHODS

All material was part of the osteological collection of the Department of Anatomy, Faculty of Medicine, University of Niš, Serbia, where the study was conducted in accordance with the Declaration of Helsinki. Fifty dry human hemimandibles from twenty-five adult subjects were included in this study. The age and sex of the subjects were unknown. Inclusion criteria were mandibles with no signs of fracture and with both the mandibular foramen and lingula. Exclusion criteria were deformed and fractured hemimandibles, and an obstructed mandibular foramen.

Measurements were obtained using a digital caliper with 0.01 mm accuracy. The presence and number of dental alveoli were noted. Shapes of the ML were observed and classified into four shapes according to Tuli et al. [5]. Morphometric landmarks were predetermined and the distances between them were measured in millimeters (Figures 1 and 2). The MF point was the lowest point of the mandibular foramen. The ML point was the tip of the lingula. The AB point was the most concave point of the anterior border. The TC point was the most concave point of the temporal crest. The PB point was the most concave point of the posterior border. The MN point was the lowest point of the mandibular notch. The G point was the lowest point at the gonion. The diameter of the mandibular foramen was measured between the anterior and posterior borders of the MF. The height of the lingula was measured as a distance from the tip of the lingula to the lowest point of the MF, along the line that was perpendicular to the plane parallel to the inferior border of the ramus. Two independent researchers cross-checked all measurements to reduce bias. Discrepancies > 10% prompted repeat measurement. The mean of two values was reported as the final value.

The dataset was analyzed using the SigmaStat 3.5 software. The normality of the data was analyzed using the Kolmogorov–Smirnov test. Levene's test was performed to determine the homogeneity of the variances. Morphometric measurements (the mean and standard deviation) were analyzed with the paired-samples Student's t-test to check symmetry between the hemimandibles, and with the one-way ANOVA to assess relationships with the presence of alveoli and the lingula shape. The ANOVA was followed by the robust Holm–Šidák post-hoc test; in case of heteroscedastic data, the ANOVA on ranks was followed

Table 1. Morphometric characteristics of the mandibular foramen and lingula according to side, in mm

Side	N		MF–TC	MF–AB	MF–PB	MF–MN	MF–G	MFD	MLH	ML–TC	ML–AB	ML–PB	ML–MN	ML–G
Left (L)	25	Mean	12.36	16.85	13.36	21.85	22.29	3.76	7.28	11.41	17.41	15.31	15.37	28.75
		SD	2.18	2.12	2.24	3.27	4.2	0.71	2.02	2.63	3.02	2.21	2.36	5.23
Right (R)	25	Mean	12.05	16.62	13.59	22.12	22.93	3.59	8.34	10.97	16.32	14.81	15	31.13
		SD	1.99	2.26	3.35	3.34	4.73	0.95	2.15	2.11	2.41	2.15	2.69	6.11
Total	50	Mean	12.21	16.74	13.47	21.99	22.61	3.67	7.81	11.19	16.86	15.06	15.19	29.94
		SD	2.07	2.18	2.82	3.27	4.44	0.83	2.13	2.37	2.76	2.17	2.51	5.76
L *vs.* R (p-value)			0.6	0.72	0.78	0.78	0.61	0.49	0.08	0.51	0.17	0.43	0.61	0.15

N – number of hemimandibles; SD – standard deviation; MF–TC – distance between mandibular foramen and temporal crest; MF–AB – distance between mandibular foramen and anterior border of ramus; MF–PB – distance between mandibular foramen and posterior border of ramus; MF–MN – distance between mandibular foramen and mandibular notch; MF–G – distance between mandibular foramen and gonion; MFD – diameter of mandibular foramen; MLH – height of mandibular lingula; ML–TC – distance between mandibular lingula and temporal crest; ML–AB – distance between mandibular lingula and anterior border of ramus; ML–PB – distance between mandibular lingula and posterior border of ramus; ML–MN – distance between mandibular lingula and mandibular notch; ML–G – distance between mandibular lingula and gonion

Table 2. Morphometric characteristics of the mandibular foramen and lingula according to the number of present dental alveoli, in mm

Alveoli	N		MF–TC	MF–AB	MF–PB	MF–MN	MF–G	MFD	MLH	ML–TC	ML–AB	ML–PB	ML–MN	ML–G
0	21	Mean	11.99	16.71	13.75	21.91	22.67	3.86	7.93	11.6	17.06	15.11	14.66	30.55
		SD	1.42	1.72	2.05	3.91	3.53	0.81	2.51	2.21	2.21	1.29	2.73	4.53
1–4	15	Mean	12.26	16.99	**14.49**[a]	21.37	22.74	3.44	7.55	11.37	17.13	**16.03**[b]	15.41	27.67
		SD	1.76	2.66	3.4	2.35	4.25	0.88	1.83	2.81	3.84	2.45	2.32	5.79
5–8	14	Mean	12.11	16.5	**11.97**[a]	22.28	22.66	3.65	7.9	11.04	16.29	**13.95**[b]	15.81	31.47
		SD	2.99	2.35	2.72	3.2	5.87	0.81	1.92	2.51	2.18	2.51	2.47	6.94

N: number of hemimandibles; SD: standard deviation; MF–TC: distance between mandibular foramen and temporal crest; MF–AB: distance between mandibular foramen and anterior border of ramus; MF–PB: distance between mandibular foramen and posterior border of ramus; MF–MN: distance between mandibular foramen and mandibular notch; MF–G: distance between mandibular foramen and gonion; MFD: diameter of mandibular foramen; MLH: height of mandibular lingula; ML–TC: distance between mandibular lingula and temporal crest; ML–AB: distance between mandibular lingula and anterior border of ramus; ML–PB: distance between mandibular lingula and posterior border of ramus; ML–MN: distance between mandibular lingula and mandibular notch; ML–G: distance between mandibular lingula and gonion;
[a]one-way ANOVA, Holm–Šidák post-hoc test; $p < 0.05$;
[b]one-way ANOVA, Dunn's post-hoc test; $p < 0.05$

Table 3. Morphometric characteristics of the mandibular foramen and lingula according to shape of lingula (ML), in mm

ML shape	N		MF–TC	MF–AB	MF–PB	MF–MN	MF–G	MFD	MLH	ML–TC	ML–AB	ML–PB	ML–MN	ML–G
Triangular	24	Mean	11.54	16.21	13.51	21.77	21.25	3.53	7.98	11.03	16.78	14.64	15.56	**27.99**[a]
		SD	1.87	2.08	2.72	3.24	4.34	0.83	1.63	2.71	2.4	2.28	2.64	5.18
Nodular	8	Mean	12.66	16.89	14.7	23.58	22.17	4.02	8.71	11.98	17.4	15.36	15.48	29.21
		SD	1.24	1.45	3.61	1.73	4.44	1.01	1.96	2.41	2.54	1.75	1.59	5.88
Truncated	16	Mean	12.87	17.58	13.12	21.42	25.45	3.61	7.22	11.57	16.84	15.78	14.85	**33.37**[a]
		SD	2.36	2.51	2.22	3.66	3.42	0.68	2.45	2.13	3.48	2.04	2.44	5.5
Assimilated	2	Mean	10.57	15.69	10.88	19.32	19.92	4.6	6.87	11.55	16.03	13.18	12.72	28.89
		SD	0.87	1.56	5.33	4.31	3.67	0.77	5.4	2.91	3.08	2.64	5.46	5

N – number of lingulae; SD – standard deviation; MF–TC – distance between mandibular foramen and temporal crest; MF–AB – distance between mandibular foramen and anterior border of ramus; MF–PB – distance between mandibular foramen and posterior border of ramus; MF–MN – distance between mandibular foramen and mandibular notch; MF–G – distance between mandibular foramen and gonion; MFD – diameter of mandibular foramen; MLH – height of mandibular lingula; ML–TC – distance between mandibular lingula and temporal crest; ML–AB – distance between mandibular lingula and anterior border of ramus; ML–PB – distance between mandibular lingula and posterior border of ramus; ML–MN – distance between mandibular lingula and mandibular notch; ML–G – distance between mandibular lingula and gonion;
[a]one-way ANOVA, Holm–Šidák post-hoc test, $p < 0.05$

by the Dunn's post-hoc test for unequal variance group sizes. Differences were considered statistically significant at $p < 0.05$.

Ethics: The study was approved by the Ethics Committee of the Faculty of Medicine, University of Niš, Serbia (No. 12-13346-1/2-5, 28 October 2025).

RESULTS

The mean values and standard deviations for distances that describe the position of the MF and ML in relation to the bony landmarks on the hemimandible are given in mm and summarized in Table 1. There were no significant differences between the left and right hemimandibles. Distances from the MF towards anteriorly positioned landmarks (the temporal crest and the anterior border of the ramus) were larger on the left side (12.36 *vs.* 12.05 mm, and 16.85 *vs.* 16.62 mm, respectively), whereas the distance from the MF to the posterior border was therefore shorter on the left side (13.36 *vs.* 13.59 mm). Both the distances from the MF to the mandibular notch and gonion were shorter on the left side (21.85 *vs.* 22.12 mm, and 22.29 *vs.* 22.93 mm, respectively). The MF diameter was lower on the right side (3.59 *vs.* 3.76 mm). The height of the lingula was larger on the right side (8.34 *vs.* 7.28 mm).

Measured from the ML, the distances towards the anteriorly positioned points were larger too (11.41 *vs.* 10.97 mm to the TC landmark, and 17.41 *vs.* 16.32 mm to the AB

point). The distances from the ML to the posterior border and mandibular notch were increased on the left ramus as well (15.31 *vs.* 14.81 mm, 15.37 *vs.* 15 mm, respectively) but decreased towards the gonion (28.75 *vs.* 31.13 mm).

Based on the number of present dental alveoli, all the hemimandibles were classified into three groups: with no alveoli, with 1–4, and with 5–8 alveoli present (Table 2). There were 21 hemimandibles, 15 hemimandibles, and 14 hemimandibles, respectively. The statistically significant difference was noted in the distances from both the MF and ML towards the posterior border in the subjects with 1–4 alveoli compared with those with 5–8 alveoli. In those with fewer alveoli, the MF–PB distance was significantly increased (14.49 *vs.* 11.97 mm, $p < 0.05$), as well as the ML–PB distance (16.03 *vs.* 13.95 mm, $p < 0.05$).

Four lingula shapes were analyzed: 24 triangular (48%), eight nodular (16%), 16 truncated (32%), and two assimilated (4%) lingulae (Table 3). All the morphometric measurements were then classified into four groups accordingly. It was the distance between the ML and gonion that was statistically significantly larger in the truncated lingula group compared with the triangular group (33.37 *vs.* 27.99 mm, $p < 0.05$).

DISCUSSION

Understanding the morphometric relationships among the MF, the ML, and adjacent landmarks may help accurately localize these structures in clinical practice [6]. The precise location of the MF and its variations is critical for the success of IAN block [11, 12]. A lack of anatomical knowledge and insufficient training to manage variations have been identified as major causes of block failure among dental students [13]. In routine practice, injections are often placed slightly anterior to the true position of the MF, which contributes to failure rates of up to 25% after the first attempt [14]. The absence of clear guidance on the appropriate depth and vertical level of needle penetration compounds this problem [9].

Endodontic therapy, extractions, and most mandibular treatments depend on block anesthesia rather than infiltration, since the dense cortical bone of the mandible prevents effective diffusion of anesthetic solutions. Because the MF is concealed by soft tissue, clinicians must inject as close as possible to its true location to avoid complications such as hemorrhage or persistent nerve injury, making reliable reference points indispensable [15]. Beyond anesthesia, the MF plays a central role in surgical planning, particularly in procedures aimed at functional or aesthetic correction of dentofacial deformities [9]. For mandibular surgeries, such as osteotomies, endodontic procedures, fracture management, and tumor resections, preoperative radiographic assessment and the use of additional anatomical guide points are recommended to minimize neurovascular injury [12].

Cone beam CT studies suggest that identifying the lingula first, and then the MF, improves the success of IAN blocks [16]. The lingula itself is widely used as a clinical marker to approximate the MF. Knowledge of its morphometric relationships is particularly valuable in situations where the lingula is absent or indistinct, or in centers where advanced three-dimensional imaging is not readily available, as clinicians must rely on anatomical reference points to guide procedures [17]. In SSRO and IVRO, precise localization of the lingula and MF is critical to avoid injury to the inferior alveolar nerve and to ensure predictable osteotomy lines [4].

The results of our study showed no significant side-to-side differences in the positions of the MF and ML relative to the bony landmarks of the mandibular ramus. The distance between these target points and the posterior border of the ramus may vary significantly, depending on the number of alveoli. We identified four shapes of the lingulae, with the triangular shape being most common (48% of subjects), whereas the assimilated shape was the rarest, observed in only 4% of samples. The distance between the lingula and the gonion was significantly larger in the truncated group than in the triangular group.

The average distance between the MF and PB in the partially edentulous subjects (14.49 mm) in our study was larger than in the other two groups and significantly greater than in those with 5–8 alveoli (11.97 mm). These mean values fall within the reported literature range (9.23–17.69 mm) [12, 18, 19]. We observed the same significance in the mean ML–PB distance between the dentate and partially edentulous subjects (13.95 *vs.* 16.03 mm), which was within the reported range of average values (13.02–18.2 mm) [4, 6, 20].

Clinical assessments in patients without morphological anomalies indicate that the MF is positioned slightly posterior to the midpoint in the anteroposterior plane and aligned with, or just above, the occlusal plane in the vertical dimension [11]. Evidence from cadaveric dissections and dry mandible studies places the MF approximately 2.75 mm behind the midpoint of the ramus and about 19 mm below the coronoid notch [11]. Radiographic analyses using panoramic imaging, together with anatomical preparations, showed the inferior alveolar foramen situated nearer to the posterior border of the mandibular ramus [18]. A CBCT study results showed that males exhibited greater distance from the MF to the posterior border, suggesting that male patients may have a relatively larger safety margin for osteotomy procedures [19]. The lingula has been described as lying further from the posterior border in males [7, 20], though Lupi et al. reported no sex-related variation [3]. Findings across studies are inconsistent: some note differences between the left and right sides, while others observe no asymmetry [20]. Population-based analysis also showed that the ML–PB distance did not distinguish Kenyans from Malays [10].

In a study that presented the same stratification as we did, a mean value of the MF–PB distance was found to be 14.66, 12.88, and 14.63 mm in the groups with no, 1–4, and 5–8 alveoli, respectively, with a significant difference between the latter two [9]. In contrast to our findings, Matveeva et al. [12] found that the PB was closer to the MF unilaterally in the edentate mandibles compared with the dentate group (9.23 *vs.* 10.69 mm on the right side). The

authors attributed these differences to resorptive alterations in the ramus due to the longer period of tooth loss. On the other hand, Menditti et al. [21] observed a remodelling of the alveolar bone with preserved surrounding soft tissue in the edentulous mandible. It was emphasized that a loss of lower teeth resulted in severe bone resorption and the mandible's structure modification [22]. This may lead to a decrease in the muscle activity and masticatory forces, which consequently may result in loss of bone tissue, which they hypothesize to be more in the region of the body of the mandible, rather than in its ramus, thus affecting the traction of the foramen towards the alveoli and further from the posterior ramus [22]. Although we found no comparable data for the ML–PB length, it is reasonable to suggest that the aforementioned changes may also affect the ML, given its close relationship with the MF.

The pioneering study on the lingual shapes in the Indian population identified the triangular lingula as the most prevalent, followed by the truncated, nodular, and assimilated [5], which was the same arrangement we found in dry mandibles as well. The same prevalence was observed in Turkish and Brazilian populations, whereas the truncated lingula was more frequent among Thais, Southern Indians, and South Africans [10]. It is suggested that the triangular shape would be the most easily identifiable and detectable during SSRO [17]. A meta-analysis of 4694 subjects postulated that the frequency differences are small, so the pooled prevalence rates were 29.33% for the triangular type, 27.99% for the nodular type, 27.62% for the truncated shape, and 10.49% for the assimilated lingulae [6]. Recent studies have used CBCT imaging to assess the shape of the human lingula. While CBCT avoids the issues associated with the preparation and preservation of dry mandibles, the interpretation of these images can be affected by reduced sharpness during software processing. In CBCT studies, the nodular type was most frequently observed, followed by truncated, triangular, and assimilated forms. These variations may relate to factors such as age, race, dentition, skeletal type, and the preparation and preservation of mandibles [23].

In the present study, the distance from the ML to the gonion was significantly larger in the truncated group than in the triangular group (33.37 *vs.* 27.99 mm). A CBCT study reported the same distance in a range of 27.3–27.8 mm [24]. It was discussed that elongated and very pointed mandibular lingulae might present with greater distances [3]. The height of the truncated lingulae in our study was lower compared with the triangular lingulae. Still, the tip of the latter pointed more posteriorly, thus being closer to the distally positioned gonion, which may explain why the ML–G distance was significantly larger in the truncated group.

This study presents several limitations. The absence of soft tissue and demographic details may overlook anatomical differences reported in studies utilizing CBCT as a rapidly advancing technology in clinical settings and contemporary morphometric and maxillofacial anatomical research. The relatively small sample size reduces the generalizability of the findings and highlights the need for larger datasets. Individual anatomical variability underscores the importance of pre-operative imaging, as dry bone data alone cannot fully guide surgical practice. Finally, validation through larger cadaveric studies, surgical trials, or correlation with imaging is required to confirm the applicability of these results *in vivo*.

CONCLUSION

The results of our study provide new information concerning the relationships of the mandibular foramen and lingula in a Serbian population sample. The morphometric results suggest that the foramen and lingula are positioned more posteriorly in dentate mandibles compared with partially edentulous mandibles. The most prevalent shape of the lingula we described was triangular (48%), followed by the truncated (32%), nodular (16%), and assimilated (4%). Depending on the shape and height of the lingula, the ML–gonion distance may be larger in the presence of a truncated lingula. The obtained data may be useful in planning and performing anesthetic blocks and surgical osteotomies.

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Морфометријска и морфолошка студија мандибуларног отвора и лингуле на доњим вилицама човека

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САЖЕТАК

Увод/Циљ Мандибуларни отвор (МО) и лингула (МЛ) представљају битне оријентире на унутрашњој страни гране мандибуле. Њихов положај у односу на ивице рамуса, дентицију и облик лингуле клинички је важан за анестетичке и хируршке процедуре. Циљ истраживања био је одредити морфолошке и морфометријске карактеристике МО и МЛ у односу на ивице гране мандибуле, присуство алвеола и облик лингуле у узорку српске популације.

Методе Материјал је чинило 50 полувилица човека из остеолошке колекције Катедре за анатомију Медицинског факултета Универзитета у Нишу. Мерена су растојања од МО и МЛ до горње, предње и задње ивице рамуса, слепоочног гребена и гониона, заједно са дијаметром МО и висином МЛ. Полувилице су класификоване по броју присутних алвеола (0, 1–4 и 5–8). Лингуле су подељене по облику на четири групе. Мерења су вршена дигиталним нонијусом, а просечне вредности са стандардним девијацијама анализиране су тестом АНОВА. Статистичка значајност је дефинисана као вредност $p < 0,05$.

Резултати Растојања између задње ивице рамуса и МО, односно МЛ као оријентира, била су статистички значајно већа код делимично безубих полувилица (14,49 *mm*, односно 16,03 *mm*) у поређењу са полувилицама са зубима (11,97 *mm* и 13,95 *mm*, по датом редоследу). Најчешћи облик лингуле био је троугласти (48%), затим зарубљени (32%), нодуларни (16%) и асимиловани (4%). Растојање од МЛ до гониона било је статистички значајно веће код мандибула са зарубљеном лингулом (33,37 *mm* према 27,99 *mm*).

Закључак Резултати студије указују на значај разматрања удаљености задње ивице мандибуле код делимично безубих пацијената, као и облика лингуле, за потребе оптималне локализације МО у анестезији и хирургији.

Кључне речи: анатомска варијација; мандибула; морфометрија; анестезија; мандибуларна остеотомија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Pharmacodynamic comparison of butylphthalide and edaravone dexborneol in acute cerebral infarction – a mechanism-based subgroup analysis focusing on culprit vessel and etiology

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SUMMARY

Introduction/Objective This study compared the pharmacodynamics of butylphthalide and sodium chloride (BSC) with those of edaravone dexborneol (ED) in middle-aged and elderly patients with acute cerebral infarction (ACI), focusing on etiology and culprit vessel stratification.

Methods A total of 138 middle-aged and elderly patients with ACI admitted to our hospital from January 2023 to June 2025 were enrolled and randomly assigned to the BSC group (n = 69) and the ED group (n = 69). Both groups received standard ACI treatment; the BSC group received BSC injection (25 mg twice daily), and the ED group received ED injection (30 mg twice daily). The main outcome measures were the National Institutes of Health Stroke Scale (NIHSS) score, modified Rankin Scale (mRS) score, and mean flow velocity (Vm) of the middle cerebral artery before and after treatment. The secondary indices included coagulation-related parameters [platelet count (PLT) and white blood cell (WBC) count] and safety evaluation. Subgroup analyses were based on culprit vessel type (large-vessel / small-vessel disease) and etiologic category (atherosclerotic infarction / cardioembolism).

Results Findings revealed that, compared with the ED group, the BSC group had a significantly lower NIHSS score ($p < 0.05$) and a significantly higher Vm in the middle cerebral artery, especially in the large-vessel disease subgroup ($p < 0.05$). Coagulation profiles showed that after seven days of treatment, platelet counts were lower in the BSC group compared with the ED group ($p < 0.05$). In the atherosclerotic infarction subgroup, BSC resulted in lower PLT counts than in the ED group ($p < 0.05$). Safety assessments indicated comparable adverse event rates and liver/kidney function between the groups ($p > 0.05$), with no cases of serious bleeding or organ damage.

Conclusion BSC exerts a pathology type-dependent therapeutic effect via its multi-target mechanism (vascular endothelial growth factor-mediated collateral augmentation and platelet-activating factor-dependent platelet inhibition), demonstrating significant etiology-specific efficacy and supporting its prioritization in large-vessel or atherosclerotic ACI subtypes.

Keywords: butylphthalide; edaravone dexborneol; acute cerebral infarction; middle-aged and elderly adults; neuroprotection; hemodynamics

INTRODUCTION

As the global population ages, the incidence of acute cerebral infarction (ACI), a leading cause of disability and mortality worldwide, continues to rise; Li et al. [1] pointed out that aging has significantly increased the global disease burden of ACI and related long-term disability. Middle-aged and elderly patients (≥ 50 years) face a particularly heightened risk of unfavorable outcomes. This vulnerability is attributed to age-related physiological decline, including reduced vascular compliance, diminished collateral circulation compensation capacity, and a higher burden of comorbidities [2]. Consequently, tailoring neuroprotective therapy to individual patients is paramount for improving clinical outcomes. Butylphthalide and sodium chloride (BSC) and edaravone dexborneol (ED) are commonly employed neuroprotective drugs. Their clinical benefits are well-established, supported by extensive clinical validation. For example, a Phase III clinical trial by Zhang et al. (2023) on BSC demonstrated its efficacy in significantly reducing the 90-day National Institutes of Health Stroke Scale (NIHSS) score and improving daily living activities in individuals with mild-to-moderate ACI [3]. Meanwhile, ED was shown in a multicenter study to suppress oxidative stress during the acute phase and shorten the time to neurological function recovery [4]. Clinical pharmacological studies have shown that BSC exerts neuroprotection by promoting collateral circulation and inhibiting glutamate excitotoxicity [5], while ED mainly targets oxidative stress [6]. Existing studies on BSC or ED, however, are predominantly conducted across broad age groups, overlooking the distinct considerations relevant to middle-aged and elderly patients [7, 8]. There is a lack of pharmacodynamic comparison between BSC and ED in middle-aged and elderly patients with ACI, especially when stratified by etiological mechanism. The diminished cerebral hemodynamic reserve commonly seen in these

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patients may alter how pharmacological agents influence cerebral arterial flow, differing from the effects observed in younger adults [9]. In addition, clinical effectiveness is considerably modulated by the high degree of heterogeneity in this group, influenced by factors such as infarct location and culprit vessel [10].

While ED primarily scavenges free radicals via Nrf2/ARE pathway activation, BSC simultaneously modulates vascular endothelial growth factor (VEGF) / notch-mediated angiogenesis and suppresses COX-2/P-selectin-dependent platelet adhesion. We hypothesize that such multi-target pharmacology confers broader efficacy in middle-aged and elderly patients with ACI with complex vasculopathy, particularly those with large artery atherosclerosis where hemodynamic rescue and thromboregulation are critical. Therefore, an innovative aspect of this investigation is its concentrated examination of heterogeneity in the middle-aged and elderly ACI population. The findings of this study will help fill the evidence gap in stratified pharmacology and provide a basis for precision medicine.

Table 1. Baseline data of the study groups

Parameter	BSC group (n = 69)	ED group (n = 69)	t or χ^2	p
Age	69.23 ± 12.81	68.45 ± 11.45	0.378	0.706
Male/Female	42/27	45/24	0.280	0.597
Time from stroke onset (h)	26.06 ± 11.59	29.38 ± 12.84	1.594	0.113
History of smoking Yes/no	34/35	30/39	0.466	0.495
History of drinking Yes/no	22/47	25/44	0.294	0.590
Large/small-vessel disease	39/30	37/32	0.117	0.732
Atherosclerotic infarction / cardiogenic embolism	42/27	46/23	0.502	0.479

METHODS

Study population

The sample size calculation was based on the expected between-group difference in the modified Rankin Scale (mRS) score, the primary outcome measure. Based on prior research, including the study by Shi et al. [11], a between-group difference of 0.5 points in mRS scores was assumed, with a standard deviation (SD) of 1.2. Given a two-sided alpha of 0.05 and 80% power (1–β), the calculation conducted with PASS 15.0 showed that 58 patients were needed per group. To allow for an estimated 20% dropout rate (including loss to follow-up, early withdrawal, or non-adherence to the protocol), the sample size was increased to 69 per group, yielding a minimum total of 138 participants. Patient selection for this study involved 138 patients with ACI, admitted to our hospital from January 2023 to June 2025. This cohort size was finalized after estimating the required sample size and implementing the specified inclusion and exclusion protocols. The clinical data of the two groups are shown in Table 1, and there was no significant difference between the two groups ($p > 0.05$).

Inclusion and exclusion criteria

Inclusion criteria: age between 50 and 90 years; time from stroke onset ≤ 48 hours; radiologically confirmed new cerebral infarction (lesion diameter ≥ 1 cm) on cranial magnetic resonance imaging (MRI) with diffusion-weighted imaging (DWI).

Exclusion criteria: presence of intracranial hemorrhage (e.g., intracerebral, subarachnoid) or tumor-related stroke; severe cerebral herniation (GCS score ≤ 8) or terminal illness (life expectancy < 3 months); significant hepatic/renal dysfunction; coagulopathy; allergy to BSC or ED; recent participation (within three months) in other therapeutic drug trials; pre-existing psychiatric or cognitive disorders that would impede study assessments.

Grouping and blinding

Using a centralized randomization system integrated with electronic medical records, participants were allocated in a 1:1 ratio to receive either BSC injection (BSC group) or ED injection (ED group). Block randomization (computer-generated sequence) was used, and assessors were blinded to minimize bias. The study adopted a single-blind design, in which both patients and outcome assessors were unaware of group assignments; statistical analysts were also blinded to treatment allocation throughout the analysis process. Baseline clinical characteristics showed no significant intergroup differences ($p > 0.05$), as summarized in Table 1.

Treatment protocols

Standard treatment for acute ischemic stroke was provided to both groups, consisting of antiplatelet agents (aspirin or clopidogrel), statins for plaque stabilization, and management of blood pressure and blood glucose. In addition, the BSC group was administered BSC injection (H20100041, CSPC-NBP Pharmaceutical Co., Ltd.) 25 mg (100 mL) per bottle, 25 mg per dose, twice daily, intravenously, with each infusion lasting ≥ 30 min. The ED group received ED injection (H20200007, Nanjing Simcere Dongyuan Pharmaceutical Co., Ltd.) 30 mg (15 mL) twice daily, intravenously, with each infusion lasting ≥ 30 minutes. All participants received treatment for 6–10 days, beginning within 48 hours after symptom onset. Concurrent use of additional neuroprotective drugs, including citicoline and oxiracetam, was not permitted. Administration of the trial drug was halted and documented if severe adverse events occurred, including elevation of liver enzymes greater than three times the upper limit of normal (ULN). The dose was based on previous pharmacokinetic studies to ensure that the blood concentration reached the therapeutic window [12, 13].

Outcome measures

The study included baseline (within 24 hours after admission) and post-treatment (within 24 hours after the end

of the 6–10-day treatment course) assessments of neurological status via the NIHSS, where higher scores signify worse neurological function [14, 15]. The peak systolic velocity (Vs), mean flow velocity (Vm), and pulsatility index (PI) of the middle cerebral artery (MCA) were measured by transcranial Doppler ultrasound (TCD) within 48 hours after admission and after treatment to evaluate improvements in cerebral blood flow. Blood samples obtained from patients were analyzed for coagulation [activated partial thromboplastin time (APTT), thrombin time (TT), international normalized ratio (INR), white blood cell (WBC) count, and platelet count (PLT)] and biochemical parameters [alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA), and urea (UREA)]. All adverse reactions during treatment were documented. Subgroup analyses further explored variations among patients categorized by culprit vessel type (large-vessel *vs*. small-vessel disease) and stroke etiology (atherosclerotic infarction *vs*. cardiogenic embolism).

Statistical analysis

The statistical evaluation was carried out using IBM SPSS Statistics, Version 31.0 (IBM Corp., Armonk, NY, USA). For categorical measures, group differences were examined via the χ^2 test or Fisher's exact test. The distribution of continuous data was verified with the Shapiro–Wilk normality test; normally distributed data were presented as mean ± standard deviation (SD), with intergroup comparisons performed using the independent-samples t-test and intragroup pre–post comparisons using the paired t-test. Non-normally distributed continuous data were presented as median (interquartile range, IQR), with intergroup comparisons performed using the Mann–Whitney U test and intragroup pre–post comparisons using the Wilcoxon signed-rank test. Results with p-values below 0.05 were deemed statistically significant.

Ethics: The investigation was performed following approval by the Lixin County People's Hospital Ethics Committee and after acquiring informed consent from all subjects.

RESULTS

Assessment of neurological improvement

Both patient cohorts had similar neurological impairment at baseline based on NIHSS and mRS scores ($p > 0.05$). The BSC intervention produced greater neurological improvement than ED treatment ($p < 0.05$), with subgroup analysis identifying this advantage specifically in the large-vessel disease subgroup ($p < 0.05$) and atherosclerotic infarction subgroup ($p < 0.05$), but not in small-vessel or cardioembolic cases ($p > 0.05$) (Figure 1 and Table 2).

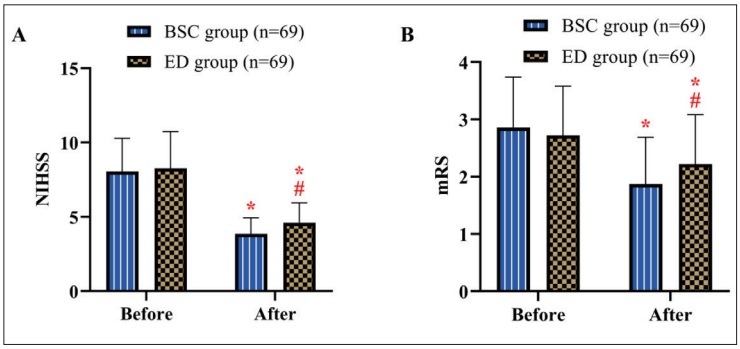

Figure 1. Comparison of neurological functions; (A) NIHSS before and after treatment; (B) mRS before and after treatment;

BSC – butylphthalide and sodium chloride; NIHSS – National Institutes of Health Stroke Scale; ED – edaravone dexborneol;
*p < 0.05 compared with before treatment;
#p < 0.05 compared with the BSC group

Table 2. Subgroup analysis of neurological function (NIHSS and mRS)

Subgroups		Groups	Before	After
NIHSS	Large-vessel disease	BSC (n = 39)	7.87 ± 2.4	3.79 ± 1.08
		ED (n = 37)	8.35 ± 2.52	5 ± 1.29#
	Small-vessel disease	BSC (n = 30)	8.30 ± 1.97	3.93 ± 1.08
		ED (n = 32)	8.16 ± 2.46	4.25 ± 1.27
	Atherosclerotic infarction	BSC (n = 42)	7.98 ± 2.45	3.81 ± 1.06
		ED (n = 46)	8.17 ± 2.59	4.89 ± 1.18#
	Cardiogenic embolism	BSC (n = 27)	8.19 ± 1.82	3.93 ± 1.11
		ED (n = 23)	8.43 ± 2.27	4.04 ± 1.46
mRS	Large-vessel disease	BSC (n = 39)	2.92 ± 0.81	1.69 ± 0.89
		ED (n = 37)	2.76 ± 0.89	2.24 ± 0.95#
	Small-vessel disease	BSC (n = 30)	2.77 ± 0.97	2.10 ± 0.66
		ED (n = 32)	2.69 ± 0.82	2.19 ± 0.74
	Atherosclerotic infarction	BSC (n = 42)	2.95 ± 0.82	1.74 ± 0.91#
		ED (n = 46)	2.76 ± 0.85	2.26 ± 0.93
	Cardiogenic embolism	BSC (n = 27)	2.7 ± 0.95	2.07 ± 0.62
		ED (n = 23)	2.65 ± 0.88	2.13 ± 0.69

BSC – butylphthalide and sodium chloride; NIHSS – National Institutes of Health Stroke Scale; mRS – modified Rankin Scale; ED – edaravone dexborneol;
#p < 0.05 compared with the BSC group

Evaluation of cerebral hemodynamics enhancement

Hemodynamic improvements were observed following treatment, with both groups demonstrating increased blood flow velocities in the MCA (increased Vs and Vm) and decreased PI compared with baseline values ($p < 0.05$). No significant between-group difference was observed in the improvement of Vs ($p > 0.05$). The PI decreased significantly in both groups after treatment ($p < 0.05$), with no significant between-group difference ($p > 0.05$). A markedly greater increase in Vm was recorded in the BSC group (52.16 ± 6.05 cm/s) compared with the ED group ($p < 0.05$). Specifically, the increase in Vm in the BSC group was significantly more pronounced in the large-vessel disease subgroup ($p < 0.05$). Given that mean flow velocity (Vm) is the most stable and representative indicator for evaluating cerebral perfusion status in ischemic stroke, which directly reflects the blood supply of

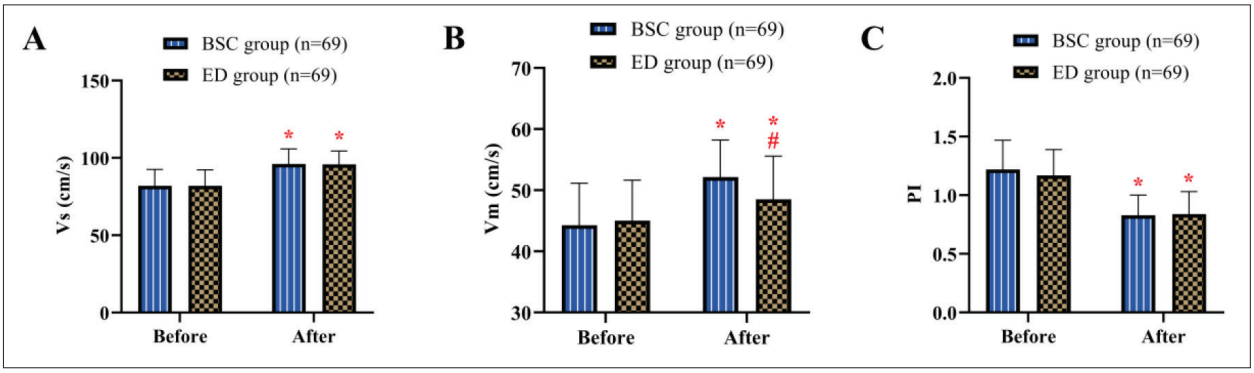

Figure 2. Comparison of cerebral hemodynamics; (A) Vs before and after treatment; (B) Vm before and after treatment; (C) PI before and after treatment;

Vs – peak systolic velocity; Vm – mean flow velocity; PI – pulsatility index;
*p < 0.05 compared with before treatment;
#indicates p < 0.05 compared with the BSC group

Table 3. Subgroup analysis of cerebral hemodynamics (Vm)

Subgroups	Groups	Before	After
Large vessel disease	BSC (n = 39)	43.64 ± 7.63	52.29 ± 5.57
	ED (n = 37)	44.11 ± 6.84	46.38 ± 7.28#
Small vessel disease	BSC (n = 30)	45.09 ± 5.76	51.99 ± 6.71
	ED (n = 32)	46.04 ± 6.34	50.95 ± 6.05
Atherosclerotic infarction	BSC (n = 42)	44.36 ± 6.75	53.04 ± 5.97
	ED (n = 46)	44.61 ± 6.25	48.22 ± 7.11#
Cardiogenic embolism	BSC (n = 27)	44.12 ± 7.19	50.8 ± 6.02
	ED (n = 23)	45.79 ± 7.43	49.06 ± 7.12

#p < 0.05 compared with the BSC group

Table 4. Subgroup analysis of coagulation function (WBC and PLT)

Subgroups		Groups	Before	After
WBC (× 10^9/L)	Large vessel disease	BSC (n = 39)	12.31 ± 1.82	8.37 ± 1.79
		ED (n = 37)	12.37 ± 2.49	10.1 ± 2.12#
	Small vessel disease	BSC (n = 30)	12.34 ± 1.72	7.83 ± 1.69
		ED (n = 32)	12.56 ± 2.37	10.6 ± 2.58#
	Atherosclerotic infarction	BSC (n = 42)	12.20 ± 8.27	8.27 ± 1.69
		ED (n = 46)	12.52 ± 2.4	10.21 ± 2.26#
	Cardiogenic embolism	BSC (n = 27)	12.53 ± 1.72	7.93 ± 1.86
		ED (n = 23)	12.32 ± 2.49	10.56 ± 2.53#
PLT (× 10^9/L)	Large vessel disease	BSC (n = 39)	291.24 ± 33.13	231.43 ± 35.29
		ED (n = 37)	287.49 ± 36.89	254.05 ± 33.45#
	Small vessel disease	BSC (n = 30)	294.38 ± 38.75	223.63 ± 24.61
		ED (n = 32)	292.93 ± 28.96	223.63 ± 21.94
	Atherosclerotic infarction	BSC (n = 42)	292.5 ± 32.41	234.23 ± 33.78
		ED (n = 46)	291.26 ± 34.54	249.1 ± 33.8#
	Cardiogenic embolism	BSC (n = 27)	292.77 ± 40.36	218.41 ± 24.04
		ED (n = 23)	287.53 ± 31.34	221.62 ± 19.16

#p < 0.05 compared with the BSC group

the ischemic penumbra, this study focused on Vm as the core hemodynamic outcome for subgroup analysis. Further analysis by subgroup demonstrated that the superiority of BSC in significantly enhancing Vm was confined to patients diagnosed with large-vessel disease and atherosclerotic infarction (p < 0.05) (Figure 2 and Table 3).

Monitoring of coagulation parameters

No intergroup differences in coagulation profiles were observed at baseline (p > 0.05). Post-intervention, parameters including PT, APTT, and INR showed no notable alterations (p > 0.05). Conversely, both WBC and PLT counts declined significantly from baseline levels (p < 0.05). A more marked decrease in PLT was noted in the BSC group versus the ED group (p < 0.05). Subgroup analysis showed that the BSC group had a significantly lower WBC count after treatment than the ED group in all subgroups (p < 0.05). However, in the large-vessel disease and atherosclerosis subgroups, the PLT count in the BSC group was lower than that in the ED group (p < 0.05) (Figure 3 and Table 4).

Comparison of hepatic and renal function

No marked variations in liver and kidney function markers (ALT, AST, CREA, UREA) were detected in either group following treatment (p > 0.05). The stability of these parameters implies minimal hepatorenal impact from both drugs (Figure 4).

DISCUSSION

ACI is a predominant contributor to disability and death among middle-aged and elderly individuals. Its pathophysiological mechanisms encompass multidimensional damage such as oxidative stress, inflammatory cascade activation, and impaired cerebral perfusion [16]. BSC and ED are known to provide neuroprotection in diverse age groups; however, older patients may respond differently due to age-related vascular stiffness, compromised collateral compensation, and multiple coexisting conditions. The central pharmacological finding of this study is that the efficacy advantage of BSC is not universal but is achieved by targeting the vascular pathology, because its platelet-inhibitory effect interacts precisely with the platelet hyperreactivity of atherosclerotic lesions, which provides a mechanistic basis for personalized medicine.

BSC, an emerging dual-target agent, provides neuroprotection via microcirculatory reconstruction – promoting collateral flow and suppressing glutamate excitotoxicity – and by boosting energy metabolism; meanwhile, BSC can not only upregulate VEGF to promote collateral circulation, but also inhibit platelet-activating factor (PAF) [5,

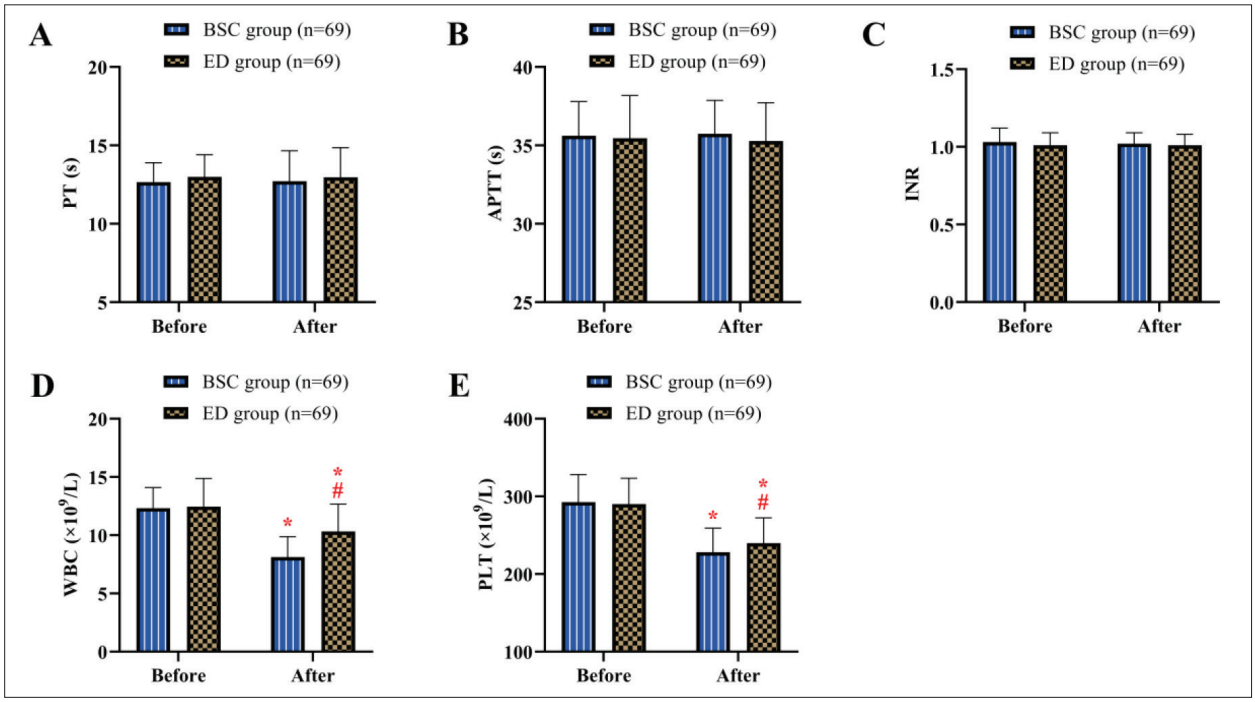

Figure 3. Comparison of coagulation function; (A) PT before and after treatment; (B) APTT before and after treatment; (C) INR before and after treatment; (D) WBC before and after treatment; (E) PLT before and after treatment;

APTT – activated partial thromboplastin time; TT – thrombin time; INR – international normalized ratio; WBC – white blood cell count; PLT – platelet count;
*p < 0.05 compared with before treatment;
#p < 0.05 compared with the BSC group

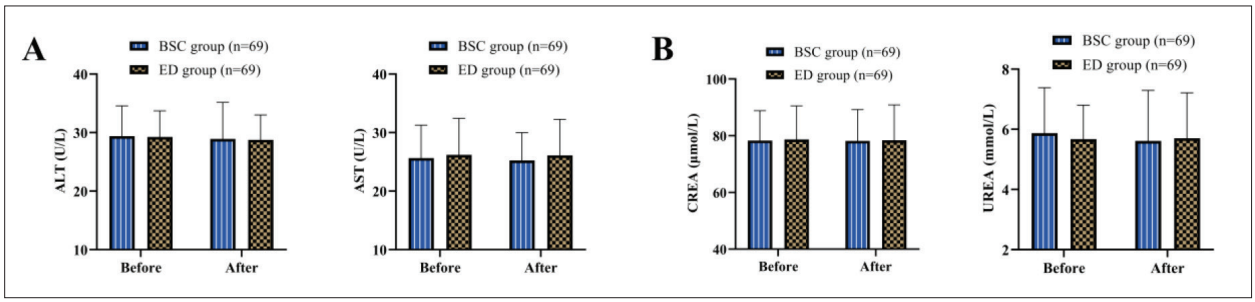

Figure 4. Comparison of liver and kidney functions; (A) ALT, AST before and after treatment; (B) CREA, UREA before and after treatment;

ALT – alanine aminotransferase; AST – aspartate aminotransferase; CREA – creatinine; UREA – urea;
*p < 0.05 compared with before treatment;
#p < 0.05 compared with the BSC group

17], which explains its advantage in large-vessel disease. This study found greater post-treatment NIHSS and mRS reductions with BSC, consistent with a previous Phase III clinical trial in mild-to-moderate ACI [12]. ED, a free-radical scavenger and inflammation suppressor, showed robust anti-inflammatory activity in previous reports [18]. However, it may lead to limited short-term neurological improvement, possibly due to delayed inflammatory modulation. Future pharmacodynamic monitoring is needed for validation. Regarding coagulation parameters, PLT was lower in the BSC group post-treatment, potentially due to BSC's inhibitory effect on platelet-activating factor (PAF)-mediated adhesion and aggregation; the decrease in PLT observed in the BSC group in this study may be related to its PAF inhibition mechanism [19]. The simultaneous improvement of Vm and PLT by BSC suggests a synergistic effect of both revascularization and thromboprophylaxis pathways, whereas the single antioxidant mechanism of ED may not address multiple pathological pathways.

Furthermore, in-depth subgroup analysis revealed significant associations between drug efficacy and specific pathophysiological features: (1) Hemodynamic-specific improvement in the large-vessel disease subgroup: Occlusions in large vessels (e.g., internal carotid artery, middle cerebral artery trunk) frequently result in significant hypoperfusion. BSC addresses this via a two-pronged mechanism: it enhances collateral circulation by upregulating vascular endothelial growth factor (VEGF), which promotes the opening of pial anastomoses [20], thus enhancing perfusion in the ischemic penumbra. Concurrently, as shown in animal experiments by Guo et al. [17], BSC modulates calcium channels to attenuate vascular smooth muscle contraction, mitigating secondary ischemia due to large-vessel disease spasm. The greater increase in Vm observed in the

large-vessel disease subgroup of the BSC group, compared with the ED group, is consistent with prior evidence demonstrating that BSC enhances intracranial large-vessel hemodynamics [21]. (2) Previous studies have confirmed that BSC can stabilize atherosclerotic plaques by suppressing MMP-9 to stabilize the fibrous cap [22], and the findings in this study may be relevant to this mechanism, which may underlie the observed differential outcomes in the atherosclerotic subgroup. (3) Targeted modulation of platelet activation: The more pronounced PLT reduction with BSC may result from its targeted inhibition of platelet activation. As noted, BSC competitively binds to the PAF receptor, suppressing collagen-induced platelet aggregation [19]. It also downregulates COX-2 and reduces thromboxane A2 synthesis [23]. Since platelet hyperactivity is common in atherosclerosis [24], BSC's platelet-modulating effects are particularly evident in these patients.

Therefore, our recommendation is to prioritize BSC in the management of middle-aged and elderly patients with ACI, especially in cases of large-vessel disease or atherosclerotic stroke, to achieve prompt restoration of cerebral perfusion. Vigilant monitoring of PLT is warranted throughout this treatment, particularly for high-risk patients, to guard against hemorrhagic tendencies. Furthermore, the complementary mechanisms of BSC and ED present a rationale for examining sequential or combined administration regimens. This approach holds promise for addressing the multifaceted pathology of ACI.

Key limitations of this work include its small sample size (n = 138) and observation period of seven days, preventing analysis of long-term prognosis (e.g., 90-day mRS) and recurrence, and possibly masking the drug's full therapeutic potential. The study also omitted quantitative evaluation of pivotal indicators such as infarct volume and collateral circulation grading, which could obscure subgroup heterogeneity. Moreover, the failure to track longitudinal changes in biomarkers related to inflammation or oxidative stress impedes a complete delineation of the drug's pharmacodynamic pathways.

Limitations

This study has several limitations. First, it was conducted at a single center with a relatively modest sample size, which may limit external validity and statistical power for subgroup analyses. Additionally, the sample size of some subgroups (e.g., cardiogenic embolism subgroup with n = 23) was relatively small, which may lead to insufficient statistical power for the corresponding subgroup analyses. Second, the follow-up was short, precluding assessment of longer-term functional outcomes and the durability of observed effects. Third, quantitative imaging metrics (e.g., infarct volume and collateral status) were not systematically incorporated, limiting evaluation of radiological heterogeneity. These indicators were not recorded systematically at the time of data collection. Finally, dynamic monitoring of inflammation- and oxidative stress-related biomarkers was not performed, which constrained mechanistic interpretation of the findings.

CONCLUSION

BSC shows a pathological type-dependent therapeutic effect in middle-aged and elderly patients with ACI through a multi-target mechanism (collateral circulation activation and platelet activity regulation). Its significant efficacy in the large-vessel disease or atherosclerosis subgroup suggests that future efficacy evaluation of neuroprotective agents should integrate vascular biology classification and pharmacokinetic parameters, rather than relying on clinical phenotype alone.

Availability of data and materials: The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest: None declared.

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Фармакодинамичко поређење бутилфталида и едаравон-дексборнеола код акутног исхемијског можданог удара – анализа подгрупа према етиологији и одговорној артерији

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САЖЕТАК

Увод/Циљ У овој студији упоређена је фармакодинамика бутилфталида и натријум-хлорида (*BSC*) са едаравон-дексборнеолом (ЕД) код средовечних и старијих болесника са акутним исхемијским можданим ударом, са фокусом на стратификацију према етиологији и одговорној артерији.

Методе Укупно 138 средовечних и старијих болесника са акутним исхемијским можданим ударом хоспитализованих у нашој болници од јануара 2023. до јуна 2025. године насумично је распоређено у *BSC* групу ($n = 69$) и ЕД групу ($n = 69$). Обе групе су примиле стандардну терапију за акутни исхемијски мождани удар; *BSC* група је примала инјекције бутилфталида и натријум-хлорида (25 *mg* два пута дневно), а ЕД група инјекције едаравон-дексборнеола (30 *mg* два пута дневно). Основне мере исхода биле су скор на Скали за мождани удар Националног института за здравље, скор на модификованој Ранкиновој скали и средња брзина протока крви кроз средњу мождану артерију пре и после лечења. Секундарни параметри укључивали су параметре коагулације (број тромбоцита, број леукоцита) и процену безбедности. Анализе подгрупа засноване су на типу одговорне артерије (болест великих крвних судова / болест малих крвних судова) и етиолошкој категорији (атеросклеротски инфаркт / кардиоемболизам).

Резултати Резултати су показали да је, у поређењу са ЕД групом, *BSC* група имала значајно нижи скор на Скали за мождани удар Националног института за здравље ($p < 0,05$) и значајно вишу средњу брзину протока крви кроз средњу мождану артерију, посебно у подгрупи са болешћу великих крвних судова ($p < 0,05$). Анализа профила коагулације показала је да је након седам дана лечења број тромбоцита био нижи у *BSC* групи у односу на ЕД групу ($p < 0,05$). У подгрупи са атеросклерозом, примена *BSC* довела је до нижих вредности броја тромбоцита у односу на ЕД ($p < 0,05$). Процене безбедности указале су на упоредиве стопе нежељених догађаја и функције јетре и бубрега међу групама ($p > 0,05$), без озбиљних крварења или оштећења органа.

Закључак *BSC* испољава терапијско дејство зависно од патолошког типа путем свог вишециљног механизма (побољшање колатералног крвотока посредовано фактором раста васкуларног ендотела и инхибиција тромбоцита зависна од фактора активације тромбоцита), показујући значајну етиолошки специфичну ефикасност, што оправдава приоритетну примену ове терапије код подтипова акутног исхемијског можданог удара повезаних са великим крвним судовима или атеросклерозом.

Кључне речи: бутилфталид; едаравон-дексборнеол; акутни исхемијски мождани удар; средовечна и старија популација; неурозаштита; хемодинамика

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Serum occludin combined with clinical features for predicting early neurological deterioration in intracerebral hemorrhage

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SUMMARY

Introduction/Objective The aim of this paper was to evaluate the incremental predictive value of 24-hour serum occludin beyond clinical features and to develop and internally validate a clinically applicable risk prediction model.

Methods This was a single-center prospective cohort study. Patients with spontaneous intracerebral hemorrhage (ICH) presenting within 24 hours of symptom onset were enrolled. The primary outcome, early neurological deterioration (END), was assessed at 72 hours post-admission. Variables for model development were selected using least absolute shrinkage and selection operator regression. A logistic regression model was constructed incorporating clinical and imaging factors and serum occludin levels measured at 24 hours post-admission. Model performance was evaluated using the area under the curve (AUC). Decision curve analysis was used to assess net clinical benefit across different risk thresholds.

Results Among the 600 enrolled patients, 210 (35%) developed END. The base model incorporating age, admission Glasgow Coma Scale score, ICH volume, intraventricular hemorrhage, location, surgical intervention, and systolic blood pressure achieved an optimism-corrected AUC of 0.78 (95% CI: 0.74–0.82). The extended model with added 24-hour serum occludin significantly improved discrimination (AUC = 0.84, 95% CI: 0.81–0.87; ΔAUC = 0.06, $p < 0.001$), with greater clinical net benefit across threshold probabilities of 10%–40%. Category-free net reclassification improvement was 0.42 (95% CI: 0.28–0.56), and integrated discrimination improvement was 0.08 (95% CI: 0.05–0.11). Risk stratification at 10% and 20% thresholds demonstrated high sensitivity (87%) and specificity (79%) for the identification of the high-risk group.

Conclusion Twenty-four-hour serum occludin significantly enhances predictive performance for END and holds potential as an improved biomarker. However, external validation is necessary before widespread implementation.

Keywords: intracerebral hemorrhage; early neurological deterioration; occludin; blood–brain barrier; prediction model; Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis

INTRODUCTION

Spontaneous intracerebral hemorrhage (ICH) accounts for 10–15% of all strokes, with 90-day mortality rates approaching 40% and only 20–30% of survivors achieving functional independence [1, 2]. Early neurological deterioration (END) typically occurs within 72 hours of onset and is a critical indicator affecting prognosis [3]. It is usually defined as a decrease in Glasgow Coma Scale (GCS) score ≥ 2 points or an increase in National Institutes of Health Stroke Scale (NIHSS) score ≥ 4 points within 24–72 hours of admission [4], with an incidence rate of 20–40% [5].

The mechanisms of END are complex, including hematoma expansion (occurring in about 30% of patients within 24 hours), perihematomal edema (PHE), intraventricular hemorrhage (IVH), and systemic complications [6, 7]. Early neurological deterioration remains common even in the absence of hematoma growth, suggesting that secondary brain injury, such as blood–brain barrier (BBB) disruption, also plays an important role [3].

Current clinical prediction models (such as the ICH score and FUNC score) are mainly based on baseline clinical and imaging variables, with limited ability to predict END [8, 9]. Although the recent SIGNALS score has improved discrimination to AUC values of around 0.78 [10], there remains scope for optimization through integration of biomarkers reflecting underlying pathophysiological processes, particularly BBB disruption.

The integrity of the BBB depends on tight junction proteins, particularly occludin, a 65-kDa transmembrane protein critical for regulating permeability [11, 12]. Following ICH, thrombin-mediated matrix metalloproteinase (MMP) activation, oxidative stress, and inflammation degrade occludin, disrupting the BBB and promoting PHE and secondary injury [13]. Experimental data show that occludin degradation is an early event: serum levels rise within hours after stroke and correlate with

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BBB damage [14], and occludin-deficient mice have worse outcomes [15]. In ICH, preliminary evidence links serum occludin to PHE volume [16], but findings are limited by small sample sizes and the lack of multivariable adjustment. Therefore, this prospective study aims to evaluate the incremental predictive value of 24-hour serum occludin beyond baseline clinical–imaging models and to develop a clinically applicable risk prediction tool for END in patients with ICH following the Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD) guidelines.

METHODS

Study design and ethical approval

This single-center prospective cohort study was conducted in accordance with the TRIPOD statement [17]. The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Baotou Medical College. Written informed consent was obtained from all participants or their legally authorized representatives before enrollment.

Study population and selection criteria

Patients presenting to the hospital's department with spontaneous ICH between February 2022 and August 2024 were screened for eligibility. The inclusion criteria were as follows: (1) spontaneous supratentorial or infratentorial ICH confirmed by non-contrast head computed tomography, (2) time from symptom onset to hospital admission ≤ 24 hours, (3) age 18–85 years, and (4) availability for blood sample collection at both baseline and 24-hour time points. The exclusion criteria were as follows: (1) secondary ICH due to arteriovenous malformation, tumor, trauma, or anticoagulation-related hemorrhage (international normalized ratio > 3 at presentation); (2) immediate postsurgical loss to follow-up or inability to assess END; (3) severe hepatic failure (Child–Pugh class C) or renal failure (estimated glomerular filtration rate < 15 mL/min/1.73 m^2); (4) concurrent enrollment in other interventional trials; (5) life expectancy < 3 months due to terminal illness; and (6) prior disability with modified Rankin Scale score > 3 before the current ICH [18].

Sample size calculation and statistical power

Based on the principle of events per predictor parameter ≥ 20 [19], the inclusion of 10–12 predictor variables would require 200–240 END events. Assuming an END incidence rate of 35% [4], the target sample size was 600 participants. According to Riley's criteria [20], a shrinkage factor ≥ 0.9 was ensured to reduce overfitting. Considering a 5% loss to follow-up, 630 participants were planned for enrollment.

Clinical data collection

All clinical variables were obtained within 24 hours of admission. Intracerebral hemorrhage volume, PHE volume, and IVH were calculated as previously described [14, 21, 22]. Intracerebral hemorrhage location was categorized as deep (basal ganglia or thalamus), lobar (involving cortical–subcortical regions), infratentorial (cerebellum or brainstem), or mixed based on epicenter determination. Computed tomography was performed using a 64-slice GE Revolution scanner (GE Healthcare Medical Systems, Slough, UK, and Milwaukee, WI). Hematoma (40–80 Hounsfield units) and PHE (15–33 Hounsfield units) volumes were measured using a validated semi-automated segmentation method with manual correction [23]. All imaging measurements were performed by two trained radiologists blinded to clinical data and biomarker results, with inter-rater reliability assessed using intraclass correlation coefficients. Serum occludin concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human Occludin ELISA Kit, catalogue #SEA145Hu, Cloud-Clone Corp., Houston, TX, USA) according to the manufacturer's instructions. Early neurological deterioration was defined as a decrease in GCS score of ≥ 2 points between baseline assessment (within six hours of admission) and the 72-hour evaluation, excluding deterioration attributable to sedation, seizures, or other reversible causes [4].

Data management and missing data handling

Data were entered into the REDCap system with logic checks and audit trails implemented. Little's missing completely at random test indicated that data were missing at random ($p = 0.18$), and multiple imputation by chained equations was performed ($m = 20$), with models including all predictor variables, outcomes, and auxiliary variables (e.g. length of hospital stay, discharge disposition). Continuous variables were imputed using predictive mean matching, whereas binary and multicategory variables were imputed using logistic and multinomial logistic regression, respectively. Imputation convergence was verified through trace plots and distribution comparisons. All analyses were performed across the imputed datasets, and results were pooled according to Rubin's rules [20, 24].

Statistical analysis

Statistical analyses followed a prespecified plan using R version 4.3.1. Descriptive statistics were used to compare END and non-END groups using appropriate parametric or non-parametric tests. Model development employed a two-stage approach: least absolute shrinkage and selection operator regression with 10-fold cross-validation for variable selection, followed by ridge regression for coefficient estimation. The base model incorporated core clinical–imaging variables, whereas the extended model additionally included 24-hour serum occludin. Internal validation was performed using bootstrap resampling (2000 iterations) to

Table 1. Baseline characteristics (n = 600)

Variable	Overall	END (n = 210)	No END (n = 390)
Age, years (mean ± SD)	64.2 ± 13.5	67.8 ± 12.4	62.3 ± 13.8
Male sex, n (%)	358 (59.7)	128 (61)	230 (59)
Time to admission, h (median [IQR])	8.4 [4.2–14.7]	7.9 [3.8–13.2]	8.7 [4.5–15.3]
Admission GCS (mean ± SD)	12.9 ± 3.2	11.2 ± 3.6	13.8 ± 2.1
Baseline ICH volume, mL (mean ± SD)	28.3 ± 23.5	38.6 ± 28.4	22.7 ± 19.3
24-hour ICH volume, mL (mean ± SD)	31.7 ± 25.8	42.1 ± 30.2	26.2 ± 21.4
24-hour PHE volume, mL (mean ± SD)	33.2 ± 26.4	42.3 ± 31.2	28.4 ± 22.7
IVH present, n (%)	247 (41.2)	122 (58.1)	125 (32.1)
Deep location, n (%)	312 (52)	118 (56.2)	194 (49.7)
Lobar location, n (%)	218 (36.3)	68 (32.4)	150 (38.5)
Infratentorial location, n (%)	70 (11.7)	24 (11.4)	46 (11.8)
Emergency surgery, n (%)	101 (16.8)	52 (24.8)	49 (12.6)
Admission SBP, mmHg (mean ± SD)	167.3 ± 28.4	172.8 ± 30.1	164.5 ± 27.2
Hypertension history, n (%)	448 (74.7)	162 (77.1)	286 (73.3)
Diabetes mellitus, n (%)	142 (23.7)	54 (25.7)	88 (22.6)
Antiplatelet use, n (%)	178 (29.7)	67 (31.9)	111 (28.5)
Anticoagulation use, n (%)	52 (8.7)	23 (11)	29 (7.4)
24-hour serum occludin, ng/mL (mean ± SD)	5.56 ± 3.12	7.82 ± 3.41	4.26 ± 2.18

END – early neurological deterioration; GCS – Glasgow Coma Scale; ICH – intracerebral hemorrhage; IVH – intraventricular hemorrhage; PHE – perihematomal edema; SBP – systolic blood pressure

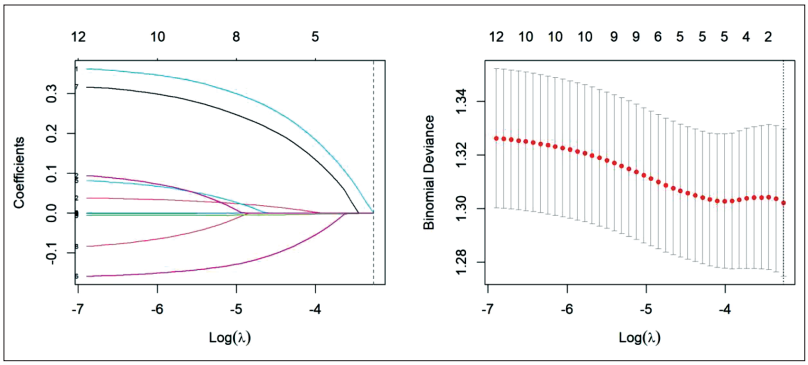

Figure 1. Variable selection process using LASSO regression; left: coefficient paths *vs.* log (lambda); optimal lambda (1-SE rule, dashed line) selected eight predictors; right: cross-validation deviance curve; numbers indicate variables retained at each lambda

obtain optimism-corrected performance estimates. Model performance was assessed using AUC with 95% CIs and Brier scores. The incremental value of occludin was evaluated through ΔAUC, net reclassification improvement (NRI), integrated discrimination improvement (IDI), and decision curve analysis. Risk stratification used prespecified cut-off points at 10% and 20% predicted probability, with sensitivity analyses examining model robustness across patient subgroups and alternative END definitions.

Ethics: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Baotou Medical College.

RESULTS

Study population and flow

A total of 687 patients with spontaneous ICH were screened, of whom 87 were excluded, leaving 600 patients included in the study. The mean age was 64.2 ± 13.5 years, 358 (59.7%) were men, and the median time from onset to admission was 8.4 (4.2–14.7) hours. Early neurological deterioration occurred in 210 patients (35%), consistent with the sample size assumptions.

Baseline characteristics and measurement reliability

Patients who developed END were older and had lower admission GCS scores, larger baseline ICH and 24-hour PHE volumes, higher rates of IVH, more frequent emergency surgery, and higher serum occludin concentrations (all $p < 0.001$) (Table 1).

Variable selection and model development

Least absolute shrinkage and selection operator regression selected eight predictors for the base model (Figure 1). Time to blood draw, antiplatelet use, and anticoagulant use were forced into the model. Occludin was added to the extended model ($\beta = 0.18$). After adjustment, 24-hour serum occludin remained independently associated with END (odds ratio per ng/mL: 1.23, 95% CI: 1.16–1.31, $p < 0.001$). Other predictive factors are shown in Table 2.

Table 2. Final model coefficients

Predictor	Base model β (OR, 95% CI)	Extended model β (OR, 95% CI)	VIF
Age (per year)	0.02 (1.02, 0.99–1.05)	0.01 (1.01, 0.98–1.04)	1.24
Admission GCS (per point)	-0.20 (0.82, 0.77–0.87)	-0.18 (0.83, 0.78–0.89)	2.18
24-hour ICH volume (per 10 mL)	0.22 (1.24, 1.16–1.33)	0.19 (1.21, 1.13–1.3)	2.45
24-hour PHE volume (per 10 mL)	0.08 (1.08, 1.02–1.15)	0.06 (1.06, 1–1.13)	1.87
IVH presence	0.78 (2.18, 1.52–3.12)	0.65 (1.92, 1.33–2.76)	1.56
Deep location	0.32 (1.38, 0.96–1.98)	0.28 (1.32, 0.92–1.9)	1.43
Emergency surgery	0.51 (1.67, 1.09–2.56)	0.42 (1.52, 0.99–2.33)	1.38
Admission SBP (per 10 mmHg)	0.05 (1.05, 0.99–1.11)	0.04 (1.04, 0.98–1.1)	1.22
24-hour serum occludin (per ng/mL)	–	0.21 (1.23, 1.16–1.31)	1.64

GCS – Glasgow Coma Scale; ICH – intracerebral hemorrhage; VIF – variance inflation factor; SBP – systolic blood pressure; all $p < 0.05$ except where OR crosses 1

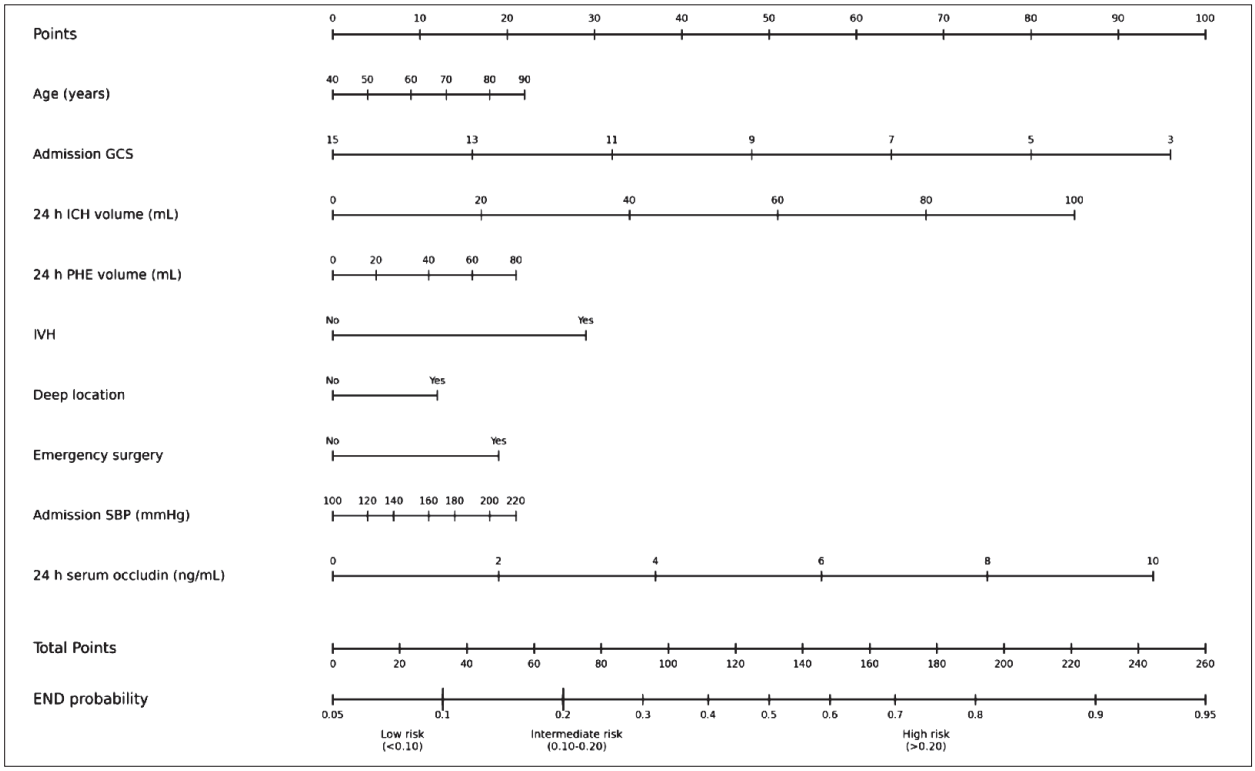

Figure 2. Nomogram for predicting END risk; risk groups may be interpreted as low risk (< 0.1), intermediate risk (0.1–0.2), and high risk (> 0.2)

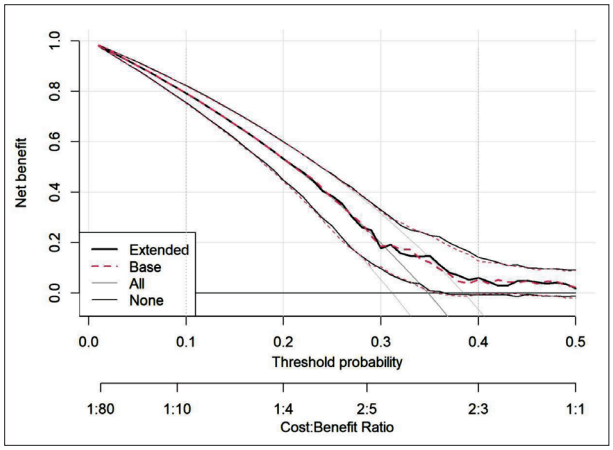

Figure 3. Decision curve analysis comparing models; net benefit across threshold probabilities (0–50%); extended model (solid black) outperforms base model (dashed gray) in clinically relevant range (10–40%), e.g., net benefit 0.22 *vs.* 0.17 at 15% threshold (equivalent to five additional true positives per 100 patients)

Model performance and validation

A nomogram (Figure 2) enables bedside risk estimation. Risk groups may be interpreted as low risk (< 0.10), intermediate risk (0.1–0.2), and high risk (> 0.2). The bootstrap-corrected AUC was 0.78 (95% CI: 0.74–0.82) for the base model, improving to 0.84 (95% CI: 0.81–0.87) with the inclusion of occludin. The Brier score decreased from 0.18 to 0.15. The addition of occludin yielded ΔAUC = 0.06 (95% CI: 0.04–0.08, p < 0.001), NRI = 0.42 (95% CI: 0.28–0.56, p < 0.001) and IDI = 0.08 (95% CI: 0.05–0.11, p < 0.001). Decision curve analysis (Figure 3) demonstrated superior net benefit for the extended model across 10–40% risk thresholds.

Sensitivity and subgroup analyses

The results remained robust in patients not undergoing surgery (AUC = 0.83 *vs.* 0.77), across different bleeding locations (deep, lobar, or subarachnoid; ΔAUC = 0.05–0.07) and blood sampling times (< 8 hours or ≥ 8 hours), in complete-case analysis (n = 581), and under alternative definitions of END (NIHSS ≥ 4, n = 542) (all p < 0.001, Table 3).

DISCUSSION

This prospective study demonstrates that 24-hour serum occludin provides substantial independent and incremental predictive value for END beyond standard clinical–imaging variables in ICH. The extended model achieved excellent performance (AUC = 0.84), improved reclassification (NRI = 0.42, IDI = 0.08), and greater net clinical benefit across relevant decision thresholds. These findings highlight the clinical value of serum occludin as a promising biomarker with demonstrable net clinical benefit across a range of decision thresholds. This enhancement in predictive accuracy supports the utility of serum occludin in improving patient stratification and clinical decision-making.

Compared with previous studies that focused only on ischemic stroke or small-sample correlation analyses, this study systematically verified the independent contribution of occludin by using a fixed 24-hour detection time point,

Table 3. Sensitivity analyses (selected subgroups); all sensitivity analyses demonstrate consistent incremental value of occludin

Subgroup	n	Events	Base AUC	Extended AUC	ΔAUC	p-value
Non-surgical patients	513	167	0.77	0.83	0.06	< 0.001
Deep location	312	118	0.76	0.82	0.06	< 0.001
Lobar location	218	68	0.79	0.84	0.05	0.002
Infratentorial location	70	24	0.75	0.82	0.07	0.041
Early blood collection (< 8 hours)	298	108	0.77	0.83	0.06	< 0.001
Late blood collection (≥ 8 hours)	302	102	0.79	0.84	0.05	0.001
Complete cases (no imputation)	581	204	0.78	0.84	0.06	< 0.001
Alternative END definition (NIHSS ≥ 4)	542	187	0.76	0.82	0.06	< 0.001

AUC – area under the curve; END – early neurological deterioration

strictly adjusting for confounding factors using multivariable correction, and applying modern evaluation methods such as NRI and IDI [15, 17]. Notably, the base model's AUC of 0.78 aligns with existing ICH prediction scores (such as the ICH score and FUNC score) [9, 10], whereas the inclusion of occludin led to a clinically meaningful improvement in predictive accuracy, emphasizing the importance of integrating biomarkers into clinical prediction models.

From a mechanistic perspective, post-ICH thrombin activation, MMP-9 activation, and oxidative stress are known to degrade occludin, leading to BBB disruption, PHE, and secondary brain injury [12, 14]. Importantly, our study shows that occludin retains predictive value even after adjustment for PHE volume, suggesting that it reflects subclinical BBB dysfunction that may not be captured by imaging alone. This finding is consistent with previous studies highlighting the role of occludin in BBB integrity and its correlation with neurological outcomes in patients with stroke [15].

The clinical applicability of our model is substantial. Patients classified as low risk (< 10%) may avoid unnecessary intensive care unit admission, whereas patients at high risk (> 20%) may be prioritized for interventions such as closer monitoring or early therapeutic strategies. The 24-hour sampling window used in this study is clinically feasible, and the nomogram developed provides a practical tool for bedside risk estimation, supporting applicability in routine clinical practice.

However, several limitations should be acknowledged. First, the single-center design of this study limits generalizability, and external validation in diverse settings is required before widespread clinical adoption. Second, the sample size in some subgroups, such as patients with subacute ICH (n = 70), was relatively small, and further studies including larger cohorts are needed to confirm these findings. Additionally, this study used a single serum occludin measurement at 24 hours post-admission, which does not capture potential dynamic changes in occludin levels over time. Longitudinal monitoring of occludin and other BBB biomarkers may provide more comprehensive insights into the progression of ICH and END. Finally, although this study focused on serum occludin, other BBB markers were not assessed, and future research should explore the synergistic effects of combining multiple biomarkers to improve predictive accuracy.

CONCLUSION

In conclusion, this prospective study demonstrates that 24-hour serum occludin significantly enhances END prediction in patients with ICH when added to comprehensive clinical–imaging models. The findings support conceptualizing occludin not as an isolated independent predictor but as an enhancement factor providing incremental value by capturing BBB disruption not fully reflected in conventional variables. External validation and impact studies are required before clinical adoption, but these results establish serum occludin as a promising biomarker warranting further investigation for personalized risk stratification in ICH management.

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Jingli Cheng: designed and conceptualized the study; drafted and revised the article.
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Серумски оклудин у комбинацији са клиничким карактеристикама у предвиђању раног неуролошког погоршања код интрацеребралног крварења

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САЖЕТАК

Увод/Циљ Циљ овог истраживања био је да се процени инкрементална предиктивна вредност серумског оклудина измереног 24 часа након пријема у односу на клиничке карактеристике, као и да се развије и интерно валидира клинички применљив модел за предвиђање ризика.

Методе Ова моноцентрична проспективна кохортна студија обухватила је болеснике са спонтаним интрацеребралним крварењем примљене у року од 24 часа од почетка симптома. Примарни исход, рано неуролошко погоршање, процењиван је 72 часа након пријема. Варијабле за развој модела изабране су применом *LASSO* регресије (енгл. *least absolute shrinkage and selection operator*). Конструисан је логистички регресиони модел који је укључивао клиничке и радиолошке параметре, као и нивое серумског оклудина измерене 24 часа након пријема. Перформансе модела процењене су коришћењем површине испод *ROC* криве (*AUC*). Анализа криве одлучивања примењена је ради процене нето клиничке користи при различитим праговима ризика.

Резултати Од укупно 600 укључених болесника, код 210 (35%) развило се рано неуролошко погоршање. Основни модел, који је укључивао старост, скор на Глазговској скали коме на пријему, волумен интрацеребралног крварења, интравентрикуларно крварење, локализацију, хируршко лечење и систолни артеријски притисак, постигао је вредност *AUC* кориговану за оптимизам од 0,78 (95% *CI*: 0,74–0,82). Додавање серумског оклудина након 24 часа значајно је побољшало дискриминацију (*AUC* = 0,84; 95% *CI*: 0,81–0,87; *ΔAUC* = 0,06; $p < 0,001$), уз већу нето клиничку корист у распону прагова ризика од 10% до 40%. Нето побољшање рекласификације без категорија износило је 0,42 (95% *CI*: 0,28–0,56), док је интегрисано побољшање дискриминације износило 0,08 (95% *CI*: 0,05–0,11). Стратификација ризика при праговима од 10% и 20% показала је високу осетљивост (87%) и специфичност (79%) у идентификацији болесника високог ризика.

Закључак Серумски оклудин измерен 24 часа након пријема значајно побољшава предиктивне перформансе за рано неуролошко погоршање и представља обећавајући биомаркер. Ипак, неопходна је спољашња валидација пре шире клиничке примене.

Кључне речи: интрацеребрално крварење; рано неуролошко погоршање; оклудин; крвно-мождана баријера; модел предикције; Транспарентно извештавање о мултиваријабилном предиктивном моделу за индивидуалну прогнозу или дијагнозу

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Hearing and balance in vestibular schwannoma – a 12-year clinical perspective

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SUMMARY

Introduction/Objective Vestibular schwannoma (VS) is a benign tumor originating from Schwann cells, predominantly affecting the vestibular portion of the eighth cranial nerve. It is the most common tumor of the cerebellopontine angle, presenting with varying degrees of hearing loss, along with tinnitus and vestibular symptoms. This paper gives a comprehensive analysis of the degree of hearing and balance impairment in individuals presenting with VS, along with a detailed clinical perspective.

Methods The research involved a retrospective analysis of the clinical database utilizing available medical records from the Audiology Department of a major tertiary care hospital in Serbia. It encompassed 83 patients diagnosed with VS between 2011 and 2023, with presenting symptoms of hearing and/or balance impairment. The analysis included basic demographic data, presenting symptoms, and results of tonal liminal audiometry, alongside specific vestibular diagnostic tests and imaging.

Results A significant hearing loss was observed in the majority of patients with VS ($p < 0.01$), with 48.7% having a severe degree of sensorineural hearing loss. Increasing age was positively associated with higher degrees of hearing impairment, as expected ($p < 0.05$). Dizziness was present in 15.6% of patients, while postural instability was reported by 29% of patients. The impairment of the vestibulo-ocular reflex of the lateral semicircular canal was observed in 62.7% of cases.

Conclusion The study provides insight into the hearing and balance of patients with VS. The results highlight the need for continuous monitoring, both pre- and postoperatively, to understand the relationships between patients' characteristics, symptoms, degree of hearing impairment, and tumor localization. These data are crucial for improving diagnostic and multidisciplinary therapeutic approaches to enhance the quality of care and function in these patients.

Keywords: vestibular schwannoma; hearing loss; dizziness; cerebellopontine angle

INTRODUCTION

Vestibular schwannoma (VS), also known in the literature as acoustic neurinoma, is the third most common benign intracranial tumor after meningioma and pituitary adenoma, with the most common localization at the level of the cerebellopontine angle (CPA) [1, 2]. It originates from nerve sheath cells (Schwann cells) and usually involves the vestibular part of the vestibulocochlear nerve, although its most common clinical presentation is cochlear, i.e., ringing in the ear and varying degrees of hearing impairment [3, 4]. Although benign, a VS, due to its localization, carries the danger of the compressive mass effect on other intracranial structures [3]. Clinically, most patients present with unilateral high-frequency sensorineural hearing loss (94%) and tinnitus (83%) [1, 3, 4]. Hearing loss in patients with VS has traditionally been associated with direct pressure and stretching of the vestibulocochlear nerve during tumor growth, causing disruption of the vascular supply [5]. However, the onset and course of hearing loss often cannot be predicted; it occurs in very small tumors, and its progression can be observed even in patients with non-growing lesions [6]. The frequency of vestibular symptoms such as vertigo and unsteadiness of gait varies widely (from 17% to 75% of patients), and it is considered that symptoms are often underreported or unrecognized [7]. Furthermore, large tumors can cause trigeminal and facial neuropathies, such as paresthesia, and can exert pressure on the brain stem, leading to hydrocephalus and increased intracranial pressure [1, 3]. Most tumors are unilateral and sporadic, while bilateral localization is rare, present in less than 5% of cases, and characteristic of the hereditary disease, neurofibromatosis type 2 [1–4]. Symptoms usually appear between the fifth and sixth decades of life, with a tendency for symptoms to appear earlier in people with a hereditary form of the disease [1, 2, 3]. The reported incidence of VSs shows an increasing trend, but there is a general consensus that this increase may be attributed to improved detection of new cases and more frequent imaging [3].

The goal of this research is to perform a comprehensive analysis of the degree of impairment of hearing and balance in individuals presenting

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with VS and to point out the importance of precise preoperative audiovestibular assessment both to improve diagnostic protocols and to better guide therapeutic approach (either "wait and scan", radiotherapy or microsurgery).

METHODS

The retrospective analysis of the available medical records of the Department of Audiology in the tertiary health care hospital was conducted. The study included 83 patients diagnosed with VS between 2011 and 2023 who had symptoms and signs of hearing and/or balance impairment.

Basic demographic data, presenting symptoms, as well as the results of specific diagnostic tests such as pure-tone audiometry (measured by AC40 clinical audiometer, Interacoustics, Middelfart, Denmark) were included. The average values of pure tone audibility thresholds for air and bone conduction were determined: air pure tone average or bone pure tone average, and the difference between the air and bone thresholds, i.e., air-bone gap (ABG). PTA and ABG values are calculated as a mean value for frequencies important for speech understanding, according to the recommendations of the American Academy of Otorhinolaryngology–Head and Neck Surgery (at 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz, where ABG values > 10 dB are defined as clinically significant due to normal test variability i.e., limitations of equipment and natural fluctuation of audiometric testing) [8]. Additionally, data on all tests performed during the neuro-otological examination, including the presence of spontaneous or gaze-evoked nystagmus, as well as provoked and induced nystagmus, were included (tested with Frenzel or videonystagmography goggles, Visual Eyes 515, Interacoustics). The presence of asymmetry in vestibular tonus was assessed using the head-shaking test, and the testing of the vestibulo-ocular reflex (VOR) of the lateral semicircular canal was performed both with low-frequency stimulus (bithermal Aqua Stim Caloric Irigator, Interacoustics) and with high-frequency stimulus with clinical head impulse test (HIT). Furthermore, the data obtained by imaging methods available in medical documentation were evaluated. Since the data in this study do not include patient-identifiable information, and no additional data collection was performed, no significant ethical concerns were identified. Descriptive and analytical statistics methods were used for analyses, while statistical hypotheses were tested at the $p < 0.05$ level of statistical significance.

Ethics: The study was formally approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade.

RESULTS

Main demographic characteristics of the sample

The average age of the patients was 52 years (SD ± 15.6), with the youngest patient being 11, and the oldest patient being 83 years old. The majority of included patients were female, comprising 51 patients (61.4%). The median time from the onset of symptoms to the first medical examination was 12 months. In four patients, VS was incidentally detected during a routine examination or imaging for other reasons. The most common initial complaint was impaired hearing (49.4%), followed by tinnitus (26.5%), while tingling in half of the face and vertigo were the least reported initial complaints, found in only two and one patient (2.4% and 1.2% respectively). For detailed patient characteristics, please refer to Table 1.

Table 1. Detailed patient's demographic characteristics

Patients' characteristics (83 overall, unless otherwise specified)	Results (%)
Age	Mean ± SD: 52.34 ± 15.62
Sex	
Male	32 (38.6%)
Female	51 (61.4%)
Duration of symptoms	Median: 12 months
Presenting symptom	
Tinnitus	22 (26.5%)
Hearing loss	41 (49.4%)
Instability	4 (4.8%)
Vertigo	1 (1.2%)
Paresthesia	2 (2.4%)
Missing data	13 (15.6%)
Affected side	
Left	42 (50.6%)
Right	39 (47%)
Bilateral	1 (1.2%)
Missing data	1 (1.2%)
Tinnitus on the affected side	
Present	58 (70%)
None	25 (30%)
Vertigo	
Yes	13 (15.7%)
No	61 (73.5%)
Missing data	9 (10.8%)
Instability	
Yes	24 (28.9%)
No	47 (56.6%)
Missing data	12 (14.5%)
Average tumor size based on MRI findings	Mean ± SD: 17.6 ± 9.6 mm
Tumor localization	
IAM	22 (26.5%)
CPA	17 (20.5%)
IAM-CPA	21 (25.3%
Missing data	23 (27.7%)

IAM – internal auditory meatus; CPA – the cerebellopontine angle

Hearing impairment

Age is positively associated with the severity of hearing impairment (right ear, $r = 0.461$, $p < 0.05$; left ear, $r = 0.220$, $p < 0.05$). As expected, there was a statistically significant relationship and high correlation between the severity of hearing impairment and the value of the air conduction thresholds at frequencies from 1000 Hz to 4000 Hz, with

Table 2. Pure tone average (PTA) in decibels for both ears and the degree of hearing impairment

Degree of hearing loss	Side affected			
	Right		Left	
	PTA in dB HL right ear	Percent of patients	PTA in dB HL left ear	Percent of patients
Normal hearing	16	17.9%	17.5	16.3%
Mild	33.75	17.9%	33.2	20.9%
Moderate	52.50	10.3%	49.4	20.9%
Severe	80.75	48.7%	76	30.2%

PTA on four frequencies important for speech comprehension (500, 1000, 2000, and 4000 Hz); dB HL – decibel hearing level; total deafness was found in 5.2% of patients with right vestibular schwannoma, and 11.7% with vestibular schwannoma localized in the left ear

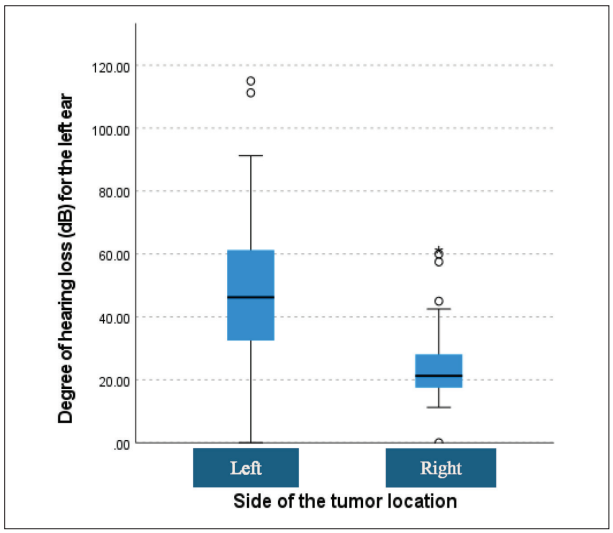

Figure 1. Average values of the degree of hearing loss in the left ear, expressed in decibel hearing level, in relation to the tumor localization

coefficients between 0.836 and 0.926 for the left ear and between 0.904 and 0.916 for the right ear ($p < 0.001$).

We examined 83 patients; however, one patient had incomplete data on the tumor side, and another had bilateral tumors. Therefore, the correct number of patients evaluated is 82, with 83 ears included in the final analyses. When the tumor was on the right, pure-tone audiometry showed that the largest number of patients (48.7%) had a severe degree of hearing impairment, and complete deafness was found in 5.2% of patients. Mild sensorineural hearing impairment was present in 17.9% of patients, while the same percentage of patients had normal hearing thresholds. Hearing in the opposite ear was preserved. In cases with a tumor on the left, slightly different patterns of hearing impairment were observed, with severe impairment in 30.2% and complete deafness in 11.7% (see Table 2 and Figure 1 and 2 for further details on hearing impairment).

Vestibular impairment

Vertigo was present in 13 patients (15.6%), while 24 patients (29%) reported instability while walking. Only one patient had spontaneous nystagmus at the initial examination. Impairment of the angular VOR of the lateral semicircular canal, confirmed by a positive HIT, was recorded in 62.7% of patients, while positive post-head-shaking nystagmus

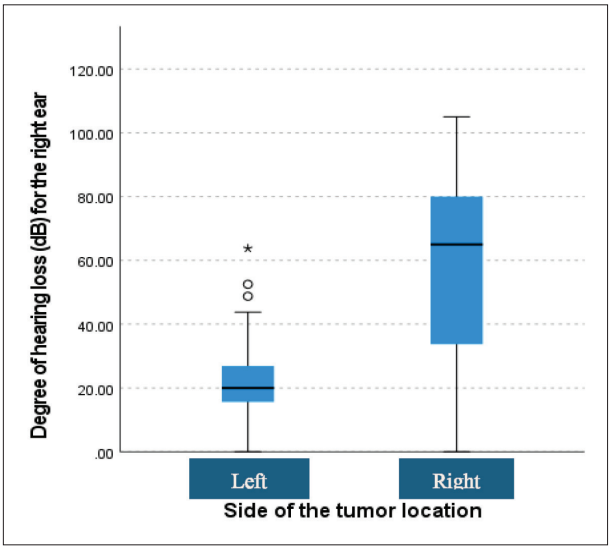

Figure 2. Average values of the degree of hearing loss in the right ear expressed in decibel hearing level in relation to the tumor localization

was present in 68.1% of the patients (as a sign of vestibular tonus imbalance). Based on the analysis of the results of the bithermal caloric test performed in 25 patients, hyporeflexia was observed on the side of the tumor in 13 patients, while areflexia was present in an additional nine patients. Symmetric (normal) findings were noted only in three patients. By analyzing the correlations between the results of different vestibular tests, a statistically significant positive correlation was observed between the positive findings in the head-shaking test and the HIT ($r = 0.451$, $p < 0.01$). However, a significant difference in the frequency of vertigo, instability while walking, results of the head-shaking test, HIT, or caloric test in relation to the degree of hearing impairment in both ears was not found ($p > 0.05$).

Tumor size and location

The average tumor size measured by magnetic resonance imaging (MRI) was 17.6 mm (SD ± 9.6 mm). There was a significant difference in the size of the tumor in relation to the localization, namely that tumors in the CPA and at the CPA-internal auditory meatus (IAM) level are larger compared to tumors localized at the level of IAM only ($p < 0.001$) (Figure 1). In our sample, no statistically significant difference was found between the size of the tumor and the degree of hearing impairment ($p < 0.05$).

DISCUSSION

It is known that VS is a slow-growing, benign tumor, usually unilaterally localized, with a tendency to involve the vestibulocochlear nerve and an overall incidence of 2.2 cases per 100,000 inhabitants per year (reaching 20.6 per 100,000 person-years for those over 70) [9, 10]. The main and most commonly reported symptom is asymmetric or unilateral hearing loss. It is also the most common initial manifestation of the disease and the main reason for diagnostic MRI in most cases [1, 3, 4, 9, 10, 11]. Similarly,

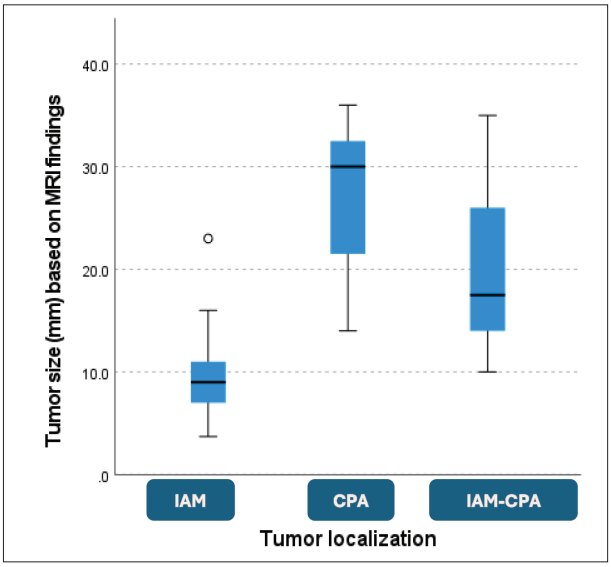

Figure 3. Average tumor dimensions (in mm) by tumor localization; IAM – internal acoustic meatus, CPA – cerebellopontine angle

impaired hearing was the most common initial complaint present in 49.4% of our patients. Wagner et al. [12] stressed that the age of patients significantly influences the degree of hearing loss before treatment. This is confirmed in our sample, where older patients had a more severe degree of hearing impairment, while tumor size had no significant effect on hearing loss. The findings of asymmetric sensorineural hearing impairment in our study meant that the largest number of patients had moderately severe and severe hearing impairment on the affected side, with preserved hearing thresholds in the other ear. The hearing level in the contralateral ear is very important when considering serviceable hearing and in deciding on best treatment modality [1, 13]. This severity of hearing impairment can be explained by the relatively long period from the onset of symptoms to visiting a doctor, which is in accordance with the results of previously published research [11].

Although VS more often involves the vestibular part of the vestibulocochlear nerve, vertigo is often not the primary symptom, and was present in only 15.7% patients included in this study, plus up to 30% of them reported gait instability. Since VS grow very slowly over the years, progressive dysfunction of the vestibular nerve is inevitable and causes a gradual increase in the use of central adaptive mechanisms, known collectively as vestibular compensation [14]. Even if patients experience acute vertigo due to axonal stretching, vestibular nerve compression, or compromised blood supply to the labyrinth induced by tumor growth, compensation develops over time and symptoms diminish [7, 14, 15]. It is important to emphasize that vestibular symptom severity does not increase linearly with size but may be most pronounced in small–medium tumors (where partial nerve injury is occurring), and in very large tumors, often near-complete vestibular loss with central compensation is present, paradoxically reducing patient's symptoms. Pathological results of the caloric test were recorded in 22 out of 25 patients who underwent the test. Borgmann et al. [16] pointed out that the caloric test should always be performed since it can be useful in predicting the origin of the VS (superior *vs*. inferior vestibular nerve) and could serve as an indirect predictor of hearing preservation after surgery (this was based on significantly smaller hearing loss in patients with tumors of the superior vestibular nerve). In addition to the caloric test, HIT is used to assess the high-frequency horizontal VOR and is currently the only bedside test that enables the identification of unilateral hypofunction of the peripheral vestibular system [15]. In our study, the results were positive in 62.7% of patients.

It remains unclear whether and how tumor size influences the degree of hearing loss in patients with VS. Some studies support this idea, while others have shown that the tumor size does not correlate well with the level of hearing impairment [17, 18]. Our results indicate that there is no statistically significant relationship between hearing impairment and tumor size. Nonetheless, there are multiple published audiometric protocols for guidance on obtaining gadolinium-enhanced MRI in patients with asymmetrical hearing loss, and an interaural difference of at least 15 dB averaging in the 0.5 and 3 kHz frequency range was recommended as optimal by the American Academy of Otolaryngology–Head and Neck Surgery (sensitivity 87.4%, specificity 65.4%) [19, 20].

Limitations of the study

In addition to being retrospective and susceptible to recall and information bias, this study has several other limitations that warrant mention. To accurately determine hearing functionality, it is essential to consider the results of the speech discrimination test, which were not included in this assessment due to the unavailability of data [12, 13]. Consequently, we relied solely on pure tone audiometry data. Furthermore, as this study was conducted at a single tertiary center, the generalizability of the data may be limited. Future studies should focus on including the results of speech discrimination tests and complete vestibular assessment to better elucidate any influence of tumor parameters on hearing and vestibular function.

CONCLUSION

This study provides insight into the initial clinical profile of patients with VS. Given that hearing loss does not necessarily correlate with tumor size and vestibular symptoms may be mild or even paradoxically subclinical in advanced cases, it is crucial to screen for VS every patient presenting with asymmetric sensorineural hearing loss (interaural difference of at least 15 dB averaging in the 0.5 and 3 kHz frequency range), unilateral tinnitus or vestibular findings. Our findings underscore the importance of detailed pre-treatment audiovestibular assessments and continued follow-up to improve diagnostics and develop personalized therapeutic approaches to preserve auditory and vestibular function, and enhance the quality of life of these patients.

Conflict of interest: None declared.

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Слух и равнотежа код вестибуларног шванома – 12-годишња клиничка перспектива

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САЖЕТАК

Увод/Циљ Вестибуларни шваном (ВШ) је бенигни тумор који потиче из Шванових ћелија и најчешће захвата вестибуларни део осмог можданог нерва. То је најчешћи тумор понтоцеребеларног угла, који се испољава различитим степеном оштећења слуха, зујањем у ушима и вестибуларним симптомима. У овом истраживању анализиран је степен оштећења слуха и равнотеже код особа којима је постављена дијагноза ВШ, уз детаљан осврт на клиничку слику.

Методе Истраживање је обухватило ретроспективну анализу базе података на основу доступне медицинске документације Одсека за аудиологију највеће установе терцијарног нивоа здравствене заштите у Србији. Обухваћена су 83 болесника са дијагнозом ВШ постављеном између 2011. и 2023. године, који су имали симптоме оштећења слуха и/или равнотеже. Анализирани су основни демографски подаци, клиничка слика и резултати тоналне лиминалне аудиометрије, као и резултати специфичних вестибулолошких дијагностичких тестова и радиолошке дијагностике.

Резултати Значајно оштећење слуха примећено је код већине болесника са ВШ ($p < 0,01$), при чему је 48,7% имало тежак степен сензоринеуралног оштећења слуха. Године старости су позитивно корелирале са тежим степеном оштећења слуха, као што се и очекивало ($p < 0,05$). Вртоглавица је била присутна код 15,6% болесника, док је постуралну нестабилност пријавило 29% болесника. Оштећење вестибуло-окуларног рефлекса латералног полукружног канала уочено је у 62,7% случајева.

Закључак Наше истраживање пружа увид у клинички профил и презентацију болесника са ВШ. Добијени резултати наглашавају потребу за континуираним праћењем и преоперативно и постоперативно, како би се боље разумели односи између одређених карактеристика болесника, присутних симптома, оштећења слуха и саме локализације тумора. Ови подаци су кључни за побољшање дијагностичких и мултидисциплинарних терапијских приступа, у циљу унапређења квалитета неге и лечења ових болесника.

Кључне речи: вестибуларни шваном; оштећење слуха; вртоглавица; понтоцеребеларни угао

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Therapeutic effect of mesotherapy on pain in patients with knee osteoarthritis

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SUMMARY

Introduction/Objective Knee osteoarthritis is a progressive, degenerative disease of the knee joint that can eventually lead to disability. Clinical mesotherapy is an intradermal therapy used for injecting diluted pharmacological substances into the superficial layer of the skin at multiple points. The objectives of this study were to determine the therapeutic effect of mesotherapy on pain in patients with knee osteoarthritis treated with a mixture of Zodol (Zodol, 30 mg/mL, Hemofarm, Vršac, Serbia) and lidocaine, compared to patients with knee osteoarthritis treated with lidocaine alone.

Methods Participants were randomly assigned into two groups. The experimental group, in which patients were treated with an injection containing a mixture of lidocaine without adrenaline (Lidocainechlorid 1%, 35 mg / 3.5 mL, Galenika, Belgrade, Serbia) and of Zodol (Hemofarm). The control group was treated with lidocaine solution without adrenaline.

Results There is a statistically significant difference in pain intensity after the second dose of mesotherapy and one month after the fourth dose of mesotherapy. Western Ontario and McMaster Universities Osteoarthritis Index scale values were significantly higher in patients in the control group. The frequency of patients experiencing moderate and severe pain was significantly higher in the control group, while the frequency of patients reporting no pain or only mild pain was significantly higher in the experimental group.

Conclusion The therapeutic effect of mesotherapy on pain in patients with knee osteoarthritis in experimental group is more effective and longer-lasting compared to patients treated with lidocaine alone.

Keywords: mesotherapy on pain; knee osteoarthritis; intradermal therapy

INTRODUCTION

Knee osteoarthritis is a degenerative joint disease that occurs as a result of progressive loss of articular cartilage. It most commonly affects older adults. It can be divided into two types: primary and secondary. Primary osteoarthritis occurs without any apparent cause, while secondary osteoarthritis can result from other conditions, most commonly including post-traumatic and post-surgical states, rickets, gout, and others. Common clinical symptoms include knee pain, which develops gradually and worsens with activity, as well as stiffness and swelling of the knee. Treatment of knee osteoarthritis begins with conservative methods, and if these do not yield results, surgical treatment is considered [1]. Nonsteroidal anti-inflammatory drugs (NSAID) are the first line of treatment for knee osteoarthritis. However, patients who cannot take these medications or do not respond to them may try intra-articular corticosteroid injections, which usually relieve pain for several weeks. When it comes to non-pharmacological treatment, patient education, weight loss (for those who are overweight), and exercise play important roles. Exercises usually focus on strengthening the muscles of the lower limbs, which helps reduce pain and improve functional status [2].

Clinical mesotherapy

Therapeutic skin injections date back to ancient Chinese and Indian medicine. In 1958, Michel Pistor introduced the term "mesotherapy" to describe the inoculation of drugs into the superficial layer of the skin [3].

Clinical mesotherapy is an intradermal therapy used to inject diluted pharmacological substances into the superficial layer of the skin at multiple points, at a depth of 3–4 mm. Specifically, this involves the use of a short needle to deposit the drug into the dermis. The intradermal microdeposit modulates the drug's kinetics by slowing absorption and prolonging the local mechanism of action [3, 4, 5]. This technique involves infiltrating a small amount of the drug into the superficial layer of the skin, observing the painful area. When injected intradermally, the drug diffuses into the tissues and joints, remaining for a longer period than with intramuscular administration. The goal of mesotherapy is to achieve the therapeutic benefit with lower drug doses when other options have failed, cannot be used, or are unavailable [6]. Many localized pain syndromes benefit from mesotherapy; in fact, mesotherapy is used to treat localized pain, resulting in improved quality of life [7]. Mesotherapy on pain is a safe method and has no adverse effects [4].

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The mesotherapy technique involves the inoculation of the drug using a 4 mm (27G) or 13 mm (30–32G) needle. The angle of the needle depends on the area being treated. The technique requires medical and pharmacological knowledge and must be performed in accordance with disinfection protocols (appropriate disinfectants are necessary) using sterile single-use devices [3]. It should be noted that despite the widespread use of mesotherapy, certain uncertainties still exist, and further preclinical and clinical research is needed to define its role in clinical practice [6]. Although it has a wide range of applications, there is still no standardized protocol. For these reasons, every study conducted in the field of mesotherapy is highly valuable [8].

METHODS

The study was designed as a prospective randomized, double-blind study and was conducted at the Clinical Hospital Center Kosovska Mitrovica, in the Department of Physical Medicine and Rehabilitation, over a period of eight weeks. The randomization code was generated used a computer-generated random number sequence, where participants was assigned in two groups in 1:1 ratio. The randomization list was prepared by an independent person not involved in participant selection. A total of 59 patients were included in the study – 16 male and 43 female patients. Although a formal statistical power analysis was not conducted prior to the start of research, the number of participants was sufficient to perform the planned statistical analysis and to monitor changes in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and visual analogue scale (VAS) at defined time points. The experimental group consisted of 31 patients, while the control group included 28 patients. The average age of patients in the experimental group was 58.7 ± 14.5 years, and in the control group 60.3 ± 15 years. The study was conducted after obtaining written informed consent from all participants. All patients were diagnosed with knee osteoarthritis prior to the study. During medical history taking, attention was paid to possible allergic reactions to the medication and the use of anticoagulant therapy, which are absolute contraindications for performing this procedure. Pain was assessed using VAS – before the start of the study, after the second dose, and one month after the fourth dose. Functional status was assessed using the WOMAC scale – at the beginning of the study, after the second dose, and one month after the fourth dose. VAS is a tool used to measure pain intensity. The scale is typically a 10-centimeter (or 100-millimeter) line marked from 0 to 10 or 0 to 100. The far-left point represents no pain (0), while the far-right point represents unbearable pain (10 or 100). Patients mark the point on the line that corresponds to their current pain intensity. The WOMAC is a questionnaire used to measure osteoarthritis symptoms in patients with knee or hip osteoarthritis. The scale covers three domains: pain, stiffness, and functional limitations. Results are measured on a Likert scale from 0 to 4, where zero indicates "none" and four indicates "extreme" symptoms. Higher scores indicate greater levels of pain, stiffness, and functional impairment.

A detailed medical history was taken from all patients, followed by a clinical examination and knee radiography. Participants were randomly assigned into two groups. The first was the experimental group, in which patients were treated with an injection containing a mixture of 1 mL of 1% lidocaine without adrenaline (Lidocainechlorid 1%, 35 mg / 3.5 mL, Galenika, Belgrade, Serbia) and 1 mL of Zodol (Zodol, 30 mg/mL, Hemofarm, Vršac, Serbia). The control group was treated with 1 mL of a Lidocaine solution without adrenaline. The mesotherapy protocol involved the use of sterile, single-use 2.5 mL syringes with a 30G × 4 mm needle, inserted at a 90° angle against the skin. Each patient received injections once a week, for a total of four doses, with the fourth dose administered one month after the third. Patients were treated using the "point-by-point" technique, targeting painful areas around the knee.

Before enrolment in the study and initiation of mesotherapy for pain management, participants were fully informed about the study protocol and declared that NSAID therapy has been discontinued at least 10 days prior to treatment and that no medications from this group were used through the duration of the study.

Descriptive methods and statistical hypothesis testing methods were used for the analysis of primary data. Among the descriptive statistical methods, measures of central tendency (arithmetic mean and median), measures of variability (standard deviation and range), and relative numbers were applied. For hypothesis testing, the Mann–Whitney test and the Friedman test were used. Statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp., Armonk, NY, USA). Statistical hypotheses were tested at a significance level of 0.05.

Ethics: The approval for conducting this study was obtained from the Ethics Committee of the Health Center in Kosovska Mitrovica.

RESULTS

A total of 59 patients were included in the study – 16 male and 43 female patients. The experimental group consisted of 31 patients, while the control group included 28 patients. The average age of patients in the experimental group was 58.7 ± 14.5 years, and in the control group 60.3 ± 15 years.

There is a statistically significant difference in pain intensity according to the WOMAC scale after the second dose of mesotherapy and one month after the fourth dose. WOMAC scores were significantly higher in patients in the control group (Table 1).

Similarly, there is a statistically significant difference in pain intensity according to the VAS scale after the second dose of mesotherapy and one month after the fourth dose. The frequency of patients experiencing moderate and severe pain was significantly higher in the control group, whereas the frequency of patients with no pain or mild

Table 1. Pain according to Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and visual analogue scale (VAS) in experimental and control group

Parameters	Experimental group n (31)	Control group n (28)	p
WOMAC scale at the start of the study, median (range)	68 (28–90)	67 (34–96)	0.843
WOMAC scale after the second dose of mesotherapy, median (range)	33 (0–64)	55 (20–96)	< 0.001
WOMAC scale one month after the fourth dose of mesotherapy, median (range)	16 (0–64)	66 (26–96)	< 0.001
VAS scaleat the start of the study, n (%)			0.602
No pain	/	/	
Mild pain	1 (3.2)	0 (0)	
Moderate pain	14 (45.2)	16 (57.1)	
Severe pain	16 (51.6)	12 (42.9)	
VAS scale after the second dose of mesotherapy, n (%)			< 0.001
No pain	5 (16.1)	0 (0)	
Mild pain	17 (54.8)	6 (21.4)	
Moderate pain	9 (29)	16 (57.1)	
Severe pain	0 (0)	6 (21.4)	
VAS scale one month after the fourth dose of mesotherapy, n (%)			< 0.001
No pain	20 (64.5)	0 (0)	
Mild pain	9 (29)	2 (7.1)	
Moderate pain	2 (6.5)	13 (46.4)	
Severe pain	0 (0)	13 (46.4)	

Table 2. Western Ontario and McMaster Universities Osteoarthritis Index scale in experimental group over time

Experimental group	n (31)	p
Start of the study, median (range)	68 (28–90)	< 0.001
After the second dose, median (range)	33 (0–64)	
After the fourth dose, median (range)	16 (0–64)	

Table 3. Western Ontario and McMaster Universities Osteoarthritis Index scale in control group over time

Control group	n (28)	p
Start of the study, median (range)	67 (34–96)	< 0.001
After the second dose, median (range)	55 (20–96)	
After the fourth dose, median (range)	66 (26–96)	

pain was significantly higher in the experimental group (Table 1).

There is a statistically significant difference in WOMAC scale values over time in patients in the experimental group. WOMAC scores decreased significantly over time (Table 2).

A statistically significant difference in WOMAC scale value over time was observe in patients in the control group. WOMAC scores decreased after the second dose, but increased again after the fourth dose (Table 3).

Also, a statistically significant difference was observed in the frequency of patients in the experimental group with varying pain intensity according to the VAS scale over time. The frequency of patients without pain increases over time. The frequency of patients with mild pain rises initially and then decreases, while the frequency of patients with moderate and severe pain decreases over time (Table 4).

Table 4. Visual analogue scale in experimental group over time

Experimental group	n (31)	p
Start of the study, n (%)		< 0.001
No pain	/	
Mild pain	1 (3.2)	
Moderate pain	14 (45.2)	
Severe pain	16 (51.6)	
After the second dose, n (%)		
No pain	5 (16.1)	
Mild pain	17 (54.8)	
Moderate pain	9 (29)	
Severe pain	/	
After the fourth dose, n (%)		
No pain	20 (64.5)	
Mild pain	9 (29)	
Moderate pain	2 (6.5)	
Severe pain	/	

Table 5. Visual analogue scale over time

Control group	n (28)	p
Start of the study, n (%)		< 0.001
No pain	/	
Mild pain	/	
Moderate pain	16 (57.1)	
Severe pain	12 (42.9)	
After the second dose, n (%)		
No pain	/	
Mild pain	6 (21.4)	
Moderate pain	16 (57.1)	
Severe pain	6 (21.4)	
After the fourth dose, n (%)		
No pain	/	
Mild pain	2 (7.1)	
Moderate pain	13 (46.4)	
Severe pain	13 (46.4)	

There is a statistically significant difference in the frequency of patients in the control group with varying pain intensity according to the VAS scale over time. The frequency of patients with mild pain rises initially and then decreases, while the frequency of patients with moderate pain stagnates then decreases, and the frequency of patients with severe pain decreases at first, but then increases over time (Table 5).

DISCUSSION

Mesotherapy is recognized as an effective alternative therapy for localized pain management [9], with studies demonstrating a reduction in neck and lower back pain by at least 50% compared to baseline levels [10]. In the context of acute conditions, Akbas et al. [11] observed that mesotherapy provides a statistically significantly greater reduction in pain intensity at 15 minutes, 30 minutes, and 24 hours post-treatment compared to intravenous dexketoprofen administration. These findings are further supported by Costantino et al. [12], who suggested that mesotherapy, using a combination of lidocaine, ketoprofen, and methylprednisolone, represents a valid alternative to conventional systemic administration of NSAIDs and corticosteroids for acute low back pain. Furthermore, it has been shown that mesotherapy can achieve therapeutic effects equivalent to systemic drug administration [12], but with a significantly more favorable safety profile. Specifically, Chen et al. [13]

noted that mesotherapy resulted in fewer adverse effects, particularly regarding hemorrhage and WOMAC scores, when compared to traditional NSAID treatments.

The efficacy of this method extends to chronic syndromes as well. In patients with chronic lumbar syndrome and chronic thoracic spine pain, studies by Pires et al. [14] and Koszela et al. [15] both demonstrated that mesotherapy with type I collagen yields statistically significant improvements compared to lidocaine alone. Similarly, Ranieri et al. [16] reported reduced pain intensity and functional improvement in patients with bilateral cervicobrachial syndrome following a six-week treatment protocol.

In our study, the application of a Zodol (Hemofarm) and lidocaine mixture produced superior results in symptom reduction for knee osteoarthritis compared to lidocaine monotherapy. This aligns with the research by Tseveendorj et al. [17], who found that a combination of meloxicam and lidocaine significantly outperformed physiological saline, with positive effects appearing within four weeks and lasting up to three months. These results are consistent with the conclusions of Farpour et al. [18], who identified piroxicam mesotherapy as an effective and safe procedure for patients with mild to moderate knee osteoarthritis. Collectively, these data underscore the clinical value of mesotherapy as a targeted, potent, and safe intervention for both spinal disorders and osteoarthritis-related pain.

CONCLUSION

The therapeutic effect of mesotherapy on pain in patients with knee osteoarthritis treated with a mixture of lidocaine and Zodol (Hemofarm) is more effective and longer-lasting compared to patients treated with lidocaine alone.

Conflict of interest: None declared.

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Терапијски ефекат мезотерапије бола код болесника са остеоартритисом колена

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САЖЕТАК

Увод/Циљ Остеоартритис колена представља дегенеративну, прогресивну болест зглоба колена која на крају може довести до инвалидитета. Клиничка мезотерапија представља интрадермалну терапију која се користи за убризгавање разблажених фармаколошких супстанци у површински слој коже у више тачака. Циљ истраживања био је да се утврди терапијски ефекат мезотерапије бола код болесника са остеоартритисом колена који су третирани мешавином Зодола (30 *mg*/*mL*, Хемофарм, Вршац, Србија) и лидокаина (лидокаин-хлорид 1%, 35 *mg* / 3,5 *mL*, Галеника, Београд, Србија), у односу на болеснике који су третирани само лидокаином.

Методе Студија је спроведена као проспективна. Учесници су насумично распоређени у две групе. Прву чини експериментална група у којој су болесници третирани инјекцијом која је садржала смешу лидокаина без адреналина и Зодола (Хемофарм). Контролна група била је третирана раствором лидокаина, без адреналина.

Резултати Утврђена је статистички значајна разлика у интензитету бола после друге дозе мезотерапије и месец дана након четврте дозе мезотерапије. Вредности Индекса остеоартритиса универзитета Западни Онтарио и Мекмастер биле су значајно више код болесника у контролној групи. Удео болесника који осећају умерен и јак бол био је значајно већи у контролној групи, док је удео болесника без бола и са благим болом значајно већи у експерименталној групи.

Закључак Примена мезотерапије бола код болесника са остеоартритисом колена у експерименталној групи показала се ефикаснијом и дуготрајнијом у поређењу са применом само лидокаина.

Кључне речи: мезотерапија бола; остеоартритис колена; интрадермална терапија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Association between endometrial heterogeneity and endometrial cancer risk stratification in postmenopausal women

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SUMMARY

Introduction/Objective The normal postmenopausal endometrium typically appears thin, homogeneous, and echogenic on transvaginal ultrasonography. In contrast, endometrial carcinoma is often associated with increased thickness, heterogeneous echogenicity, irregular endometrial–myometrial interface, and enhanced Doppler vascularization. The objective of this study was to evaluate the association between heterogeneous endometrial echogenicity and the risk of endometrial malignancy in postmenopausal women.

Methods This prospective clinical study included 120 postmenopausal women treated at the University Clinic for Gynecology and Obstetrics in Skopje. Participants were divided into a control group (n = 40) and an examined group (n = 80). The examined group was further stratified according to uterine bleeding status and endometrial thickness (5–8 mm, > 8–11 mm, and > 11 mm). Endometrial echogenicity was assessed by transvaginal ultrasonography and classified as homogeneous or heterogeneous. Binary logistic regression analysis was performed to determine independent predictors of malignancy.

Results Heterogeneous endometrial echogenicity was significantly more frequent in the examined group compared with controls ($p < 0.001$). It was identified as an independent predictor of endometrial malignancy (OR = 4.938; 95% CI: 1.24–19.62; $p = 0.023$). No statistically significant association was observed between echogenicity and endometrial thickness subgroups ($p = 0.49$) or uterine bleeding status ($p = 0.82$).

Conclusion Heterogeneous endometrial echogenicity represents a significant independent sonographic predictor of endometrial malignancy in postmenopausal women and should be incorporated into routine ultrasound risk assessment.

Keywords: postmenopause; endometrial echogenicity; endometrial cancer; transvaginal ultrasonography; malignancy risk

INTRODUCTION

Endometrial carcinoma is the most common gynecologic malignancy in developed countries, with a continuously increasing incidence worldwide [1, 2]. According to recent global cancer statistics, endometrial cancer represents a significant public health burden, particularly in postmenopausal women [1]. The rising prevalence has been associated with metabolic syndrome, obesity, and increased life expectancy [3].

Current international guidelines, including ESGO/ESTRO/ESP and ESMO recommendations, provide standardized approaches for diagnosis, staging, and management of endometrial carcinoma [4, 5]. The 2023 FIGO staging revision introduced important refinements in disease stratification, further emphasizing the prognostic heterogeneity of this malignancy [6, 7].

Transvaginal ultrasonography remains the first-line diagnostic modality in the evaluation of postmenopausal bleeding. Traditionally, endometrial thickness has been considered the primary screening parameter [4]. However, recent evidence suggests that reliance solely on thickness measurement may not provide optimal diagnostic accuracy [8, 9].

Contemporary ultrasound practice incorporates structured reporting systems such as the ISUOG consensus recommendations and IETA terminology, which emphasize qualitative morphological assessment including echogenicity, endometrial–myometrial interface, and vascular patterns [10, 11]. Advanced imaging techniques, including microvascular flow imaging and ultrasound-based predictive scoring systems, have demonstrated improved diagnostic performance in identifying high-risk endometrial lesions [12–15].

Moreover, modern molecular classification has revealed substantial biological heterogeneity in endometrial carcinoma, including mismatch repair deficiency and microsatellite instability [16, 17]. Emerging translational approaches such as cfDNA fragmentomics and radiomics further support the integration of imaging and molecular biomarkers in risk stratification [18, 19].

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The objective of this study was to evaluate the association between heterogeneous endometrial echogenicity and the risk of endometrial malignancy in postmenopausal women.

METHODS

This prospective clinical study included 120 postmenopausal women treated at the University Clinic for Gynecology and Obstetrics in Skopje. Participants were divided into two groups: a control group (n = 40) and an examined group (n = 80). The control group consisted of postmenopausal patients hospitalized and surgically treated for benign urogenital pathology.

The examined group was further stratified according to the presence or absence of uterine bleeding. Based on ultrasound-verified endometrial thickness, patients were categorized into three subgroups: 5–8 mm, > 8–11 mm, and > 11 mm.

Transvaginal ultrasonography was performed in all participants. Endometrial echogenicity was classified as homogeneous or heterogeneous. Histopathological verification was obtained following fractional exploratory curettage.

Exclusion criteria included: women of reproductive age, inability to undergo fractional curettage, prior or current malignant disease, ovarian tumors, breast cancer treated with tamoxifen, and previous pelvic surgery for other gynecological conditions.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0. Categorical variables were analyzed using the Pearson chi-square test or Fisher's exact test, as appropriate. Continuous variables were assessed for normality using the Shapiro–Wilk test. Student's t-test, Mann–Whitney U test, and Kruskal–Wallis ANOVA were applied as indicated.

Binary logistic regression analysis was performed to determine independent predictors of endometrial malignancy. Statistical significance was defined as $p < 0.05$.

Ethics: Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje (Approval No. 03-1997/6). All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

RESULTS

According to endometrial echogenicity, the study population was divided into two categories: homogeneous and heterogeneous (Table 1). In the examined group, 43 patients (53.7%) had homogeneous endometrial echogenicity, whereas 37 patients (46.3%) had heterogeneous echogenicity. In the control group, all 40 patients (100%) had homogeneous endometrial echogenicity.

Table 1. Descriptive analysis of the sample according to groups and endometrial echogenicity; data are presented as counts (n) and percentages (%)

Endometrial echogenicity	Examined group n (%)	Control group n (%)	Total n (%)
Homogeneous	43 (53.75)	40 (100)	83 (69.17)
Heterogeneous	37 (46.25)	0 (0)	37 (30.83)
Total	80 (66.67)	40 (33.33)	120 (100)

Table 2. Analysis of the examined group according to endometrial thickness and echogenicity; data are presented as counts (n) and percentages (%)

Endometrial echogenicity	5–8 mm n (%)	> 8–11 mm n (%)	> 11 mm n (%)	Total n (%)
Homogeneous	21 (58.33)	10 (58.82)	12 (44.44)	43 (53.75)
Heterogeneous	15 (41.67)	7 (41.18)	15 (55.56)	37 (46.25)
Total	36 (45)	17 (21.25)	27 (33.75)	80 (100)

Table 3. Analysis of the examined group according to uterine bleeding and endometrial echogenicity; data are presented as counts (n) and percentages (%)

Endometrial echogenicity	No bleeding n (%)	Uterine bleeding n (%)	Total n (%)
Homogeneous	21 (52.5)	22 (55)	43 (53.75)
Heterogeneous	19 (47.5)	18 (45)	37 (46.25)
Total	40 (50)	40 (50)	80 (100)

A statistically significant difference was observed between the examined and control groups regarding endometrial echogenicity (Fisher's exact two-tailed test, $p < 0.001$).

Analysis of the examined group according to endometrial thickness and endometrial echogenicity

In the subgroup with endometrial thickness of 5–8 mm, 21 patients (58.3%) had homogeneous echogenicity and 15 (41.7%) had heterogeneous echogenicity.

In the subgroup with endometrial thickness > 8–11 mm, 10 patients (58.8%) had homogeneous echogenicity and 7 (41.2%) had heterogeneous echogenicity (Table 2).

In the subgroup with endometrial thickness > 11 mm, 12 patients (44.4%) had homogeneous echogenicity, while 15 (55.6%) had heterogeneous echogenicity (Table 2).

No statistically significant association was observed between endometrial thickness categories and echogenicity (Pearson $\chi^2 = 1.42$; df = 2; $p = 0.491$).

Analysis of the examined group according to uterine bleeding and endometrial echogenicity

Among patients without uterine bleeding, 21 (52.5%) had homogeneous echogenicity and 19 (47.5%) had heterogeneous echogenicity. Among patients with uterine bleeding, 22 (55%) had homogeneous echogenicity and 18 (45%) had heterogeneous echogenicity.

There was no statistically significant difference between bleeding status and echogenicity (Pearson $\chi^2 = 0.05$; df = 1; $p = 0.823$) (Table 3).

Table 4. Analysis of the non-bleeding group according to endometrial thickness and echogenicity; data are presented as counts (n) and percentages (%)

Endometrial thickness	Homogeneous n (%)	Heterogeneous n (%)	Total n (%)
5–8 mm	11 (52.38)	8 (42.11)	19 (47.5)
> 8–11 mm	6 (28.57)	5 (26.32)	11 (27.5)
> 11 mm	4 (19.05)	6 (31.58)	10 (25)
Total	21 (52.50)	19 (47.50)	40 (100)

Table 5. Analysis of the bleeding group according to endometrial thickness and echogenicity; data are presented as counts (n) and percentages (%)

Endometrial thickness	Homogeneous n (%)	Heterogeneous n (%)	Total n (%)
5–8 mm	10 (45.45)	7 (38.89)	17 (42.5)
> 8–11 mm	4 (18.18)	2 (11.11)	6 (15)
> 11 mm	8 (36.37)	9 (50)	17 (42.5)
Total	22 (55)	18 (45)	40 (100)

Combined analysis: bleeding status, thickness, and echogenicity

An analysis of endometrial thickness and endometrial echogenicity was performed in the group without uterine bleeding (Table 4). The analysis indicated that homogeneous endometrial echogenicity was most common, occurring in 11 (52.4%) patients with an endometrial thickness of 5–8 mm, followed by six (28.6%) patients with endometrial thickness > 8–11 mm. Regarding heterogeneous endometrial echogenicity, most patients, 8 (42.1%), had an endometrial thickness of 5–8 mm, followed by six (31.6%) patients with endometrial thickness > 11 mm. In patients without uterine bleeding, for $p > 0.05$, there is no statistically significant difference between the groups with homogeneous or heterogeneous echogenicity of the endometrium relative to endometrial thickness (Pearson $\chi^2 = 0.867$; df = 2; $p = 0.6483$).

In patients with uterine bleeding, an analysis was performed according to endometrial thickness and endometrial echogenicity (Table 5). Homogeneous endometrial echogenicity was most common, occurring in 10 (45.5%) patients with an endometrial thickness of 5–8 mm, followed by eight (36.4%) patients with endometrial thickness > 11 mm. Among patients with heterogeneous endometrial echogenicity, most patients, nine (50%), had endometrial thickness > 11 mm, followed by seven (38.9%) patients with endometrial thickness of 5–8 mm.

In the uterine bleeding group, for $p > 0.05$, there is no statistically significant difference between subgroups with homogeneous or heterogeneous endometrial echogenicity relative to endometrial thickness (Pearson $\chi^2 = 0.863$; df = 2, $p = 0.6493$) (Table 5).

Endometrial echogenicity is a significant predictor of endometrial malignancy ($p < 0.05$). Women with heterogeneous endometrial echogenicity have a 4.938-fold higher likelihood ($p = 0.023$, 95% CI = 1.243–19.619) of endometrial cancer compared to women with homogeneous endometrial echogenicity (Table 6).

Table 6. Binary logistic regression analysis of the predictive role of certain parameters in relation to endometrial malignancy – examined group

Variable	B	S.E.	Wald	df	Sig.	Exp (B)	95% CI for Exp (B)
Echogenicity – homogeneous *vs.* heterogeneous	1.597	0.704	5.149	1	0.023*	4.938	1.243–19.62

B – regression coefficient; S.E. – standard error; df – degrees of freedom; Sig. – significance; Exp (B) – odds ratio;
*statistically significant, $p < 0.05$

Logistic regression analysis

Binary logistic regression analysis demonstrated that heterogeneous endometrial echogenicity was a significant independent predictor of endometrial malignancy.

Women with heterogeneous echogenicity had 4.938-fold higher odds of endometrial cancer compared with women with homogeneous echogenicity (OR 4.938; 95% CI 1.24–19.62; $p = 0.023$) (Table 6).

DISCUSSION

Our results showed that heterogeneous endometrial echogenicity is a significant independent predictor of endometrial malignancy, with nearly a fivefold increase in odds (OR 4.94; 95% CI 1.24–19.62; $p = 0.023$). This finding emphasizes the importance of qualitative ultrasonographic assessment, beyond conventional measurement of endometrial thickness.

These findings are in agreement with the study by Yan et al. [20], who reported that endometrial echogenic heterogeneity is strongly associated with malignancy and significantly improves diagnostic accuracy when incorporated into predictive nomograms. Their model demonstrated that combining echogenicity with other ultrasound parameters enhances risk stratification compared to single-parameter approaches. Notably, while their study integrated multiple variables, our results confirm that echogenicity alone already has substantial predictive value.

Similarly, Ai et al. [21] developed a nomogram for predicting endometrial cancer in postmenopausal women and demonstrated that incorporating ultrasonographic features significantly improves diagnostic performance. In comparison to their multifactorial model, our study provides additional evidence that even a single qualitative parameter – echogenicity – can independently discriminate between benign and malignant conditions.

In contrast to these findings, our study did not demonstrate a statistically significant association between endometrial thickness and echogenicity ($p = 0.491$), nor between thickness and malignancy risk within echogenicity subgroups. This observation is consistent with the meta-analysis by Chee et al. [8], which concluded that endometrial thickness alone has limited specificity, particularly in symptomatic postmenopausal women. Their analysis highlighted that fixed cut-off values may not adequately capture the complexity of endometrial pathology, leading to diagnostic uncertainty.

Our findings also showed no statistically significant association between uterine bleeding status and endometrial echogenicity ($p = 0.823$). This result is in line with the guideline by Wolfman et al. [9], which emphasizes that although postmenopausal bleeding is a key clinical symptom, it should not be interpreted in isolation. Imaging findings, particularly qualitative ultrasound features, provide important complementary information and may improve diagnostic accuracy.

Importantly, the significant difference in echogenicity between the examined and control groups ($p < 0.001$) in our study reinforces the discriminatory value of this parameter. This is comparable to findings from multicenter analyses such as the study by Colombi et al. [22], which demonstrated correlations between ultrasonographic features and histopathological as well as molecular characteristics of endometrial cancer. While their work focused on broader imaging-pathology correlations, our study narrows this relationship specifically to echogenicity as a practical and easily applicable marker.

Our findings regarding the predictive value of heterogeneous endometrial echogenicity are supported by earlier sonographic studies reporting that morphologic characteristics enhance malignancy discrimination. In a seminal work by Opolskiene et al. [23], heterogeneous endometrial echogenicity demonstrated strong diagnostic performance with an AUC of 0.83 and, when combined with other grey-scale and Doppler parameters, yielded an even higher discriminative ability in predicting endometrial malignancy. This aligns with our observation that qualitative ultrasonographic features – particularly echogenicity – provide valuable diagnostic information beyond endometrial thickness alone.

A key strength of our study is the detailed subgroup analysis according to endometrial thickness and bleeding status. Unlike some previous studies, we demonstrated that echogenicity retains its predictive role even when stratified by these variables, further supporting its independence as a diagnostic factor. However, the lack of statistical significance in subgroup analyses suggests that echogenicity may operate as a global marker rather than being strongly influenced by individual clinical parameters.

From a clinical perspective, our findings support a shift from a purely quantitative to a more integrative ultrasonographic approach. While endometrial thickness remains a useful screening tool, it should be complemented by qualitative assessment, particularly echogenicity patterns. This aligns with contemporary trends in gynecologic imaging, where multiparametric evaluation is increasingly recognized as essential for accurate diagnosis.

Nevertheless, several limitations must be acknowledged. The relatively small sample size and single-center design may limit external validity. In addition, the absence of molecular classification restricts comparison with modern endometrial cancer subtypes, as highlighted in recent literature. Future studies should aim to integrate imaging findings with histopathological and molecular data in large, multicenter cohorts.

CONCLUSION

The observed odds ratio of 4.938 in our study further supports the clinical relevance of echogenicity as a risk stratification parameter. Heterogeneous endometrial echogenicity represents a clinically relevant independent sonographic predictor of malignancy in postmenopausal women. Incorporating qualitative ultrasound assessment into routine diagnostic algorithms may enhance early detection and improve individualized risk stratification.

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Повезаност хетерогености ендометријума и ризика од карцинома ендометријума код жена у постменопаузи

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САЖЕТАК

Увод/Циљ Нормалан ендометријум у постменопаузи ултрасонографски се приказује као танак, хомоген и ехоген. Карцином ендометријума често је повезан са повећаном дебљином, хетерогеном ехогеношћу и неправилном границом између ендометријума и миометријума. Циљ рада био је да се испита повезаност хетерогене ехогености ендометријума и ризика од малигнитета код жена у постменопаузи.

Методе Проспективна клиничка студија обухватила је 120 жена у постменопаузи лечених на Универзитетској клиници за гинекологију и акушерство у Скопљу. Испитанице су подељене на контролну групу (n = 40) и испитивану групу (n = 80). Испитивана група је даље стратификована према присуству крварења и дебљини ендометријума (5–8 *mm*, > 8–11 *mm* и > 11 *mm*). Ехогеност ендометријума процењена је трансвагиналном ултрасонографијом и класификована као хомогена или хетерогена. Примењена је бинарна логистичка регресија.

Резултати Хетерогена ехогеност ендометријума била је значајно чешћа у испитиваној групи (p < 0,001). Представљала је независни предиктор малигнитета (*OR* = 4,938; 95% *CI*: 1,24–19,62; p = 0,023). Није утврђена статистички значајна повезаност између ехогености и дебљине ендометријума (p = 0,49) нити присуства крварења (p = 0,82).

Закључак Хетерогена ехогеност ендометријума представља значајан независни ултрасонографски предиктор малигнитета код жена у постменопаузи и треба да буде укључена у рутинску процену ризика.

Кључне речи: постменопауза; ехогеност ендометријума; карцином ендометријума; трансвагинална ултрасонографија; ризик од малигнитета

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Validation of the Serbian version of the Breastfeeding self-efficacy scale-short form

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SUMMARY

Introduction/Objective Breastfeeding prevalence in Serbia remains low compared with global estimates. Breastfeeding self-efficacy scale-short form is widely recognized as a key, modifiable determinant of breastfeeding outcomes, with growing evidence that improving it is associated with higher breastfeeding rates. This study aimed to translate and culturally adapt the scale for use in Serbian language, establish its psychometric adequacy within a population of breastfeeding mothers throughout the first postpartum year, and examine differences in scores by selected sociodemographic and obstetric characteristics.

Methods A total of 70 breastfeeding mothers who delivered at the Clinic for Gynecology and Obstetrics, University Clinical Center of Vojvodina participated in this cross-sectional study. The validated Croatian version served as the basis for the adaptation process, which followed standardized cross-cultural procedures. Internal consistency was evaluated using item-total statistics and Cronbach's α. Group differences were compared using the Mann–Whitney U test.

Results The participants' mean age was 32.19 years (SD = 5.17). The instrument showed strong internal consistency (Cronbach's α = 0.81; 95% CI: 0.73–0.87). Corrected item–total correlation coefficients ranged from 0.13 to 0.73. The mean score was 54.11 ± 10.81, indicating high breastfeeding self-efficacy. Lower scores were observed among mothers with cesarean delivery, shorter intended breastfeeding duration, early cessation, lack of early initiation, and insufficient breastfeeding support.

Conclusion The Serbian version of the scale exhibited good internal consistency and preliminary validity as an instrument for assessing breastfeeding self-efficacy.

Keywords: breast feeding; self-efficacy; surveys and questionnaires; cross-sectional studies; mothers; postpartum period

INTRODUCTION

In line with World Health Organization guidance, infants are recommended to receive only breast milk for the first six months, after which complementary foods are introduced while breastfeeding continues for up to two years [1]. A substantial body of clinical research demonstrates that breastfeeding reduces the risk of both short-term and long-term adverse health outcomes in infants. Children who were breastfed for longer durations display lower incidence of infectious illnesses and reduced mortality [2]. Breastfeeding confers numerous health benefits, including a decreased risk of type 1 diabetes, asthma, respiratory illnesses, gastrointestinal infections and celiac disease, and sudden infant death syndrome in infants [3]. The composition of human breast milk dynamically adapts to the infant's changing nutritional and developmental needs during the early stages of life. Beyond providing essential nutrients, it also contributes to non-nutritive functions through a wide range of bioactive components, including hormones, antioxidants, secretory immunoglobulin A (IgA), lactoferrin, and numerous other protective substances. Furthermore, the protein profile of breast milk is specifically suited to the physiological immaturity of neonatal ureagenesis and renal excretory function, while its low antigenicity and high biological compatibility support the establishment and maintenance of immune tolerance in the infant [4]. Moreover, ensuring an optimal balance of essential nutrients and micronutrients in human milk is fundamental for subsequent skeletal growth and development during infancy [5]. In mothers, breastfeeding has been associated with a reduced risk of cardiometabolic disease, ovarian cancer, and type 2 diabetes [6]. Human milk is widely regarded as the optimal form of infant

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feeding, meeting both the physical and psychological needs of newborns during the early months of life [7, 8]. Despite well-established benefits and existing recommendations, breastfeeding initiation and continuation rates remain suboptimal among mothers [9]. Multiple factors are associated with early cessation of breastfeeding, such as maternal age, educational attainment, and psychosocial status. Additional influences on successful and sustained exclusive breastfeeding include maternal knowledge and attitudes, the level of support received, socioeconomic conditions, and breastfeeding self-efficacy [10]. UNICEF Belgrade data indicate that early initiation of breastfeeding occurs in only 8% of newborns in Serbia, with 6% in urban areas and 10% in non-urban areas. Exclusive breastfeeding is reported in 24% of infants aged 0–5 months nationwide [11]. Maternal breastfeeding self-efficacy is recognized as a key influencing factor of initiation, duration, and exclusiveness of breastfeeding [6]. Breastfeeding self-efficacy scale (BSES), developed by Dennis and Faux (1999), was created to measure maternal sense of competence in breastfeeding. Dennis (2003) later revised the instrument, reducing it from 33 to 14 items and renaming it the BSES-short form (BSES-SF) [12]. Self-efficacy refers to the women's perceived capability to breastfeed successfully [13, 14]. The BSES-SF allows healthcare professionals to identify women at higher risk of not initiating or sustaining breastfeeding and to pinpoint specific areas of reduced confidence, thereby facilitating the development of individualized breastfeeding promotion strategies [11]. Several factors contribute to breastfeeding self-efficacy, such as prior personal experience, exposure to breastfeeding practices, maternal well-being, and verbal support. Efforts to improve self-efficacy are important because they are associated with more favorable breastfeeding outcomes and better infant health [15]. The BSES-SF has demonstrated strong psychometric properties and has been translated into several languages, but a Serbian version is still lacking. For this reason, validating a Serbian version and using it within efforts to improve breastfeeding rates is an important step. Grounded in Bandura's social cognitive theory, the BSES-SF measures maternal confidence in breastfeeding ability. This psychometric assessment supports the reliability of the Serbian version of the BSES-SF and quantifies maternal breastfeeding self-efficacy throughout the postpartum. The absence of a clear link between mothers demographic characteristics and overall scale score suggests that the BSES-SF captures a distinct construct, pointing to a modifiable factor that may help identify mothers at higher risk [16]. Beyond measurement, the BSES-SF can indicate which mothers may need more support during breastfeeding. Lower scores, in this context, are usually associated with a greater need for intervention. Accordingly, BSES-SF may be used as a tool to reveal specific support needs among mothers and suggest lactation support programs. Based on BSES-SF results, specific strategies to enhance maternal confidence can be implemented.

METHODS

This cross-sectional study included breastfeeding mothers who had delivered at a tertiary care hospital in Novi Sad in 2025. Women could take part if they were 18 years or older, had delivered after 37 gestational weeks, spoke Serbian, were able to give informed consent, and had reached at least six months postpartum. We included only mothers whose postpartum course did not involve intensive care, since early contact between mother and infant is considered important for establishing breastfeeding. Those with medical conditions or ongoing therapies that made breastfeeding unfeasible were not included. Participants were informed of the study objectives before enrollment, and written informed consent was then obtained. Participants then completed the study questionnaire either in person or online.

Of the 100 women invited to take part, 22 declined. Among those who responded, eight were later excluded for incomplete questionnaires, leaving a final sample of 70 participants. Sample size was estimated using general guidance for psychometric validation, which typically recommends including several participants per item. For a 14-item questionnaire, this resulted in an expected range of 70 to 140 participants. A larger sample was preferred in order to strengthen the analysis. The BSES-SF is a unidimensional instrument comprising 14 items. All items are positively worded and introduced by the phrase "I can always," and are rated on a five-point Likert-type scale covering the range from 1 ("not at all confident") to 5 ("always confident"). Total scores are based on the sum of item responses, ranging from 14 to 70, with higher scores indicating greater breastfeeding self-efficacy. In addition to the BSES-SF, data on demographic characteristics (maternal age, education level, employment, and marital status) were collected. The questionnaire also involved information about nursing practices and obstetric and neonatal characteristics, such as gestational age, parity, mode of delivery, prior breastfeeding experience, and timing of lactation initiation.

The Serbian version of the BSES-SF was developed through a standardized cross-cultural adaptation process based on the previously validated Croatian version. Permission for translation and use of the instrument was obtained from the author of the Croatian version. The translation into Serbian was carried out by two independent certified court interpreters, with the aim of maintaining conceptual equivalence with the original version. The draft was reviewed by healthcare professionals working in nursing and pediatric care to suggest changes to improve the wording and structure. It was then revised further with the original author to ensure it remained consistent with the original instrument and to resolve any remaining issues. The final version was established after repeated comparison with the original scale, with particular attention to both conceptual clarity and linguistic suitability for use in the Serbian setting.

A pilot study was conducted with 10 participants who satisfied the study eligibility criteria, to evaluate how

Table 1. Results of breastfeeding self-efficacy scale-short form according to demographics

Variable	Category	Mean ± SD	Test (statistic)	p
Parity	Primipara	53.13 ± 10.99	532.5	0.422[a]
	Multipara	54.85 ± 10.75		
Marital status	Married	54.28 ± 11.02	225.5	0.389[a]
	Not married	53 ± 9.80		
Education level	Primary school	58 ± 7	2.64	0.619[b]
	Secondary school	52.22 ± 11.06		
	College	47.33 ± 21.94		
	University degree	55.96 ± 9.33		
	Master/magister/PhD	55.31 ± 10.97		

SD – standard deviation;
[a]Mann–Whitney U test;
[b]Kruskal–Wallis test

Table 2. Results of breastfeeding self-efficacy scale-short form according to obstetric characteristics

Variable	Category	Mean ± SD	Test (statistic)	p
Number of pregnancies	1	53.70 ± 10.31	4.05	0.256[b]
	2	54.81 ± 11.45		
	3	56.92 ± 8.27		
	4	43 ± 13.04		
	> 4	59 ± 5.66		
Infant sex	Male	51.68 ± 12.47	496	0.172[a]
	Female	56.42 ± 8.52		
Type of delivery	Cesarean section	49.35 ± 14.02	353.5	0.040[a]
	Vaginal delivery	56.02 ± 8.68		
Breastfeeding duration	Up to six months	37.50 ± 19.60	66.5	0.040[a]
	More than six months	55.12 ± 9.40		
Breastfeeding cessation during pregnancy	< 6 months	45.88 ± 12.28	199	< 0.001[a]
	> 6 months	58.98 ± 5.89		
Breastfeeding initiated in maternity ward	Yes	57.81 ± 8.35	300.5	0.001[a]
	No	48.57 ± 11.82		
Adequate breastfeeding support	Yes	57.47 ± 8.99	407	0.018[a]
	No	51.29 ± 11.50		
Community midwife visit	Yes	53.53 ± 10.96	558	0.555[a]
	No	54.61 ± 10.81		

SD – standard deviation;
[a]Mann–Whitney U test;
[b]Kruskal–Wallis test

clear and easy to understand the translated instrument was. Participants were asked to identify any difficulties in understanding the items; all reported that the questions were clear and easy to understand. Based on the outcomes, the final Serbian version of the BSES-SF was established.

Statistical analysis was performed using JASP, Version 0.97.1 (University of Amsterdam, Amsterdam, The Netherlands). Continuous variables were presented as mean ± standard deviation (SD), while categorical variables were expressed as frequencies and percentages. The internal consistency of the BSES-SF was assessed using Cronbach's α coefficient and corresponding 95% confidence intervals (CIs), with values greater than 0.70 considered acceptable. Corrected item–total correlations were calculated for each item, and the effect of item removal on overall reliability was evaluated. Differences between two independent groups were analyzed using the Mann–Whitney U test, while comparisons across more than two groups were performed using the Kruskal–Wallis test. All statistical tests were two-tailed, and a $p < 0.05$ was considered statistically significant.

Ethics: The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University Clinical Center of Vojvodina (Decision No. 00-44/22.).

RESULTS

The mean participant age was 32.19 years (SD = 5.17). The instrument showed strong internal consistency and reliability (α = 0.81; 95% CI: 0.73–0.87; $p < 0.001$). Corrected item–total correlation coefficients varied from 0.133 to 0.726 (Table 1).

The exclusion of individual items did not result in any substantial improvement in the scale's internal consistency. Overall, participants reported high levels of breastfeeding self-efficacy, reflected by a mean BSES-SF score of 54.11 (SD = 10.81), indicating considerable confidence in their breastfeeding abilities. No significant differences in BSES-SF scores were observed across obstetric variables (Table 2). However, significantly lower self-efficacy scores were identified among mothers who underwent cesarean delivery, intended to breastfeed for less than six months, ceased breastfeeding before six months postpartum, did not initiate breastfeeding during their maternity hospital stay, or reported insufficient breastfeeding support (Table 3, Figure 1).

DISCUSSION

The study evaluated psychometric characteristics of the Serbian version of BSES-SF, a globally accepted and widely used tool. So far, no such study has been conducted in Serbia. Previous studies have shown that BSES-SF is associated with relevant sociodemographic and obstetric characteristics. The scale showed strong internal consistency, with a Cronbach's α of 0.81, which falls within the range reported in earlier validation studies (0.74–0.97) [7, 9, 12, 16–19]. Mothers confirmed high level of self-efficacy, with a mean total score of 54.11 (SD = 10.81). Similar findings have been reported in other populations, with mean scores ranging from 34.39 to 63.31. The present result is closer to the upper range of reported values, suggesting relatively high breastfeeding confidence in this sample, while lower scores in some studies may be explained by differences in population characteristics and postpartum conditions.

Table 3. Breastfeeding self-efficacy scale-short form item-total correlations and reliability analysis

Questions	Corrected item-total correlation	Cronbach's α if item deleted
I can always determine that my baby is getting enough milk	0.325	0.808
I can always successfully cope with breastfeeding like I have with other challenging tasks	0.572	0.787
I can always breastfeed my baby without using formula as a supplement	0.360	0.807
I can always ensure that my baby is properly latched on for the whole feeding	0.528	0.791
I can always manage the breastfeeding situation to my satisfaction	0.565	0.789
I can always manage to breastfeed even if my baby is crying	0.173	0.814
I can always keep wanting to breastfeed	0.525	0.797
I can always comfortably breastfeed with my family members present	0.279	0.813
I can always be satisfied with my breastfeeding experience	0.726	0.777
I can always deal with the fact that breastfeeding can be time-consuming	0.212	0.814
I can always finish feeding my baby on one breast before switching to the other breast	0.133	0.824
I can always continue to breastfeed my baby for every feeding	0.687	0.780
I can always manage to keep up with my baby's breastfeeding demands	0.676	0.781
I can always tell when my baby is finished breastfeeding	0.506	0.794

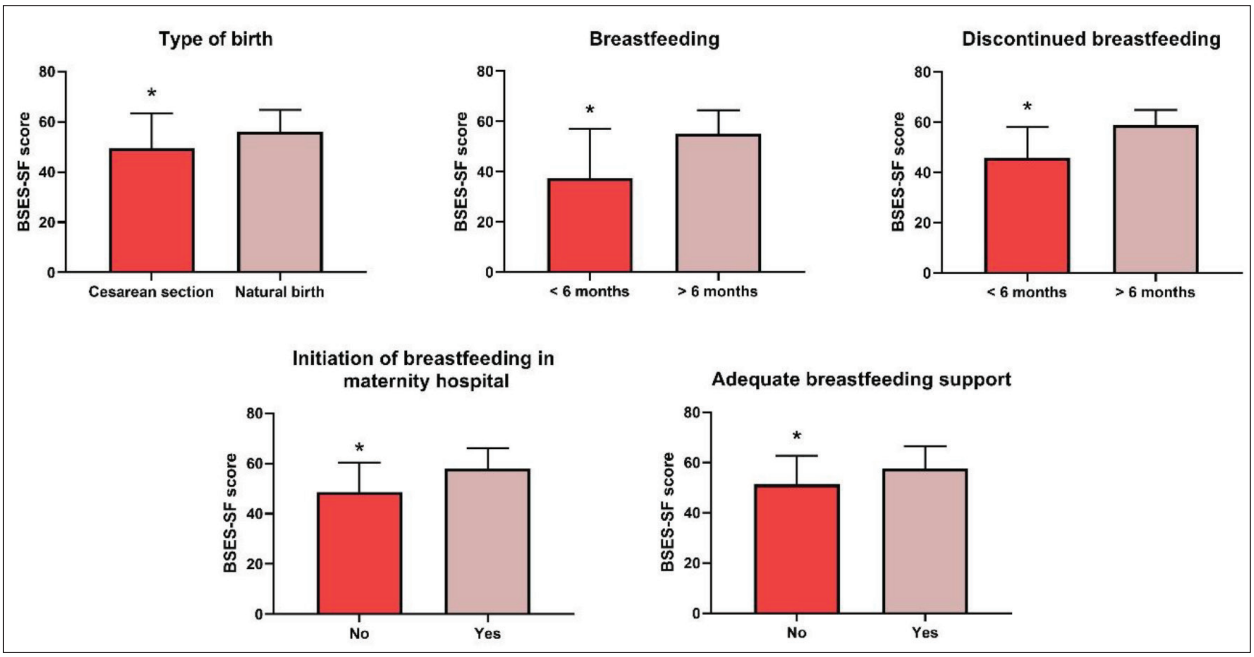

Figure 1. Breastfeeding self-efficacy scale-short form score according to obstetric characteristics

As in other studies, there were no significant associations between total score and the mother's and infant's age [20]. Respondents who completed only primary school had the highest scores. Among respondents with higher educational attainment, those with a university education scored higher than those with secondary education, which is in line with Amini et al. [20]. Similar results were found by Mazúchová et al. [12] where better scores were noted in women with lower educational attainment, in comparison with women with tertiary-level education. Rarely, in some studies there were no significant differences in relation to educational level [18, 21]. On the other hand, Gizaw et al. [22] showed better attitude for breastfeeding associated with educational level, particularly among mothers with secondary education or higher. Respondents who are married (or living with a partner) had greater success in breastfeeding compared to single mothers. The multiparous had a better score compared to the respondents with only one child, even though the questions related to the experience with the first child [7, 12]. There are studies that show even that multiparous mothers who had previously breastfed for more than six months scored significantly better than those with shorter nursing experience [17, 18, 22]. The number of pregnancies correlated positively with the outcome of the survey, regardless of the number of live births. A total of 83% of respondents delivered vaginally and had higher scores than those who underwent cesarean section, consistent with previous findings [9, 15]. Many evidence indicates that mothers who deliver by cesarean section are less likely to intend to breastfeed, which may be related to the absence of early breastfeeding support practices such as lactation initiation in the first hour after birth and skin-to-skin contact [7]. This is in line with studies suggesting that cesarean delivery may be associated with short-term psychological effects. All respondents reported during pregnancy that they intended to breastfeed for more than

six months. Among them, 55% achieved this goal and had higher scores, suggesting that greater breastfeeding self-efficacy may be associated with both the intention to breastfeed and its successful continuation. These findings are in line with previous studies highlighting self-efficacy as an important factor in breastfeeding duration. A woman's breastfeeding perception is influenced by multiple factors including prior experience, breastfeeding intention, commitment, and emotional well-being [21]. A total of 70% of participants established lactation during their stay in the maternity ward and achieved higher scores, regardless of delivery method. About 50% of the participants believe that the support in the maternity hospital is adequate in terms of establishing lactation, they also had better score values than the respondents who were not satisfied with the support. Greater social support from spouses, family members, nurses, and midwives during breastfeeding period has been associated with higher overall scores [9, 21].

In Serbia, as part of primary health care, there is an outpatient service that automatically assigns a nurse to the mother in the first days after leaving the maternity hospital. Yet, almost 40% of women state that they hired private breastfeeding counselors to improve their breastfeeding experience. Recent studies suggest that postnatal breastfeeding support interventions appear more effective than antenatal interventions in improving breastfeeding self-efficacy, which coincides with the impressions of the mothers who participated in our study.

In recent World Health Organization and UNICEF communications, particular emphasis has been placed on the development of sustainable breastfeeding support systems, recognizing that breastfeeding success depends not only on maternal factors but also on family, community, workplace, and health-care support. These factors are also known determinants of breastfeeding self-efficacy. Also, new guidelines from the Association of Anesthetists from March 2026 are suggesting that the breastfeeding status of women scheduled for procedures requiring anesthesia or sedation should be assessed during preoperative planning. Available data suggest that most perioperative drugs are excreted into human milk in minimal concentrations, with no demonstrated clinically significant adverse effects on nursing infants. Accordingly, interruption of breastfeeding or discarding expressed milk after anesthesia is not routinely recommended. Instead, breastfeeding can usually be continued once the mother is awake and clinically stable [23].

Recent International Council for Harmonization recommendations advocate for the appropriate inclusion of pregnant and breastfeeding individuals in clinical trials in order to generate evidence-based data on the safety and efficacy of medicinal products during pregnancy and lactation. This approach may improve clinical decision-making and reduce unnecessary interruption of breastfeeding [24].

According to a clinical report published by the American Academy of Pediatrics in January 2026, feeding with human milk is associated with improved clinical outcomes among very low birth weight infants and has been correlated with a lower risk of several major neonatal morbidities, such as necrotizing enterocolitis, late-onset sepsis, bronchopulmonary dysplasia, retinopathy of prematurity, and neurodevelopmental deficits. Therefore, healthcare professionals should ensure that parents are adequately informed about the significant role of human milk in promoting infant health and provide continuous breastfeeding support during the entire neonatal intensive care stay to help families meet their lactation goals [25].

CONCLUSION

In this study, the Serbian version of the BSES-SF demonstrated good reliability and validity for assessing breastfeeding self-efficacy among Serbian-speaking mothers. These findings are similar to those reported in earlier validation studies.

Further research is needed to examine the scale in mothers with medical conditions that may affect breastfeeding. It would also be useful to include more heterogeneous populations to confirm these findings and to better understand how breastfeeding self-efficacy can be improved.

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Валидација српске верзије кратке форме скале самоефикасности у дојењу

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САЖЕТАК

Увод/Циљ Преваленција дојења у Србији и даље је ниска у поређењу са глобалним проценама. Самоефикасност у дојењу представља важан и променљив фактор који утиче на исход дојења, при чему све више студија указује на то да њено унапређење доприноси квалитету и трајању дојења. Циљ ове студије био је да се кратка форма скале самоефикасности у дојењу преведе и културолошки прилагоди српском језику, да се процене њене психометријске карактеристике код дојиља до 12 месеци након порођаја, као и да се испитају разлике у скоровима у односу на релевантне социодемографске и опстетричке карактеристике.

Методе Ова студија пресека обухватила је 70 дојиља порођених на Клиници за гинекологију и акушерство Универзитетског клиничког центра Војводине. Скала је прилагођена на основу претходно валидиране хрватске верзије, уз примену стандардизованих процедура културне адаптације. Унутрашња конзистентност је процењена помоћу статистике ставка-укупно (*item-total*) и Кронбахове α. Разлике између група анализиране су применом Ман–Витнијевог *U*-теста.

Резултати Просечна старост испитаница износила је 32,19 ± 5,17 година. Скала је показала добру унутрашњу конзистентност (Кронбахова $\alpha = 0,81$; 95% *CI*: 0,73–0,87). Кориговане корелације између ставки и укупног скора кретале су се од 0,13 до 0,73. Просечан скор износио је 54,11 ± 10,81, што указује на висок ниво самоефикасности у дојењу. Нижи скорови забележени су код мајки порођених царским резом, са краћим планираним трајањем дојења, раном обуставом дојења, касним започињањем дојења и недовољном подршком за дојење.

Закључак Српска верзија скале показала је задовољавајућу унутрашњу конзистентност и прелиминарну валидност за мерење самоефикасности у дојењу.

Кључне речи: дојење; самоефикасност; упитници; студије пресека; мајке; постпартални период

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Knowledge and attitudes of biomedical science students about antibiotic resistance

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SUMMARY

Introduction/Objective Antibiotics are essential drugs for treating bacterial infections; however, inappropriate use contributes to antibiotic resistance. This study aimed to assess the knowledge and attitudes of biomedical science students regarding risks associated with antibiotic use and the emergence of antibiotic resistance.

Methods This cross-sectional study was conducted using a questionnaire-based survey. A total of 195 biomedical science students participated, including 65 students each from medicine, pharmacy, and dentistry at the Faculty of Medical Sciences, University of Kragujevac. Participants anonymously completed a 15-item questionnaire covering sociodemographic characteristics and knowledge and attitudes related to antibiotic use and resistance. Data were analyzed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA).

Results The mean age of participants was 24.06 ± 1.1 years, and most respondents were female (80.5%). Nearly all students were familiar with the definition of antibiotics (98.5%), types of antibiotics (89.2%), and the concept of antimicrobial resistance (99.5%). The majority (75.9%) recognized antibiotic resistance as a major global health problem. Statistically significant differences were observed between medical and dental students, as well as between pharmacy and dental students. Concern about the impact of antibiotic resistance on personal health varied significantly among groups; 55.4% reported concern, while 33.3% were not concerned. Most respondents (96.4%) strongly agreed that responsible antibiotic use is a collective responsibility.

Conclusion Students across all study programs demonstrated good knowledge of antibiotic use and resistance but expressed a need for additional education. Curriculum revision during clinical training may improve application of knowledge in future clinical practice.

Keywords: antibiotics; resistance; students

INTRODUCTION

Antimicrobial resistance (AMR) is a significant public health problem worldwide [1, 2], defined as the ability of microorganisms to resist antimicrobial agents to which they were previously susceptible [3]. AMR represents a growing global public health challenge, arising from a range of complex processes such as genetic mutations, horizontal gene transfer, and bacterial adaptations including biofilm development and increased efflux pump activity. These processes hasten the evolution and dissemination of resistant strains, further shaped by genetic context, mobile genetic elements, and environmental pressures [4]. Various factors contribute to the spread of AMR, including self-medication or the use of antibiotics without a prescription and the over prescription of antibiotics [5, 6]. Studies have shown a global increase in antibiotic prescribing in recent decades [1–7]. This problem is often attributed to inappropriate guidelines for antibiotic prescribing and the empirical use of antibiotics without identifying the causative agent of the infection. The issue is particularly prevalent in low- and middle-income countries [8]. Additionally, insufficient knowledge regarding the indications for specific antibiotic groups coupled with the lack of clear protocols during and after the COVID-19 pandemic, has exacerbated the irrational use of antibiotics, potentially leading to a further rise in antibiotic resistance [9]. AMR has numerous consequences, including severe illness, increased hospital admissions, higher costs of second-line drugs, elevated overall healthcare expenses, and increased mortality rates [10]. AMR ranks among the major global causes of mortality, disproportionately affecting low-resource regions [11].

Serbia is among the European countries with the highest rates of antibiotic resistance and consumption [12, 13]. According to the 2020 Central Asia and Europe Antimicrobial Resistance Surveillance Network (CAESAR) report, which includes data from 18 countries and territories outside the European Union, high resistance rates in *P. aeruginosa*, *Acinetobacter spp.*, and *E. faecium* in Serbia are particularly concerning. Additionally, there is moderately high resistance to third-generation cephalosporins, aminoglycosides,

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and fluoroquinolones in *E. coli*, as well as to penicillin and macrolides in *S. pneumoniae* [12]. With an antibiotic consumption rate of 31.57 defined daily doses (DDD) per 1000 inhabitants per day, Serbia ranks among the three European countries with the highest total antibiotic consumption, alongside Greece (33.85 DDD) and Turkey (38.18 DDD), according to the European Antimicrobial Network Report on Medicines Consumption for the period 2016–2018 [13]. The Republic of Serbia has implemented a National Antimicrobial Resistance Control Program for 2019–2023, which emphasizes, among other strategies, raising awareness among healthcare professionals who prescribe antimicrobials [14]. As a middle-income country, Serbia reports that 80% of all antibiotics are prescribed for outpatient conditions [15]. Serbia is also a member of the CAESAR network and it ranks among the European countries with the highest percentage of resistant isolates [16]. More robust interventions are essential to facilitate the prudent and evidence-based use of antibiotics. Additionally, research is needed to explore and understand the knowledge, attitudes, and behaviors of the general population regarding antibiotic use [17].

Considering that the knowledge, attitudes, and behaviors of individuals are crucial for establishing and ensuring the rational use of antibiotics, the indications for their prescription must be defined responsibly [16]. In Serbia, the medical education curricula for future doctors, dentists, and pharmacists include courses in pharmacology and microbiology but lack dedicated courses on antimicrobial use and resistance.

Recent global reports continue to highlight AMR as one of the leading threats to public health, with projections indicating a further increase in morbidity and mortality if current trends persist [18]. In addition, growing evidence emphasizes the importance of educating future healthcare professionals as a key strategy in antimicrobial stewardship programs [19]. Therefore, assessing knowledge and attitudes among biomedical students remains essential for designing targeted educational interventions.

The aim of this study was to assess the knowledge and attitudes of biomedical science students at the Faculty of Medical Sciences, University of Kragujevac, regarding their perception of risks associated with antibiotic use and the emergence of antibiotic resistance.

METHODS

Study design

The study was designed as a cross-sectional study based on the use of a questionnaire. It was conducted at the Faculty of Medical Sciences, University of Kragujevac from November to December 2023. The study population comprised a total of 195 biomedical science students (65 final year students of medicine, 65 final year students of pharmacy and 65 third, fourth and final year students of dentistry). The selected groups of students are future healthcare professionals who will be allowed to prescribe or sell antibiotics in the Republic of Serbia. Inclusion criteria: students of biomedical sciences (medicine, pharmacy, and dentistry) who have passed the pharmacology exam. Exclusion criteria: students of other faculties and students who have not passed the pharmacology exam yet.

Questionnaire design

The questionnaire was adapted from previously published instruments originally developed by Golhar et al. [20] and aligned with the World Health Organization recommendations on AMR awareness. Prior to the study, the questionnaire was reviewed by experts in pharmacology and public health to ensure content validity. A pilot test was conducted on a small group of students to assess clarity and comprehensibility, and minor modifications were made accordingly.

The final questionnaire consisted of 15 items divided into two domains: knowledge (two questions) and attitudes (13 questions). All questions were close-ended with three response options. The questionnaire also included sociodemographic variables such as sex, year of birth, study program, and year of study.

Data analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as frequencies, percentages, means, and standard deviations. The normality of data distribution was assessed using the Kolmogorov–Smirnov test. As the data did not follow a normal distribution, non-parametric tests were applied.

Differences between two independent groups were analyzed using the Mann–Whitney U test, while comparisons among three groups were performed using the Kruskal–Wallis test. Categorical variables were analyzed using the χ^2 test. A $p < 0.05$ was considered statistically significant.

Ethics: This study was approved by the Ethics Committee of the University of Kragujevac, Medical Sciences (No: 09-666/3, February 13, 2025).

RESULTS

A total of 195 biomedical sciences students were included in the study, with 65 participants in each academic group. Female students predominated in all three groups, accounting for 80.5% of the total sample. Demographic characteristics are presented in Table 1. A statistically significant difference in age was observed among the groups ($p < 0.001$), with the highest mean age recorded in medical students and the lowest in dental students.

The overall distribution of respondents' knowledge and attitudes toward antibiotic use and antibiotic resistance is presented in Table 2, whereas between-group comparisons are shown in Table 3. Overall, the respondents demonstrated a high level of knowledge regarding antibiotics. Almost

Table 1. Demographic data of the respondents

Variables		IASM n (%)	IASPH n (%)	IASD n (%)
Sex n (%)	Male	15 (23.1)	7 (10.8)	16 (24.6)
	Female	50 (76.9)	58 (89.2)	49 (75.4)
Total		65 (100)	65 (100)	65 (100)
*Agea (X ± SD)		25.1 ± 0.1	24 ± 0.1	23.1 ± 0.1

IASM – integrated academic studies of medicine; IASPH – integrated academic studies of pharmacy; IASD – integrated academic studies of dentistry; n – number of the respondents; % – percent of the respondents; X – mean value; SD – standard deviation;
*$p < 0.001$;
[a]Kruskal–Wallis test

all participants correctly identified that antibiotics may exert both bacteriostatic and bactericidal effects (98.5%), 89.2% correctly recognized the group consisting exclusively of antibiotics, and 99.5% reported being familiar with the term antibiotic resistance.

With respect to antibiotic-use practices, 69.7% of respondents reported having used antibiotics without a prescription, with no significant differences among the academic groups ($p = 0.528$). In addition, 86.7% stated that they always take antibiotics exactly as prescribed, and 89.2% reported that they do not discontinue antibiotic therapy once symptoms begin to improve; neither variable differed significantly between groups ($p = 0.627$ and $p = 0.577$, respectively). Furthermore, 62.1% considered it unacceptable to take an antibiotic from a family member or friend without prior consultation with a physician, although this attitude did not significantly differ across groups ($p = 0.261$).

Regarding recent antibiotic exposure, 54.4% of participants reported not having used antibiotic therapy in the previous six months, with no statistically significant difference among the groups ($p = 0.182$). In relation to agriculture, 59.5% of respondents believed that antibiotics are used extensively in this sector, while 59% believed that such use should be reduced. Although perceptions of extensive agricultural use did not differ significantly between groups ($p = 0.056$), attitudes toward reducing antibiotic use in agriculture did show a significant difference ($p = 0.009$). Agreement with reducing such use was higher among medical and pharmacy students (66.2% in both groups) than among dental students (44.6%).

Most respondents (76.9%) believed that antibiotic resistance does not receive sufficient attention at universities, in professional literature, or in the media, with no significant between-group difference ($p = 0.345$). In total, 75.9% agreed that antibiotic resistance is one of the leading global problems; however, this perception differed significantly across groups ($p < 0.001$), with greater agreement among pharmacy students (92.3%) and medical students (83.1%) than among dental students (52.3%). A significant between-group difference was also observed regarding concern about the impact of antibiotic resistance on personal and family health ($p = 0.030$), with pharmacy students expressing the highest level of concern (66.1%) and dental students the lowest (41.5%). Finally, 96.4% of respondents agreed that the responsible use of antibiotics is a collective responsibility, with no statistically significant difference among the groups ($p = 0.087$).

DISCUSSION

This is the first study to examine this topic among students at the University of Kragujevac, rather than the general population in Serbia. Our research revealed that the surveyed students possess good knowledge about antibiotics and antibiotic resistance, with the percentage of students demonstrating adequate knowledge being slightly higher than that observed in the general population in Serbia [17]. A total of 89.23% of the surveyed students reported not discontinuing antibiotics as soon as they noticed improvement, indicating adherence to the prescribed duration of therapy. These findings align with those of a study conducted in the USA, where nearly all interviewed medical students were aware that the inappropriate use of antibiotics could harm patients and contribute to the spread of resistant bacterial strains [21]. In contrast, a study by Horvat et al. [17] found that approximately 22% of dental and veterinary students and 10% of medical students discontinued antibiotics once their symptoms resolved.

A study by Sobierajski et al. [22] demonstrated that students are aware of the dangers of antibiotic resistance, identifying the misuse of antibiotics as its primary cause. Additionally, students emphasized the need for more classes on antibiotic therapy to be integrated into the curriculum of the Medical University of Warsaw, and this viewpoint was shared by 76.92% of our respondents. In a study by Jackson et al. [23], the majority of participants, Nigerian students (94.3%), exhibited above-average knowledge about antibiotics and antibiotic resistance and recognized AMR as a global problem, though their understanding of proper antibiotic use was inadequate. These findings are consistent with our results, which showed that students had strong theoretical knowledge, with 98.46% correctly identifying the definition of antibiotics and 89.23% providing the correct response to the question about which drug belongs to the group of antibiotics. Compared to similar studies conducted in Europe and developing countries, our findings indicate a relatively high level of theoretical knowledge; however, important gaps remain in practical attitudes and behaviors, particularly regarding self-medication and inappropriate antibiotic use [24, 25].

Kose et al. [26] examined the knowledge of final-year medical students and doctors in their study. Doctors were more hesitant during the undergraduate period and more confident in the postgraduate period in their decision to start antibiotic treatment. One of the greatest concerns was that doctors tend to forget their theoretical knowledge about antibiotics over time and are unable to keep up with current developments after graduation. The most significant concern during the undergraduate period was the choice of antibiotics from the wrong group, while in the postgraduate period, the fear of an unproven infection being present was more prominent. Doctors' habits, attitudes, and behavior in prescribing antibiotics vary before

Table 2. Knowledge and attitudes of the respondents related to antibiotic use and resistance

Question	n (%)	p^a
The mechanism of action of antibiotics is:		
bacteriostatic effect	0 (0)	
bactericidal effect	3 (1.5)	
bacteriostatic and bactericidal effect	192 (98.5)	0.000*
Select the response that includes exclusively antibiotics:		
tafluprost, amikacin, clarithromycin	3 (1.5)	
gentamicin, ciprofloxacin, favipiravir	18 (9.2)	
azithromycin, doxycycline, teicoplanin	174 (89.2)	0.000*
Have you ever used antibiotics without a prescription?		
yes	136 (69.7)	
no	18 (9.2)	
not sure	8 (4.1)	0.000*
Do you always take antibiotics exactly as prescribed (dose, dosing schedule, etc.)?		
yes	169 (86.7)	
no	25 (12.8)	
not sure	1 (0.5)	0.000*
Do you discontinue antibiotic therapy once your symptoms begin to improve?		
yes	20 (10.3)	
no	174 (89.2)	
not sure	1 (0.5)	0.000*
Do you consider it acceptable to take an antibiotic from a family member or friend, if it is indicated for your condition, without prior consultation with a doctor?	6	
yes	5 (33.3)	
no	121 (62.1)	
not sure	9 (4.6)	0.000*
Have you used antibiotic therapy in the last six months?		
yes	86 (44.1)	
no	106 (54.4)	
not sure	3 (1.5)	0.000*
Are you familiar with the term antibiotic resistance?		
yes	194 (99.5)	
no	0 (0)	
not sure	1 (0.5)	0.000*
Do you think that antibiotics are used extensively in agriculture (primarily in animal farming)?		
yes	116 (59.5)	
no	6 (3.1)	0.000*
not sure	73 (37.4)	
Do you think that antibiotic resistance receives enough attention (at university, in professional literature, and in the media)?		
yes	35 (17.9)	
no	150 (76.9)	
not sure	10 (5.1)	0.000*
Antibiotic resistance is one of the leading global problems		
yes	148 (75.9)	
no	15 (7.7)	0.000*
not sure	32 (16.4)	
I think healthcare professionals should prescribe antibiotics only when it is truly necessary		
yes	184 (94.4)	
no	10 (5.1)	
not sure	1 (0.5)	0.000*
I believe that the use of antibiotics in agriculture should be reduced		
yes	115 (59)	
no	11 (5.6)	
not sure	69 (35.4)	0.000*
I am concerned about the impact of antibiotic resistance on my health and the health of my family		
yes	108 (55.4)	
no	65 (33.3)	
not sure	22 (11.3)	0.000*
Responsible use of antibiotics is a collective responsibility		
yes	188 (96.4)	
no	3 (1.5)	
not sure	4 (2.1)	0.000*

n – number of respondents; % – percent of respondents; *p* – significance;
a – χ^2 test; * – statistical significance

and after graduation. Continuous education on antibiotic use for doctors after graduation could positively contribute to reducing the rate of AMR and increasing awareness of rational antibiotic use.

Knowledge, attitudes, and practices vary greatly depending on the university, training cycle, and socioeconomic status.

A significant proportion of students consider the standard of education on antibiotics and bacterial resistance at their university to be poor or mediocre. These findings indicate that there is a need to strengthen the curriculum and programs for medical students on antibiotics, the mechanisms of antibiotic resistance, and the prudent use of antibiotics. This is an important strategy to combat the public health problem of resistance, especially in endemic countries [27].

The findings from the study, conducted by Mayers et al. [28], demonstrate that short-term international programs integrating hands-on microbiology training with AMR-focused education can effectively enhance students' technical competencies, awareness of AMR, and professional confidence. Moreover, the workshop's cross-cultural and English-based collaborative framework highlights its value in fostering global perspectives and partnerships essential for addressing AMR as a shared public health challenge [28].

The systematic review and thorough meta-analysis by Jahromi et al. [29] reveal considerable deficiencies in healthcare workers' knowledge, attitudes, and practices concerning AMR worldwide. Overall, it appears that the levels of knowledge and attitudes and consequently good practice among healthcare workers, including students, particularly in less developed countries, remain far from satisfactory [29].

The majority of students in the study by Baddal et al. [30] had sufficient basic knowledge about antibiotics, but there were areas of concern. All groups of students were aware of how antibiotic resistance develops and their roles as healthcare professionals in implementing measures against resistance. These results are consistent with our findings, where the majority of respondents believe that antibiotic resistance is one of the leading global problems.

This study has several limitations, including being conducted at a single institution, which may limit the generalizability of the findings, and the use of self-reported questionnaires that may introduce response bias. Additionally, the cross-sectional design prevents establishing causal relationships between knowledge and attitudes.

Nevertheless, the study provides important insights into the current level of awareness

Table 3. Knowledge and attitudes between groups of the respondents related to antibiotic use and resistance

Question	IASM n (%)	IASPH n (%)	IASD n (%)	p^a
The mechanism of action of antibiotics is:				
bacteriostatic effect	0 (0)	0 (0)	0 (0)	
bactericidal effect	1 (1.5)	0 (0)	2 (3.1)	
bacteriostatic and bactericidal effect	64 (98.5)	65 (100)	63 (96.9)	0.447
Select the response that includes exclusively antibiotics:				
tafluprost, amikacin, clarithromycin	1 (1.5)	0 (0)	2 (3.1)	
gentamicin, ciprofloxacin, favipiravir	7 (10.8)	0 (0)	11 (16.9)	
azithromycin, doxycycline, teicoplanin	57 (87.7)	65 (100)	52 (80)	0.159
Have you ever used antibiotics without a prescription?				
yes	46 (70.8)	44 (67.7)	46 (70.8)	
no	18 (27.7)	19 (29.2)	14 (21.5)	
not sure	1 (1.5)	2 (3.1)	5 (7.7)	0.528
Do you always take antibiotics exactly as prescribed (dose, dosing schedule, etc.)?				
yes	57 (87.7)	53 (81.6)	59 (90.8)	
no	8 (12.3)	11 (16.9)	6 (9.2)	
not sure	0 (0)	1 (1.5)	0 (0)	0.627
Do you discontinue antibiotic therapy once your symptoms begin to improve?				
yes	8 (12.3)	7 (10.8)	5 (7.7)	
no	56 (86.2)	58 (89.2)	60 (92.3)	
not sure	1 (1.5)	0 (0)	0 (0)	0.577
Do you consider it acceptable to take an antibiotic from a family member or friend, if it is indicated for your condition, without prior consultation with a doctor?				
yes	21 (32.3)	16 (24.6)	28 (43.1)	
no	41 (63.1)	46 (70.8)	34 (52.3)	0.261
not sure	3 (4.6)	3 (4.6)	3 (4.6)	
Have you used antibiotic therapy in the last six months?				
yes	24 (36.9)	29 (44.6)	33 (50.8)	
no	40 (61.5)	26 (55.4)	30 (46.2)	
not sure	1 (1.5)	0 (0)	2 (3.1)	0.182
Are you familiar with the term antibiotic resistance?				
yes	65(100)	65 (100)	64 (98.5)	
no	0 (0)	0 (0)	0 (0)	
not sure	0 (0)	0 (0)	1 (1.5)	0.221
Do you think that antibiotics are used extensively in agriculture (primarily in animal farming)?				
yes	43 (66.2)	41 (63.1)	32 (49.2)	
no	1 (1.5)	3 (4.6)	2 (3.1)	
not sure	21 (32.3)	21 (32.3)	31 (47.7)	0.056
Do you think that antibiotic resistance receives enough attention (at university, in professional literature, and in the media)?				
yes	11 (16.9)	14 (21.5)	10 (15.4)	
no	52 (80)	49 (75.4)	49 (75.4)	
not sure	2 (3.1)	2 (3.1)	6 (9.2)	0.345
Antibiotic resistance is one of the leading global problems				
yes	54 (83.1)	60 (92.3)	34 (52.3)	
no	5 (7.7)	3 (4.6)	7 (10.8)	
not sure	6 (9.2)	2 (3.1)	24 (36.9)	0.000*
I think healthcare professionals should prescribe antibiotics only when it is truly necessary				
yes	60 (92.3)	63 (96.9)	61 (93.8)	
no	4 (6.2)	2 (3.1)	4 (6.2)	
not sure	1 (27.7)	0 (0)	0 (0)	0.502
I believe that the use of antibiotics in agriculture should be reduced				
yes	43 (66.2)	43 (66.2)	29 (44.6)	
no	4 (6.2)	3 (4.6)	4 (6.2)	
not sure	18 (27.7)	19 (29.2)	32 (49.2)	0.009*
I am concerned about the impact of antibiotic resistance on my health and the health of my family				
yes	38 (58.5)	43 (66.1)	27 (41.5)	
no	21 (32.3)	18 (27.7)	26 (40)	0.030*
not sure	6 (9.2)	4 (6.2)	12 (18.5)	
Responsible use of antibiotics is a collective responsibility				
yes	64 (98.5)	63 (97)	61 (93.9)	
no	1 (1.5)	1 (1.5)	1 (1.5)	
not sure	0 (0)	1 (1.5)	3 (4.6)	0.087

IASM – integrated academic studies of medicine; IASPH – integrated academic studies of pharmacy; IASD – integrated academic studies of dentistry; n – number of respondents; % – percent of respondents; p – significance; a – χ^2 test; * – statistical significance

among future healthcare professionals and highlights the need for continuous education and curriculum improvement.

CONCLUSION

Based on our results, we can conclude that students from all study groups are educated about antibiotic resistance, as well as the use and application of antibiotics in treatment, but students expressed a desire for additional education. A possible solution to this problem is to change the curriculum in the clinical years of the faculty in order for students to expand their knowledge that they will implement in their daily clinical practice after their studies. This way, they would more clearly define the indications for the use of antibiotics to prevent improper prescribing of antibiotics and the development of AMR with global economic and health consequences.

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Знања и ставови студената биомедицинских наука о антибиотској резистенцији

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САЖЕТАК

Увод/Циљ Антибиотици су значајни лекови у борби против бактеријских инфекција, међутим неадекватна употреба доприноси развоју антибиотске резистенције. Циљ ове студије био је да се процене знања и ставови студената биомедицинских наука о ризицима повезаним са употребом антибиотика и појавом антибиотске резистенције.

Методе Студија је дизајнирана као студија пресека базирана на коришћењу упитника. У истраживању је учествовало укупно 195 студената биомедицинских наука, по 65 студената медицине, фармације и стоматологије са Факултета медицинских наука Универзитета у Крагујевцу. Испитаници су анонимно попунили упитник од 15 питања који је обухватао социодемографске карактеристике, као и питања о знању и ставовима у вези са употребом антибиотика и резистенцијом. Подаци су статистички обрађени у програму *IBM SPSS Statistics for Windows*, верзија 22.0. (*IBM Corp*., Армонк, Њујорк, САД).

Резултати Просечна старост испитаника била је 24,06 ± 1,1 година, а већину су чиниле жене (80,5%). Готово сви студенти познавали су дефиницију антибиотика (98,5%), врсте антибиотика (89,2%) и појам антимикробне резистенције (99,5%). Већина испитаника (75,9%) сматрала је да је антибиотска резистенција велики глобални здравствени проблем. Утврђене су статистички значајне разлике између студената медицине и стоматологије, као и између студената фармације и стоматологије. Забринутост због утицаја антибиотске резистенције на лично здравље показала је статистички значајну разлику; 55,4% је изразило забринутост, док 33,3% није било забринуто. Већина испитаника (96,4%) у потпуности се сложила да је одговорна употреба антибиотика колективна одговорност.

Закључак Студенти свих студијских програма показали су добро знање о употреби антибиотика и антибиотској резистенцији, али су нагласили потребу за додатном едукацијом. Измена информатора предмета на клиничким годинама студија могла би унапредити примену стеченог знања у будућој клиничкој пракси.

Кључне речи: антибиотици; резистенција; студенти

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Workplace violence – health institutions in Serbia in focus

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SUMMARY

Introduction/Objective Health workers are five times more likely to be exposed to workplace violence than other workers are. The goal of this paper is to present an overview of the situation related to violence against health workers and other employees in health institutions, before amendments to the Criminal Code of the Republic of Serbia by the end of 2024.

Methods This cross-sectional study was carried out over a three-month period, from July 10, 2024, to October 10, 2024, in the form of a purpose-designed online survey, voluntarily completed by 125 respondents.

Results The survey showed that 74.4% of participants had experienced violence. Psychological abuse dominated. Verbal abuse was experienced by 68.8% of workers, psychological-nonverbal by 64.4%, and physical violence by 12.8% of participants in the survey.

There was no statistically significant difference in experienced workplace violence in relation to sex, place of residence, nationality, marital status, number of children, or level of education, type of specialization, type of institution, presence of security staff, activity on social networks, number of their workplaces, and trade union membership. Workplace violence decreased as the length of service increased. Managers experienced less violence. The dominant cause of violence in general hospitals is communication problems and a patient's psychological disorder, while in health centers it is the non-acceptance of organizational limitations.

Conclusion We provide proposals that would contribute to the overall prevention of violence at workplaces and to the protection of healthcare workers.

Keywords: workplace violence; health; aggression; survey; law

INTRODUCTION

Workplace violence (WPV) is any act or threat of physical violence, harassment, intimidation, or other threatening or disruptive behavior that occurs in the workplace, as defined in guidelines from organizations such as OSHA (Occupational Safety and Health Administration) [1].

While there is no universal definition of workplace violence (WPV), differing methods of categorizing WPV are found, based on the nature of the aggression or based on associated intent [2].

Workplace violence (WPV) against health workers has been a global problem for decades [3]. Health workers are five times more likely to be exposed to violence at work than other workers [1, 4, 5]. Non-physical violence was reported at rates two to ten times higher than physical violence [6, 7].

According to WHO, between 8% and 38% of health workers suffer physical violence at some point in their careers [6]. The prevalence of physical violence is reported to be as high as 65% and there is evidence that it has increased significantly over the last 30 years relative to the change in non–physical violence [7]. The prevalence of aggressive behavior on psychiatric wards varied (8–76%) [8]. In the meta-analysis by Lu et al. [8], the overall prevalence of workplace violence against healthcare professionals was 62.4%, with verbal abuse accounting for the largest share (61.2%), followed by psychological violence (50.8%), threats (39.5%), physical violence (13.7%), and sexual harassment (6.3%). Despite this, a large number of cases of workplace violence remain unregistered, which probably makes the true prevalence of this problem higher [7, 9, 10, 11].

The consequences of violence at the workplace are personal and organizational, as well as being a large financial burden on the state. In institutions, a lack of trust between workers and executives and a toxic atmosphere emerge, which affects work processes and is manifested through absenteeism, low productivity, or complete loss of productivity and dissatisfaction with work. The consequences of a worker being exposed to violence at the workplace may be acute and chronic and are seen as physical injuries, psychological trauma, and sometimes even death [1, 7, 12].

The nature of violence perpetrated by colleagues and superiors was found to differ particularly from that perpetrated by patients or visitors [13, 14].

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The role of the media in creating public distrust toward the health service is very important, and it contributes to aggression toward health workers [15], while violence is often manifested through lynching on social media [13].

Even though this problem is widespread even in our country, the amount of research on violence against health workers in Serbia is limited [16].

The goal of this paper is to provide a quick overview of the situation related to violence against workers in health institutions before the adoption of amendments to the Criminal Code of the Republic of Serbia, which were due to be adopted by the end of 2024 [17]. The research was conducted independently and considered the causes, perpetrators, victims' reactions, consequences, and needs.

METHODS

This cross-sectional study was carried out in a three-month period, from July 10, 2024, to October 10, 2024, in the form of a purpose-designed online survey conducted by Hatorum LLC – Centre for Education and Counselling with the support of the Trade Union of Employees in Health and Social Care Institutions of Serbia. All healthcare workers and healthcare employees in the Republic of Serbia, without the obligation to be union members, were invited to complete an online survey, which was located on the website www.hatorum.com.

The invitation to participate in the survey was located on the websites www.zdravko.org.rs and www.hatorum.com, and the advertisement with the invitation was distributed through Union representatives for placement on the bulletin boards of the Union of Healthcare and Social Welfare Employees of Serbia, in all state healthcare institutions in the Republic of Serbia. In addition, the invitation was sent through three Facebook groups that gather healthcare workers from Serbia, though not exclusively, https://www.facebook.com/groups/519782948126826/ (32.4 thousand members), https://www.facebook.com/groups/730899947580244/ (3.6 thousand members), https://www.facebook.com/groups/39503031067/ (33.2 thousand members).

The survey was completed voluntarily. It was not anonymous and was completed by 125 respondents. It was not possible to predict how many healthcare workers would receive information about the survey via Facebook, nor to verify whether advertisements about the survey were posted and how long they were on the Union's bulletin boards.

The survey was created for the purposes of this research. It consisted of 72 questions divided into four areas: demographic and general data, type of aggression experienced, consequences, and needs. Participants could select multiple answers to one question.

Survey participants were informed that the data obtained in the survey were protected in accordance with the Law on the Protection of Personal Data (Official Gazette of the Republic of Serbia, No. 87/2018) and that they could be used exclusively in connection with the task and objectives of the survey, and in the interests of employees. Regarding the security of the data obtained, data were collected from the site using WPForms version 1.9.6.1. The form was removed after the survey was completed.

The data were collected in Microsoft Excel, adjusted, and imported into IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA), for analysis. The statistical methods applied were frequencies, percentages, and the χ^2 test. The results are shown in Tables 1–5. Statistical significance was determined at a type I error level of 0.05.

Ethics: The study was previously approved by decision No. 1264/22 of the Ethics Committee of the University Clinical Center of Serbia.

RESULTS

Among 125 respondents, 74.4% experienced violence during their healthcare work in the last three years.

The perpetrators of violence were mostly patients (66.3%), their families (53.7%), or people connected to them (34.7%); communication problems and a patient's psychological disorder as causes of violence were reported with high statistical significance by respondents from general hospitals; non-acceptance of organizational limitations was reported as a cause with statistical significance in health centers and general hospitals.

Psychological-verbal abuse dominated by 68.8%, psychological-nonverbal abuse by 37.6%, physical violence was experienced by 12.8% of respondents, but violence took other forms as well. Our respondents reported repeated violence in the last three years.

There was no statistically significant difference between experienced violence and sex, place of residence, nationality, marital status, number of children, level of education, whether the physician was a specialist, type of specialization, type of institution where the respondent is employed, whether there are security guards in the institution, panic buttons and video surveillance, activity on social networks or the number of respondents' workplaces, as well as trade union membership.

A decrease in violence was observed as the length of service increased, with a highly statistically significant difference. Beginners with 0–2 years of service were the most exposed group and the least exposed were senior employees with 31–40 and over 40 years of service.

A statistically significant difference was found: individuals holding leadership positions in an institution reported less violence when performing their work duties.

In 77.8% of cases, respondents received no medical assistance and, in 74.7%, no psychotherapeutic assistance in their institution.

Among the respondents, 77.7% suffered psychological injuries, 40% reported damage to their reputation, 11.6% sustained minor physical injuries, and 1.1% sustained serious physical injuries, as well as other types of harm. Changes in body weight and sleep disturbance, daily

Table 1. Descriptive sample analysis

Sex								
85.6% Female			13.6% Male			0.8% Undefined		
Age								
3.2% (18–24)		18.4% (25–34)		32% (35–44)		28.8% (44–45)		17.6% (55–65)
Marital status								
55.2% Married		20% Single		12.8% Divorced		8.8% Extramarital union		3.2% Widow/widower
Number of children								
40.8% Two		30.4% None		19.2% One		8% Three		1.6% Four and more
Location								
60% Vojvodina			20.8% Belgrade			19.2% Central Serbia		
Nationality								
81.6% Serbs		11.2% Hungarians		1.6% Slovaks		0.3% Bosnians		4.8% Others
Level of education								
47.6% Faculty	37.1% Secondary school		7.3% High school		4% PhD	2.4% Master		1.6% Elementary school
Profession								
36% Doctors	35.2% Nurses	4.8% Physiotherapist	4% Dentist	4% Medical technician	3.2% Laboratory assistant		3.2% Dental nurse	1.6% Psychologist	8% Others
Specialization								
11.8% General medicine	7.8% Emergency medicine	5.9% Psychiatrist	5.9% Gynecologist	3.9% Pediatrician	3.9% Clinical biochemistry	2% Medical microbiology	2% Physical medicine and rehabilitation		54.9% I have no specialization
Length of service								
6.4% (0–2)	25.6% (3–10)	25.6% (11–20)		24.8% (21–30)		15.2% (31–40)		2.4% Over 40	
Institution								
56.4% Health centers		16% Clinical center		14.4% General hospital		4% Special hospital		2.4% Emergency service	6.8% Other
Position in institution								
72% Full-time employed			19.2% Leading position			8.8% Employed for a definite time			
Number of healthcare institutions where you work								
76% One		12.8% Two		4% Three		7.2% Several			
Union membership								
39.6% Yes, passive			28% No	23.2% Yes, active		9.6% Yes, management			
Presence on social networks								
64% Yes, active			29.6% Yes, passive			6.4% No			
Religiosity								
77.6% Yes			12.8% No			9.6% I don't know			

medication use and sick leave indicate short- and long-term health consequences.

The results show other losses after WPV in the last three years and feelings of disenfranchised grief in 56.8% of participants; 37.9% of participants are not motivated to go to work, 16.8% plan to move to another country, and 9.5% plan to change their occupation. Of the overall number of respondents, 88% think that health workers should get the official status under Article 112 of the Criminal Code, 98.4% of all respondents think that punishments for violence against health workers should be more severe.

With high statistical significance, 45.7% of respondents who experienced violence stated that they needed educational courses aimed at psychological self-defense and 49.5% needed courses on physical self-defense.

DISCUSSION

Even though the number of respondents represents a sufficiently large group for the research, it is a small number of respondents compared to the overall number of health workers and other workers in health in Serbia. Consequently, the results of the survey can be interpreted only for this group and in light of the fact that the survey was not anonymous.

For those whose names are attached to these data, each decimal has a special meaning in both pain and the recovery process. Leaving personal data while taking safety measures and respecting the General Data Protection Regulation was done with the aim of providing the respondents who had experienced violence with the opportunity to express their statements openly, which was also supposed to have a therapeutic effect rather than concealing them.

Table 2. Perpetrators of violence, setting

From whom have you experienced violence related to the performance of healthcare activities in the last three years?							
66.3% Your patient	53.7% Your patient's family	34.7% Another person related to your patient	26.3% Someone else's patient	12.6% Another person's patient's family	27.4% Employees	23.2% Superiors	25.3% Unknown persons
3.2% Professional association	2.1% Health inspection	3.2% Ministry of Health	5.3% Politician	1.1% Member of own family	5.3% Media	5.3% Unknown group	3.2% Other
Where have you experienced violence related to your health-related work in the last three years?							
97.9% At the workplace	11.6% Outside the workplace		5.3% Via social networks		4.2% Through the media	3.2% At home	3.2% In another place
What protective measures do you have against workplace violence?							
23.2% Yes, security 24 hours	2.4% Yes, security before noon		1.6% Yes, security, only in the evening		46.4% Yes, video surveillance	5.6% Yes, panic buttons	72.8% No
What was the cause of violence?							
8.4% Acceptance of the diagnosis	13.7% Diagnostic process	22.1% Treatment Implementation	7.4% Treatment outcome	55.8% Communication problem	52.6% Unfulfilled expectation	0% Ethical problems	0% Fatigue
22.1% Weak organization	20% Slander	35.8% Patient's psychological problems	15.8% I don't know	9.5% Personal conflict	50.5% Restrictions and rules are not accepted	0% Professional mistake	7.4% Other reasons
Has violence against you been repeated by the same perpetrator over time?							
28.8% Yes		51.2% No		18.4% This question does not apply to me		1.8% I don't want to answer	

Table 3. Victims, reactions

Have you experienced any type of violence related to your healthcare work in the last three years?									
12.8% Physical	68.6% Psychological verbal	37.6% Psychological nonverbal	10.4% Financial	1.6% Sexual	4.6% Destruction of personal property	8% Destruction of medical documentation	5.6% Destruction of medical equipment	6.4% Destruction of property of a health institute	
How many times have you experienced violence related to your work in the last three years?									
24.8% None	5.6% Once	29.6% From two to five times		15.2% More than five times		20.8% More than 10 times		4% I don't want to answer	
Was the violence reported to the police?									
14.9% Yes, by me		4.3% Yes, by the institution			5.3% Yes, by me and by the institution			75.5% No	
Were legal proceedings initiated against the perpetrator?									
7.4% Yes, by me		2.1% Yes, by my institution			1.1% Yes, the institution and I sued the perpetrator			89.4% No	
Did you ask for help?									
15.8% Yes, I sought medical help		16.8% Yes, I sought psychotherapy help		64.9% Reporting to Superiors		28.7% Yes, I sought legal assistance		14.9% Yes, I reported it to the police	7.4% Initiated legal proceedings
Where did you receive medical help?									
12.4% In your institution		4.5% In another state institution		3.4% In your institution and another state institution		2.2% In private practice		77.8% I didn't get help	
Where did you receive psychological/psychotherapy help?									
12.1% In your institution		3.5% In another state institution		4.4% In your institution and another state institution		5.5% In private practice		74.7% I didn't get help	
From whom did you get support after workplace violence?									
31.6% Superiors	81.1% Colleagues	24.2% Patients	4.2% Legal service	2.1% Professional chamber	2.1% Other professional associations	0% Union	3.2% Media	1.1% Priest	1.1% Political party
55.8% Family	49.5% Friends	4.5% Acquaintances	7.4% Unknown people	3.2% Ministry of Health	1.1% Prosecutor's office	13.7% Lawyer	4.2% Police	4.2% I didn't get support	1.1% Others

Table 4. Victims, consequences

Direct damage								
11.6% Minor physical injury	1.1% Serious physical injury	77.7% Psychological Injury	40% Social injury	13.7% Inability to perform the work				
41.1% Difficulty performing work	15.8% Financial loss	2.1% Loss of title	1.1% Termination of research	1.1% Loss of research	20% Damage to others related to you			
Do you have later consequences after experiencing aggression while performing health activities?								
10.6% Worsening of an existing illness	4.3% New physical illness	1% Infection	4.3% Physical injury	10.6% I feel physically ill	22.3% I feel mentally ill	51.1% I don't want to answer	0% Disability	0% Operation
Did you change your body weight after you experienced workplace violence?								
7.4% Yes, up to 5 kg	11.6% Yes, over 5 kg	9.5% Yes, up to 5 kg	7.4% Yes, over 5 kg	46.3% No, I didn't	13.7% I don't know	4.2% I don't want to answer		
Do you have trouble sleeping after experiencing workplace violence?								
22.1% I can't sleep	31.6% I wake up often	9.5% I wake up early	20% I sleep less than 6 hours	1.1% I sleep longer than 9 hours	3% I have nightmares	33.7% No, I don't have sleep problems	10.5% I don't want to answer	
Are you currently taking medication daily?								
7.4% Psychiatric	26.3% Cardiology	6.7% Pulmonary	7.4% Gastroenterological	1.1% Hematological				
2.1% Immunological	12.6% Analgesics	5.3% Other	53.7% I don't take medications.	4.2% I don't want to answer				
Do you drink alcohol (at least two glasses of spirits, two glasses of wine, or two beers)? Do you take drugs?								
4.2% Yes, during the week	21% Yes, during the month	72.6% I don't drink	2.1% I don't want to answer	5.3% I got drunk in the last month	100% No, I don't take drugs			
Have you been on sick leave in the last three years?								
40% No	41.1% Yes, up to 30 days	5.3% Yes, up to 60 days	6.3% Yes, more than two months	3.2% Yes, more than six months	3.2% I am on sick leave now	1.1% I don't want to answer		

In our sample there was a much greater percentage of respondents (74.4%) who experienced violence during their healthcare work compared to the findings of Victimology Society of Serbia, which pointed out a rate increase from 48.7% in 2008 to 64.2% in 2010, and compared these data with the findings related to employees in primary health protection in Belgrade in 2015, mentioned by Fišeković et al. [16], where 52.6% of employees experienced abuse, and 18.3% experienced physical violence.

In our study 68.8% of respondents experienced psychological-verbal abuse, 37.6% experienced psychological-nonverbal abuse, and 12.8% experienced physical violence.

A decrease in experiencing violence happens as the length of service increases. This statistically significant finding can be interpreted by seniors' greater experience in working with patients.

With high statistical significance, managers experienced less aggression compared to other employees, so we can assume that they are the most experienced in their job, more effective in solving communication problems, protected by their position, not on the front line of contact with patients, or have a tendency to present facts and circumstances more favorably than they are, in order to remain in a leading position and present themselves as capable.

However, more than a quarter of violent incidents (26.3%) were perpetrated by patients whom health workers did not treat and 25.3% were perpetrated by unknown people, which may hypothetically be explained by social factors or completely irrational reasons connected to a patient's psychological problems in 35.8% of cases. Similarly, more than a quarter of respondents (27.4%) said that they suffered from aggression from other employees in the medical institution, while 23.2% said they were attacked by their superiors, which may be hypothetically interpreted by the perpetrator's and victim's personal reasons (50.5% unaccepted restrictions and rules, 20% slander, 7.4% other reasons), as well as weak organization (22.1%), where there are not enough health professionals, leading to employees being exposed to burnout syndrome [18].

Violence that did not occur only in the workplace may be explained by the role of social networks (5.3%) as well as some media (4.2%), which sometimes actively participate in the lynching of healthcare workers, so the intensity of such violence, its duration and long-term consequences are not adequately represented by a small percentage.

Our survey results indicate that communication problems play a crucial role in generating violence. We have established mechanisms and procedures for resolving medical disputes, ways to report adverse events, and obligations to inform the police, but the respondents point out that in their cases and many other cases these procedures were not used. Without informing the police (75.5%) and legal punishment (89.4%), the repetition of violence by the same perpetrator is not surprising.

Table 5. *Secondary losses, other consequences, plans and needs*

Have you suffered significant losses in the last three years as a result of workplace violence against you in connection with your healthcare activities?													
71.6% No, I haven't	1.1% Divorce	3.2% Love breakup	1.1% Loss of custody	1.1% Abortion	11.6% Financial loss	7.4% Loss of friendship							
5.3% Loss of important person	2.1% Loss of job	1.1% Loss of license	0% Loss of patient	0% Loss of number of patients	0% Prison sentence	8.4% Other loss	5.3% I don't want to answer						
How is your attitude towards work after experiencing workplace violence?													
45.3% The same attitude towards work	36.8% The same attitude towards patients	35.8% The same attitude towards colleagues	5.3% I do my job better	3.2% I do my job worse	0% I make mistakes at work	25.3% I do my job more carefully	37.9% I'm not motivated to go to work						
12.6% My attitude towards patients has changed permanently	9.5% My attitude towards patients has changed permanently	17.9% My relationship with people has changed permanently	4.2% I'm afraid to go to work	2.1% I'm afraid I'll make a mistake	1.1% The question does not apply to me	5.3% I don't want to answer							
Do you feel disenfranchised grief?													
56.8% Yes			27.4% No		15.8% I am not sure								
Are you planning any significant changes after experiencing workplace violence?													
4.2% Transfer to another workplace within the same institution	15.8% Moving to another institution	1.1% Moving to another town	16.8% Moving to another country	9.5% Change of occupation	0% Change in physical appearance	88.4% No, I am not							
Proposals													
88% Healthcare workers should be given official status			49.5% Healthcare workers should receive additional education in physical self-defense		45.7% Healthcare workers should receive additional education on psychological self-defense								

People who experienced psychological abuse were seven times more likely to become victims of physical violence [19]. In the study on violence in hospitals in China, the frequency of violence in hospitals reached as much as 95%, which points to the fact that physical and verbal harassment of medical staff happened often [20].

The finding that 77.5% of the attacked respondents did not receive medical aid and 74.7% did not receive psychotherapy in their institution is worrying.

The finding of disenfranchised grief (56.8%) and the health consequences for victims of WPV are worrying, as is the percentage of employees who do not want to talk about it (51.1%).

Overall, 54.7% of respondents had changed their attitude towards work; 64.2% have changed their attitude towards colleagues, and 63.2% who experienced violence had changed their attitude towards patients.

With high statistical significance, respondents who were the victims of aggression feel disenfranchised grief and say that they need psychological training to protect themselves. However, with a higher statistical significance, there are more of those who say they need to learn physical self-defense. Keeping in mind communication problems (55.8%) in our results, it is crucial to implement a competent program for the prevention and suppression of workplace violence and de-escalation courses taking into account all risk factors that may contribute to this issue [4, 21].

Limitations of the study

Given the limited number of respondents and the fact that the survey was not anonymous, responses denying any responsibility of the health worker as the cause of the violence or that might question the personal credibility of the health worker at the time of the violence and afterwards should be interpreted with caution.

In the future, it would be necessary to repeat the research on a larger sample, to do it anonymously, and then compare the results.

CONCLUSION

In our survey and the literature, psychological abuse was present in a larger percentage than physical and other types of violence. Unfortunately, it was not covered by the new amendments to the Criminal Code of the Republic of Serbia, which provide harsher penalties only for acts of physical violence against medical professionals.

Even though our research has not shown statistical significance, it is necessary to introduce

security measures in all health institutions and monitor the effects of these changes.

We suggest the establishment of ethics hubs at the level of healthcare institutions, which would include a mandatory timely reporting system, post-incident procedures, and services that include trauma-crisis counselling, critical-incident stress debriefing, and employee assistance programs, safety and health training in order to ensure that all staff members are aware of potential hazards and how to protect themselves and their co-workers through established policies and procedures, provide legal and medical aid with clearly defined powers supported and delegated by the adopted protocols.

If violence, related to the work in a health institution, led to the deterioration and worsening of the employee's physical and psychological health and a new diagnosis of a chronic disorder within three years after having experienced workplace violence, we suggest the employee who suffered violence be provided with a 100% compensation during sick leave.

It is necessary for health institutions to commit to transferring healthcare employees who experienced workplace violence to another workplace if they request it.

In cases where it has been proven in court that the act of violence was perpetrated by an employee of the health institution against another employee, it is necessary to establish a mechanism for transferring the perpetrator to another workplace and depriving them of a leadership position.

At least once a year, it is necessary to organize preventive anti-stress training for all healthcare employees who experienced any kind of physical or psychological trauma, with the aim of detecting individuals who have a complicated reaction to a traumatic event and loss, and who are at risk of later developing a psychiatric or psychosomatic disorder or addiction.

It is necessary for healthcare employees to be specifically insured against physical and psychological injuries, and material and non-material damage inflicted during work. Cooperation is necessary between healthcare institutions and professional associations in incorporating codes of practice and ethics, and clauses concerning the unacceptability of any form of workplace violence, and support from the media, non-governmental organizations (NGOs) and other relevant community bodies in actively advocating awareness and training against workplace violence. When determining a fine or other penalties for the perpetrator who committed the act of violence in the Criminal Code, all consequences related to the deterioration of physical and mental health and losses that are connected to a concrete event should be assessed, whether physical or online violence, as well as material and non-material damage inflicted on an employee, their family, institution or another person or legal entity that are connected to them. In this regard, we hope that this work will contribute to additional improvement of the current legal regulations.

During their education, healthcare workers do not learn that workplace violence is a frequent occurrence in healthcare institutions. In this regard, while learning how to protect and help others, the educational program should also include the skills needed by healthcare workers to protect themselves.

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In addition to intellectual curiosity about the topic of violence against healthcare workers, which is not sufficiently researched in our environment, and decades of professional experience at the Clinic for Psychiatry University Clinical Center of Serbia in connection with this problem, the first author has personal experience of surviving lynching, initiated by the media, due to conducting professional healthcare activities, after the mass murder at the Vladislav Ribnikar Elementary School in Belgrade in 2023, and, in this regard, has personal reasons and motivation for publishing and discussing various aspects of this problem in the professional community. In the presentation of the results of the survey, an objective distance was achieved in the interpretation of the results. The work on the survey, the trust of the survey participants, and the development of ideas related to the prevention of workplace violence helped the author's personal recovery.

The research was conducted under the auspices of the Hatorum LLC – Centre for Education and Counselling, of which the first author is the founder and director, and with the support of the Trade Union of Health and Social Care Employees. Hatorum LLC was founded in 2008 and has been engaged in violence prevention and health protection for many years. At the time of the research, the author was only able to post the survey online on the institution's website. The survey was not posted on the union website because we wanted to avoid political connotations and invite all healthcare workers to participate, not just members of one union. No financial benefit was realized in connection with the research.

The cooperation of the Trade Union of Health and Social Welfare Employees of Serbia and Hatorum LLC is in accordance with the Program and Statute of the trade union, where the primary task of the trade union is to achieve labor and legal protection, improve the working environment, and the conditions under which work is performed, which implies the absence of any form of violence, whether physical or psychological, as well as mutual respect and tolerance, collegiality and solidarity.

The research results did not influence the final version of the amendments to the Criminal Code.

Conflict of interest: None declared.

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Насиље на радном месту – у фокусу здравствене установе у Србији

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САЖЕТАК

Увод/Циљ Здравствени радници су пет пута више изложени насиљу на радном месту него остали радници. Циљ овог рада је да прикаже преглед стања у вези са насиљем над здравственим радницима и другим запосленима у здравственим установама у Републици Србији, пре усвајања измена Кривичног законика Републике Србије до краја 2024. године.

Методе Ова студија попречног пресека је спроведена током три месеца, од 10. 7. 2024. до 10. 10. 2024. године, у облику онлајн упитника који је циљано направљен и који је добровољно попунило 125 учесника.

Резултати Од укупног узорка, 74,4% испитаника је доживело насиље. Доминирало је психолошко злостављање. Вербално злостављање доживело је 68,8% здравствених радника, психолошко-невербално 64,4% и физичко насиље 12,7% учесника анкете.

Није утврђена статистички значајна разлика у доживљеном насиљу на радном месту у односу на пол, место становања, националност, брачни статус, број деце, ниво образовања, врсту специјализације, врсту установе, присуство обезбеђења, активност на друштвеним мрежама, број радних места и чланство у синдикату.

Насиље на радном месту смањивало се са повећањем радног стажа. Руководиоци су ређе доживљавали насиље. Доминантни узроци насиља у општим болницама су проблеми у комуникацији и психолошки поремећаји пацијената, док је у домовима здравља то неприхватање организационих ограничења.

Закључак У раду су изнети предлози који би допринели укупној превенцији насиља на радном месту и заштити запослених у здравству.

Кључне речи: насиље на радном месту; здравље; агресија; анкета; закон

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PRELIMINARY COMMUNICATION / ПРЕТХОДНА И КРАТКА САОПШТЕЊА

Platelet transfusion refractoriness in patients with acute myeloid leukemia – the role of antiplatelet antibodies

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SUMMARY

Introduction/Objective Platelet transfusion refractoriness represents a common issue in the treatment course of acute myeloid leukemia (AML) patients who require chronic transfusion support. Antibodies to human platelet antigens could cause immune-mediated transfusion refractoriness. The main goal of this study was to examine the presence and quantity of antibodies to GP IIb/IIIa, GP Ib/IX, and GP Ia/IIa in AML patients refractory to platelet transfusion in comparison to healthy male subjects.

Methods This prospective study involved 22 adult AML patients who demonstrated resistance to platelet transfusion. Antibody titers for the following platelet antigens: GP IIb/IIIa, GP Ib/IX, and GP Ia/IIa were measured in these patients before and two hours after platelet transfusion as well as in healthy male controls.

Results The antibodies to platelet antigens taken before the platelet transfusion were significantly increased in all patients compared with healthy untransfused controls ($p = 0.001$). In all three groups of antibodies, the titers were significantly higher after platelet transfusion ($p = 0.001$) than before. There was no statistically significant difference between males and females, platelet sources (buffy-coat *vs.* apheresis-derived) in the examined initial antibody levels, as well as in the antibody titer increments after the transfusion. No significant correlation between platelet count increment (absolute numbers) and initial levels of antiplatelet antibodies was found.

Conclusion Platelet transfusion refractoriness in AML patients could be caused by the antibodies to platelet antigens, since their levels are significantly higher in the examined patients than in healthy male untransfused controls.

Keywords: platelet transfusion refractoriness; acute myeloid leukemia; human platelet antigens; AML; HPA

INTRODUCTION

Treating acute myeloid leukemia (AML) often involves high-intensity chemotherapy treatment, which results in bone marrow aplasia and (pan)cytopenia. Moreover, cytopenia in AML patients could be a manifestation of the disease itself as well [1]. Consequently, repeated allogeneic transfusions of red blood cells and platelets are frequently required. Prophylactic transfusion of platelets is recommended to maintain platelet count (PC) above 10×10^9/L [2]. However, an adequate post-transfusion PC increment cannot always be achieved.

A PC increment of less than 10×10^9/L after administration of an aphaeresis unit (or 1.75×10^9/L per random donor platelet concentrate) is indicative of refractoriness [3]. Platelet refractoriness may be the result of immune and non-immune factors. Non-immune causes of refractoriness are more common, and they are usually associated with splenomegaly, infection (sepsis), fever, administration of drugs, disseminated intravascular coagulation, and bleeding [4]. On the other hand, immune-mediated refractoriness is caused by antibodies to human leucocyte antigens (HLA) and human platelet antigens (HPA). Previous transfusions or pregnancy are considered risk factors for such alloimmunization. While HLAs are widely present in human tissues, HPAs are platelet-specific [5]. HLA class I antigens are present on the platelet membrane. These molecules are known for their very high polymorphism, which makes them extremely immunogenic [6]. For that reason, HLA-matched platelets, antigen-restricted platelets, or cross-matched platelets are increasingly used, especially among those patients who require chronic transfusions [7]. On the other hand, HPA polymorphisms occur mostly due to single amino acid changes in glycoproteins (GPs) [8]. Numerous platelet-specific antigens have been characterized, and those that are considered the most common polymorphic are GPIa, GPIb, GPIIb, GPIIIa, and CD109 [8, 9]. However, their role in transfusion refractoriness is still controversial [4]. It was observed that leukoreduction does not affect the incidence of HPA [10].

The goal of this study was to assess the presence and levels of antibodies to GP IIb/IIIa, GP Ib/IX and GP Ia/IIa, which carry major HPA epitopes, in AML patients refractory to platelet transfusion in comparison to healthy male subjects.

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METHODS

This prospective study, conducted from 2012 to 2015, included adult patients diagnosed with acute myeloid leukemia at the Clinical Center of Serbia, who were platelet transfusion-refractory. All patients received intensive chemotherapy regimens, which resulted in chemotherapy-induced thrombocytopenia (platelet count < 100×10^9/L) and required platelet transfusions. A transfusion-related allergic reaction (e.g., chills, fever, and skin manifestations such as erythema and urticaria) was observed in all included patients, but it was not used as an inclusion criterion. Refractoriness was defined as a posttransfusion platelet count increment equal to or lower than 10×10^9/L. Due to the lack of precise data on transfused platelet dose and body surface area, platelet count increment was used instead of corrected count increment. Platelet transfusion refractoriness accompanied by PC < 10×10^9/L is a life-threatening condition and the main reason for our investigation was to identify its cause and determine whether it could be prevented. All patients had received multiple platelet transfusions before inclusion in the study (more than five). Seven patients were transfused with buffy coat-derived platelet concentrates since apheresis-derived platelet concentrates were not available, and 15 patients were transfused with apheresis-derived platelet concentrates. Apheresis units minimize donor exposure and have been shown to allow for more prolonged survival of platelets compared to pooled platelets [11]. Age, sex, and history of pregnancies and labor in female subcohort were collected. Patients with potential non-immune causes of refractoriness (splenomegaly, fever/sepsis, disseminated intravascular coagulation or other consumptive coagulopathy at the time of screening) were excluded from this study. The control group consisted of 22 untransfused healthy male donors. Female donors were not included in order to avoid confounding by pregnancy-related alloimmunization. Other AML patients who did not meet the platelet transfusion refractoriness criteria were not used as controls because of the previous exposure to platelet transfusions.

Antibody titers were measured by enzyme-linked immunosorbent assay (ELISA) before (up to 30 minutes before) and two hours after platelet transfusion for the following platelet antigens: GP IIb/IIIa, GP Ib/IX, and GP Ia/IIa. Post-transfusion samples were analyzed to evaluate potential immediate changes in circulating antibody levels following platelet exposure. The antiplatelet antibodies were measured using a PakAuto assay® (Immucor, Norcross, GA, USA), and the results are expressed as optical density (OD) values measured at 405 nm. To determine the presence of antibodies, the OD ratio (sample OD divided by the mean OD of the negative control) was used. In our study, a sample was considered positive if the OD value was equal to or greater than twice the mean OD of the negative control. The procedure was conducted in accordance with the manufacturer's instructions.

IBM SPSS Statistics, Version 23.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. The methods of descriptive and inferential statistics were used. For continuous variables with normal distribution, the mean ± standard deviation (SD) was used, while for those that did not follow a normal distribution, median and interquartile ranges were used. The frequencies are presented as relative (percentages) and absolute numbers. Histograms and the Shapiro–Wilk test were used to assess the normality. The Wilcoxon test was used for numerical paired variables, and the Mann–Whitney U test was used for numerical independent variables, with the level of significance of 5%. The Spearman rank-order correlation coefficient was used to examine the correlation between platelet count increment and initial antibody levels.

Ethics: The study protocol received ethical approval from the Ethics Committee of the University Clinical Center of Serbia (No. 1435/10, September 8, 2011).

RESULTS

During the study period 22 adult patients were included in the study (9 (40.91%) males). The mean age of the patients was 52.1 ± 10.8. Patients' demographic and clinical data are shown in Table 1.

Table 1. Patients' characteristics

Characteristics		Total (N = 22 pts)
Sex N (%)	Male	9 (40.91)
	Female	13 (59.09)
Age mean ± SD		52.1 ± 10.8
Platelet source N (%)	Buffy coat-derived	7 (31.82)
	Apheresis-derived	15 (68.18)
Pregnancy* N (%)		11 (84.61)
PC increment median (IQR)		10 (5–10)
PC increment N (%)	< 10 × 109/L	9 (40.91)
	10 × 109/L	13 (59.09)

N – number; pts – patients; IQR – interquartile range; SD – standard deviation; PC – platelet count
*applies only to female subjects

Table 2. Antibody titers before and after platelet transfusion

Target antigens	Time point	Total (N = 22 pts)	p*
GP IIb/IIIa median (IQR)	before	0.180 (0.121–0.268)	0.001
	after	0.369 (0.268–0.421)	
GP Ib/IX median (IQR)	before	0.180 (0.123–0.239)	0.001
	after	0.325 (0.260–0.370)	
GP Ia/IIa median (IQR)	before	0.145 (0.114–0.227)	0.001
	after	0.320 (0.248–0.397)	

N – number; pts – patients; GP – glycoprotein;
*Wilcoxon test; significance level of 5%

The initial antibody titers (before transfusion) to platelet antigens (GP IIb/IIIa, GP Ib/IX, and GP Ia/IIa) were significantly higher in all patients compared with healthy untransfused donors (Wilcoxon matched-pairs signed-rank test: Z = -3.408, p = 0.001).

Antibody titer values before and after platelet transfusion for the following platelet antigens: GP IIb/IIIa, GP Ib/IX, and GP Ia/IIa are shown in Table 2. In all three

Table 3. Anti-GP IIb/IIIa titers before and antibody titers' increment after platelet transfusion – differences among sexes, platelet source and levels of platelet increment

Parameters		Anti-GP IIb/IIIa Ab initial levels		Anti-GP IIb/IIIa Ab increment after-before PT	
		Median	p*	Median	p*
Sex	Male	0.180	0.624	0.189	0.462
	Female	0.182		0.147	
Platelet source	Buffy coat-derived	0.187	0.346	0.168	0.906
	Apheresis-derived	0.180		0.162	

GP – glycoprotein; Ab – antibody; PT – platelet transfusion;
*Mann–Whitney U test; significance level of 5%

Table 4. Anti-GP Ib/IX titers before and antibody titers' increment after platelet transfusion – differences among sexes, platelet source and levels of platelet increment

Parameters		Anti-GP Ib/IX Ab initial levels		Anti-GP Ib/IX Ab increment after-before PT	
		Median	p*	Median	p*
Sex	Male	0.179	0.540	0.153	0.129
	Female	0.180		0.106	
Platelet source	Buffy coat-derived	0.179	0.637	0.106	0.724
	Apheresis-derived	0.180		0.140	

GP – glycoprotein; Ab – antibody; PT – platelet transfusion
*Mann–Whitney U test; significance level of 5%

Table 5. Anti-GP Ia/IIa titers before and antibody titers' increment after platelet transfusion – differences among sexes, platelet source and levels of platelet increment

Parameters		Anti-GP Ia/IIa Ab initial levels		Anti-GP Ia/IIa Ab increment after-before PT	
		Median	p*	Median	p*
Sex	Male	0.210	0.713	0.119	0.902
	Female	0.130		0.150	
Platelet source	Buffy coat-derived	0.137	0.953	0.134	0.859
	Apheresis-derived	0.205		0.150	

GP – glycoprotein; Ab – antibody; PT – platelet transfusion
*Mann–Whitney U test; significance level of 5%

Table 6. Correlation between platelet count increment and initial levels of antiplatelet antibodies (Spearman rank-order correlation coefficient)

Parameters	Correlation coefficient	p
Anti-GP IIb/IIIa Ab initial levels	0.314	0.254
Anti-GP Ib/IX Ab initial levels	0.099	0.726
Anti-GP Ia/IIa Ab initial levels	0.243	0.383

GP – glycoprotein; Ab – antibody

groups of antibodies, the titers were significantly higher after platelet transfusion (0.180 *vs.* 0.369, 0.180 *vs.* 0.325, 0.145 *vs.* 0.320, respectively, p = 0.001).

No significant differences were observed in baseline antibody levels between male and female patients (anti-GP IIb/IIIa: p = 0.624, anti-GP Ib/IX: p = 0.540, anti-GP Ia/IIa: p = 0.713). Similarly, no differences were found between patients receiving buffy coat-derived *vs.* apheresis-derived platelet concentrates (anti-GP IIb/IIIa: p = 0.346, anti-GP Ib/IX: p = 0.637, anti-GP Ia/IIa: p = 0.953) (Tables 3, 4, and 5).

Changes in antibody titers were measured for all three groups and compared across sexes (anti-GP IIb/IIIa: p = 0.462, anti-GP Ib/IX: p = 0.129, anti-GP Ia/IIa: p = 0.902), and different types of transfusion products (anti-GP IIb/IIIa: p = 0.906, anti-GP Ib/IX: p = 0.724, anti-GP Ia/IIa: p = 0.859) with no differences found between the groups (Tables 3, 4, and 5).

No significant correlation between platelet count increment (median 10×10^9/L; IQR 5–10) and initial levels of antiplatelet antibodies was found (anti-GP IIb/IIIa Ab: p = 0.254; anti-GP Ib/IX Ab: p = 0.726; anti-GP Ia/IIa Ab: p = 0.383) (Table 6).

DISCUSSION

Platelet transfusion is an inevitable part of the treatment course of acute myeloid leukemia. Patients with acute myeloid leukemia often require chronic transfusion support, which presents an obstacle in the form of alloimmunization and consequential transfusion refractoriness. The presence of platelet refractoriness complicates the management of AML patients [12]. Additionally, AML patients with platelet refractoriness have shorter overall survival than those without [13]. Platelet-specific antibodies are common in patients with a history of possible previous immunization, particularly if patients were refractory to platelet transfusions [14]. In our study, we used healthy untransfused male donors as controls in order to avoid previous immunization caused by transfusions (frequent in AML patients) or pregnancies. Our results have shown that in all of our transfusion-refractory patients, levels of antiplatelet antibodies were significantly higher than those measured in controls. The prevalence of baseline autoantibodies was higher than in some previous studies [15, 16]. The reason for such a high prevalence of anti-HPA antibodies may be explained by the strict inclusion criteria. Moreover, acute leukemia patients are found to be alloimmunized more frequently than patients with other malignant hematological diagnoses [17]. On the other hand, this could be an explanation for the post-transfusion low platelet increment in our patients, bearing in mind that previous studies have shown that in post-transfusion purpura, antigen-negative transfusion recipients are sensitized against antigen-positive donor platelets, which leads to accelerated clearance of both donor and recipient platelets [18]. Interestingly, antibody titers were observed to be higher after the subsequent transfusion in all subjects. It should be noted that the observed increase in antibody levels two hours post-transfusion likely reflects redistribution or immediate immune complex formation, rather than de novo antibody production, which requires several days. However, our study did not observe any significant correlation between the increment in platelet count and the initial levels of antiplatelet antibodies.

Regarding post-transfusion platelet increment, previous studies have shown that single-donor platelets were not

superior to pooled-donor platelets [18]. Moreover, in our study, it has been found that the level of examined antiplatelet antibodies was not significantly different between patients who received buffy coat-derived and apheresis-derived platelets.

Comont et al. [19] showed that platelet transfusion refractoriness is more common among female subjects. In our study, there was no difference in titers between sexes.

We are aware of the limitations of this study. First and foremost, the small number of participants in the study may limit the accuracy of correlations and conclusions. Nevertheless, the observed differences compared with the control group and the effects of transfusion on antibody titers warrant further investigation in larger cohorts, and would potentially inform better transfusion protocols. Moreover, we have not taken into account the presence of anti-HLA antibodies, which could be an important component of the antiplatelet alloreactivity, and it is often noted that those who develop anti-HPA antibodies develop anti-HLA antibodies at the same time [20]. Additionally, we engaged male-only controls in order to avoid potential alloimmunization in the control group. Finally, clinically relevant factors associated with platelet transfusion, such as storage duration (age) of platelet concentrates, were not taken into account [21].

CONCLUSION

We showed the presence of antiplatelet antibodies in all examined platelet transfusion-refractory patients, which supports the hypothesis that antiplatelet antibodies may contribute to platelet transfusion refractoriness. However, no significant connection was found between antibody titers and subjects' sex and source of platelets. Future studies should include larger cohorts, assessment of anti-HLA antibodies, and genetic analysis of HPA polymorphisms to further clarify immune causes of platelet transfusion refractoriness.

Conflict of interest: None declared.

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Улога антитромбоцитних антитела у развоју рефрактарности на трансфузије тромбоцита код болесника са акутном мијелоидном леукемијом

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САЖЕТАК

Увод/Циљ Рефрактарност на трансфузије тромбоцита представља чест проблем у лечењу болесника са дијагнозом акутне мијелоидне леукемије (АМЛ), код којих је неопходна дуготрајна трансфузиона потпора. Основни циљ овог истраживања био је испитивање присуства и титра антитела на хумане тромбоцитне антигене *GP IIb/IIIa*, *GP Ib/IX* и *GP Ia/IIa* код болесника са АМЛ рефрактарних на трансфузије тромбоцита, у поређењу са здравим мушким, претходно нетрансфундованим контролним субјектима.

Методе Спроведена је проспективна студија у коју су укључена 22 пунолетна болесника са АМЛ код којих је доказана рефрактарност на трансфузије тромбоцита. Титар антитела на следеће тромбоцитне антигене: *GP IIb/IIIa*, *GP Ib/IX* и *GP Ia/IIa* процењиван је применом ензимског имуноесеја код ових болесника пре и два сата након трансфузије тромбоцита, као и код здравих мушких контролних субјеката.

Резултати Нивои антитела на тромбоцитне антигене из крви узорковане пре трансфузије тромбоцита били су статистички значајно виши код свих болесника у односу на здраве нетрансфундоване контролне субјекте ($p = 0,001$). У све три групе антитела титар је био значајно виши након трансфузије тромбоцита ($p = 0,001$). Није утврђена статистички значајна разлика у иницијалном титру антитела, као ни у његовом порасту након трансфузије, у односу на пол или извор тромбоцита (*buffy-coat* / аферезни) ($p > 0,05$). Није утврђена статистички значајна корелација између пораста броја тромбоцита (израженог у апсолутним бројевима) и иницијалног нивоа антитромбоцитних антитела.

Закључак Рефрактарност на трансфузије тромбоцита код болесника са АМЛ може бити посредована антителима на тромбоцитне антигене, имајући у виду да је титар ових антитела значајно виши код испитиваних болесника него код здравих, мушких нетрансфундованих контролних субјеката.

Кључне речи: рефрактарност на трансфузије тромбоцита; акутна мијелоидна леукемија; хумани тромбоцитни антигени; АМЛ; ХТА

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PRELIMINARY COMMUNICATION / ПРЕТХОДНА И КРАТКА САОПШТЕЊА

Prevalence and socio-demographic factors associated with self-medication with antibiotics in the municipality of Nikšić, Montenegro

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SUMMARY

Introduction/Objective Self-medication (SM) and self-initiated treatment of others (STO) with antibiotics contribute to antimicrobial resistance and represent a significant public health concern. This study aimed to assess their prevalence and association with socio-demographic factors within the primary healthcare setting of Nikšić, Montenegro.

Methods A cross-sectional study was conducted among 356 adults who visited a general practitioner or family medicine specialist at the Nikšić Primary Healthcare Center between March 1 and May 31, 2024. Data were collected through interviews using a structured questionnaire and analyzed using SPSS 25. Associations were assessed using chi-square and Fisher's exact tests, with Spearman's correlation used to assess relationship strength. Logistic regression analyses were performed to explore factors associated with SM and STO.

Results The prevalence of SM was 43%, while 16.6% of respondents reported STO. A significant positive association was found between these practices ($p < 0.001$). SM was more common among individuals aged 35–49, employed, and with higher education, while STO was more frequent among those living in family or community settings. In univariate analysis, age 50–64, higher education, and employment were associated with SM, while being retired was associated with STO; however, no variables remained significant in multivariate analysis.

Conclusion SM and STO are common and significantly associated behaviors in the studied population in Nikšić and may represent important public health challenges, including the development and spread of antimicrobial resistance, adverse health outcomes, and unnecessary costs. The absence of independent socio-demographic factors associated with these behaviors indicates a complex, multifactorial underlying structure, warranting further investigation.

Keywords: antibiotics; self-medication; socio-demographic factors; Montenegro

INTRODUCTION

Modern medicine – including surgical, invasive diagnostic, and transplant procedures – relies heavily on antibiotics. According to some authors, their widespread use has increased average human lifespan by approximately 23 years [1]. However, this success has been accompanied by the growing threat of antimicrobial resistance (AMR), now recognized as a major global public health challenge. The increase in antibiotic consumption worldwide, particularly in low- and middle-income countries, along with their irrational use in human medicine, agriculture, and trade [2, 3], is a key driver of AMR [4]. Multidrug-resistant microorganisms are estimated to cause around 1.2 million deaths annually, with projections rising to 10 million by 2050 [5].

In Montenegro, outpatient antibiotic use increased by 71% between 2000 and 2022, with self-medication (SM) identified as a contributing factor [6]. Compared to EU/EEA countries, the use of most Anatomical Therapeutic Chemical (ATC) subgroups was higher, with a statistically significant difference observed for cephalosporins [6, 7].

The World Health Organization defines SM as the selection and use of medicines (including herbal and traditional products) by individuals to treat self-recognized illnesses or symptoms [8]. In practice, this also includes the use of medicines without a prescription, as well as sharing medicines with family members or friends [9]. While acceptable for approved over-the-counter drugs used appropriately [10], antibiotic SM is problematic. It contributes to AMR, delays proper diagnosis and treatment, and increases the risk of adverse effects and drug interactions.

A 2025 systematic review of 71 studies (63,251 participants) reported a global SM prevalence ranging 0.65–92.2%, with a pooled estimate of 43% [11]. Prevalence was highest

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in sub-Saharan Africa (55.2%), followed by the Middle East and North Africa (48.3%), and Europe (34.7%). In Europe, lower rates were reported in northern countries such as Denmark (4.5%) and Sweden (0.43%) [12, 13], while higher rates were observed in Southern and Eastern Europe, ranging from 18.9% in Portugal [14] to over 40% in Bulgaria and Greece [15, 16]. Common reasons for SM include perceived knowledge, previous experience, mild symptoms, and easy access to antibiotics [11].

Antibiotics are often used for conditions where they are not indicated, such as cough, common cold, fever, headache, or dizziness [17, 18]. Inappropriate practices – such as early discontinuation, incorrect dosing, and sharing antibiotics – further worsen outcomes [16].

Determinants of SM operate at patient, healthcare professional, and system levels [19]. According to Lescure et al. (2018), patient-level factors include socio-demographic characteristics (e.g., age, sex, education), treatment-related factors (e.g., lack of knowledge, storing antibiotics at home), and health- and disease-related factors (e.g., presence of chronic illness) [19]. Understanding these determinants is essential for designing effective public health interventions.

This study aimed to assess the prevalence and socio-demographic factors associated with SM and self-initiated treatment of others (STO) with antibiotics in a primary healthcare setting in Nikšić, Montenegro.

METHODS

Study setting and design

This cross-sectional, questionnaire-based descriptive study was conducted in the municipality of Nikšić, the second largest municipality in Montenegro. According to the recent 2023 census, the municipality had a population of 65,705, which is about 10% of the total population in the country [20]. Data were collected between March 1 and May 31, 2024, in the Nikšić Primary Healthcare Center Public Health Institution.

Sample

A convenience sample of 356 participants who visited a general practitioner or family medicine specialist was recruited. Inclusion criteria were age ≥ 18 years, ability to understand the questions and provide clear responses, and signed informed consent.

Although the sample was not strictly random with respect to the general population, it reflects individuals seeking healthcare services. Based on the total population of Nikšić (65,705) and the proportion of adults (78.45%), the estimated adult population is approximately 51,500 [20]. Using national data indicating that around 3% of citizens use antibiotics daily [21], and adjusting for a three-month period, it is estimated that between 1,500 and 2,500 adults received antibiotic therapy in this setting. With a sample size of 356, the margin of error at the 95% confidence level is approximately ± 4.5% to ± 4.8%, supporting the statistical reliability of the findings for the defined population of healthcare users, although the findings primarily apply to healthcare-seeking individuals.

Data collection procedures

The questionnaire was created based on high-quality studies on similar topics published to date, including questions that we considered relevant to our conditions [22, 23, 24].

The questionnaire had two parts. Part one included socio-demographic characteristics such as sex, age, place of residence, educational level, employment status, living arrangement, and marital status. Part two included questions to assess SM and STO with antibiotics ("Do you use antibiotics at your own discretion, without a doctor's prescription?" and "Do you administer antibiotics to other people at your own discretion, without a doctor's prescription?"). The answers offered to both questions were: "No, never," "Yes, occasionally," "Yes, often," and "Yes, whenever I think it is necessary."

The data were collected through face-to-face interviews conducted by two previously trained medical doctors, during their mandatory internship at the Nikšić Primary Healthcare Center.

Statistical analysis

Data were analyzed using IBM SPSS Statistics, Version 25.0 (IBM Corp., Armonk, NY, USA). Both descriptive and inferential statistical methods were applied. Descriptive statistics were used to present the prevalence of SM and STO, as well as participants' socio-demographic characteristics.

Associations between SM and STO were assessed using the chi-square (χ^2) test, while the χ^2 test for linear trend (linear-by-linear association) was used to examine trend relationships. The strength and direction of associations were additionally evaluated using Spearman's rank correlation coefficient. Fisher's exact test was applied when expected cell counts were fewer than 5.

Associations between SM, STO, and socio-demographic variables were examined using the χ^2 test, while Fisher's exact test was used when assumptions for the χ^2 test were not met.

SM and STO were treated as dependent variables in separate analyses, while socio-demographic characteristics (sex, age, education, employment status, place of residence, living arrangement, and marital status) were included as independent variables.

Univariate logistic regression analysis was performed to assess the association between each independent variable and the outcomes (SM and STO), with odds ratios (OR) and 95% confidence intervals (CIs) reported.

Variables that were statistically significant ($p < 0.05$) or showed borderline significance ($p < 0.1$) in univariate analysis were included in the multivariate logistic regression model to identify independent predictors while controlling for potential confounders. Model fit was assessed using the Nagelkerke R^2 coefficient. Statistical significance was set at $p < 0.05$.

Ethics: The study was approved by the Nikšić Primary Healthcare Center PHI Ethics Committee (Protocol No. 883, February 27, 2024).

RESULTS

SM with antibiotics was reported by 43% of participants, and 16.6% reported STO (Table 1).

A statistically significant association between SM and STO was observed ($\chi^2 = 95.369$, df = 9, $p < 0.001$). A χ^2 test for linear-by-linear association indicated a significant positive trend between increasing frequency of SM and STO ($p < 0.001$). Fisher's exact test confirmed the statistical significance of the association ($p < 0.001$). Additionally, Spearman's rank correlation confirmed a moderate positive association between these variables ($\rho = 0.343$, $p < 0.001$) (Table 2).

SM was significantly associated with age ($p = 0.008$), employment status ($p = 0.003$), and education level ($p = 0.026$, Fisher's exact test). The highest prevalence of SM was observed among participants aged 35–49 years (56.5%), employed individuals (53%), and those with higher education (53.8%).

Although SM was more frequent among women (44.1%) and urban residents (44.4%) compared to men (40.7%) and rural residents (34.6%), these differences were not statistically significant.

Table 1. Frequency of self-medication (SM) and self-initiated treatment of others (STO) with antibiotics

Frequency	SM		STO	
	N	%	N	%
"No, never"	203	57	297	83.4
"Yes, occasionally"	119	33.4	46	12.9
"Yes, often"	33	9.3	9	2.5
"Yes, whenever I think it is necessary"	1	0.3	4	1.1
Total	356	100	356	100

Table 2. Association between self-medication (SM) and self-initiated treatment of others (STO) with antibiotics

SM/STO	No, never	Occasionally	Often	Whenever	Total
No, never	190 (93.6)	11 (5.4)	2 (1)	0 (0)	203 (100)
Occasionally	88 (73.9)	27 (22.7)	1 (0.8)	3 (2.5)	119 (100)
Often	19 (57.6)	8 (24.2)	5 (15.2)	1 (3)	33 (100)
Whenever	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)
Total	297 (83.4)	46 (12.9)	9 (2.5)	4 (1.1)	356 (100)

Table 3. Distribution of self-medication (SM) and self-initiated treatment of others (STO) with antibiotics across socio-demographic characteristics

Socio-demographic categories	Total N (%)	SM			STO		
		Yes (%)	No (%)	p-value	Yes (%)	No (%)	p-value
Sex							
Male	118 (33.1)	48 (40.7)	70 (59.3)	0.537	14 (11.9)	104 (88.1)	0.093
Female	238 (66.9)	105 (44.1)	133 (55.9)		45 (18.9)	193 (81.1)	
Age, years							
18–34	105 (29.5)	49 (46.7)	56 (53.3)	0.008*	19 (18.1)	86 (81.9)	0.430
35–49	69 (19.4)	39 (56.5)	30 (43.5)		14 (20.3)	55 (79.7)	
50–64	93 (26.1)	40 (43)	53 (57)		17 (18.3)	76 (81.7)	
65–79	73 (20.5)	21 (28.8)	52 (71.2)		7 (9.6)	66 (90.4)	
> 80	16 (4.5)	4 (25)	12 (75)		2 (12.5)	14 (87.5)	
Place of residence							
Urban	304 (85.4)	135 (44.4)	169 (55.6)	0.187	52 (17.1)	252 (82.9)	0.514
Rural	52 (14.6)	18 (34.6)	34 (65.4)		7 (13.5)	45 (86.5)	
Education							
No formal education	5 (1.4)	0 (0)	5 (100)	0.026*	0 (0)	5 (100)	0.338
Primary school	52 (14.6)	18 (34.6)	34 (65.4)		8 (15.4)	44 (84.6)	
Secondary school	221 (62.1)	93 (42.1)	128 (57.9)		33 (14.9)	188 (85.1)	
Faculty/college	78 (21.9)	42 (53.8)	36 (46.2)		18 (23.1)	60 (76.9)	
Employment							
Employed	160 (44.9)	84 (53)	76 (47.5)	0.003*	31 (19.4)	129 (80.6)	0.086
Unemployed	93 (26.1)	39 (41.9)	54 (58.1)		19 (20.4)	74 (79.6)	
Retired	86 (24.2)	26 (30.2)	60 (69.8)		8 (9.3)	78 (90.7)	
Other	17 (4.8)	4 (23.5)	13 (76.5)		1 (5.9)	16 (94.1)	
Living arrangement							
In a family / community setting	310 (86.5)	135 (43.5)	175 (56.5)	0.572	56 (18.1)	254 (81.9)	0.049*
Alone	46 (13.5)	18 (39.1)	28 (60.9)		3 (6.5)	43 (93.5)	
Marital status							
Single	115 (32.3)	51 (44.3)	64 (55.7)	0.106	19 (16.5)	96 (83.5)	0.884
Married / in partnership	194 (54.5)	84 (43.3)	110 (56.7)		34 (17.5)	160 (82.5)	
Divorced	6 (1.7)	5 (83.3)	1 (16.7)		0 (0)	6 (100)	
Widowed	41 (11.5)	13 (31.7)	28 (68.3)		6 (14.6)	35 (85.4)	

*Statistically significant ($p < 0.05$);
Fisher's exact test was used when expected cell counts were < 5.

STO with antibiotics was significantly associated with living arrangement (p = 0.049), with higher prevalence observed among individuals living in family or community settings (18.1%) compared to those living alone (6.5%). No statistically significant differences were found according to sex, age, education, employment, or place of residence (Table 3).

In univariate logistic regression analysis, age, education, and employment status were significantly associated with SM with antibiotics. Participants aged 50–64 years were more likely to report SM compared to those aged 18–34 years (OR = 3.9, 95% CI: 1.14–13.31, p = 0.030). Higher education was associated with lower odds of SM (OR = 0.4, 95% CI: 0.19–0.81, p = 0.011), while employed individuals had higher odds compared to unemployed participants (OR = 2.55, 95% CI: 1.46–4.44, p = 0.001). No significant associations were observed for sex, place of residence, living arrangement, or marital status (Table 4).

Table 4. Univariate logistic regression with self-medication (SM) with antibiotics as a dependent variable and socio-demographic characteristics as independent variables

Variable	OR (95% CI)	p-value
Sex		
Female *vs.* male	0.87 (0.56–1.36)	0.537
Age (years)		
35–49 *vs.* 18–34	2.63 (0.8–8.67)	0.113
50–64 *vs.* 18–34	3.9 (1.14–13.31)	0.030*
65–79 *vs.* 18–34	2.26 (0.68–7.55)	0.183
≥ 80 *vs.* 18–34	1.21 (0.35–4.19)	0.762
Education		
Secondary *vs.* low	0.62 (0.37–1.05)	0.074
Higher *vs.* low	0.4 (0.19–0.81)	0.011*
Employment		
Employed *vs.* unemployed	2.55 (1.46–4.44)	0.001*
Retired *vs.* unemployed	1.48 (0.81–2.7)	0.199
Residence		
Urban *vs.* rural	0.66 (0.36–1.23)	0.189
Living arrangement		
Family *vs.* alone	0.83 (0.44–1.57)	0.573
Marital status		
Single *vs.* married	1.23 (0.64–2.36)	0.534
Other *vs.* married	1.28 (0.64–2.57)	0.480

*p < 0.05 was considered statistically significant

Table 5. Multivariate logistic regression with self-medication (SM) with antibiotics as a dependent variable and relevant socio-demographic characteristics as independent variables

Variable	Adjusted OR (95% CI)	p-value
Age (years)		
35–49 *vs.* 18–34	2.41 (0.58–10.01)	0.228
50–64 *vs.* 18–34	3.06 (0.7–13.36)	0.137
65–79 *vs.* 18–34	2 (0.5–7.95)	0.327
≥ 80 *vs.* 18–34	1.19 (0.34–4.13)	0.781
Education		
Secondary *vs.* low	0.83 (0.47–1.46)	0.511
Higher *vs.* low	0.68 (0.3–1.52)	0.342
Employment		
Employed *vs.* unemployed	1.17 (0.44–3.12)	0.751
Retired *vs.* unemployed	0.84 (0.33–2.18)	0.725

In multivariate logistic regression analysis, none of the examined variables remained independently associated with SM. Although the overall model was statistically significant (χ^2 = 17.749, p = 0.023), individual socio-demographic factors including age, education, and employment were not significant after adjustment. This suggests that the associations observed in univariate analysis may be confounded by other variables. The model explained a small proportion of variance (Nagelkerke R^2 = 0.065) (Table 5).

Regarding STO with antibiotics (Table 6), female sex (OR = 0.58, p = 0.095) and living in a family/community setting (OR = 0.32, p = 0.061) showed borderline associations, although neither reached statistical significance. Additionally, being retired was significantly associated with higher odds of STO compared to unemployed individuals (OR = 2.34, p = 0.043).

Multivariate logistic regression analysis showed that none of the examined variables were independently associated with STO. However, female sex (OR = 0.57, p = 0.088), living in a family/community setting (OR = 0.36, p = 0.102), and being retired (OR = 2.13, p = 0.078) showed borderline associations with STO with antibiotics (Table 7).

Table 6. Univariate logistic regression with self-initiated treatment of others (STO) with antibiotics as a dependent variable and socio-demographic characteristics as independent variables

Variable	OR (95% CI)	p-value
Sex		
Female *vs.* male	0.58 (0.3–1.1)	0.095
Age (years)		
35–49 *vs.* 18–34	1.78 (0.36–8.77)	0.477
50–64 *vs.* 18–34	1.57 (0.33–7.54)	0.576
65–79 *vs.* 18–34	1.57 (0.33–7.54)	0.576
≥ 80 *vs.* 18–34	0.74 (0.14–3.96)	0.727
Education		
Secondary *vs.* low	0.59 (0.31–1.11)	0.103
Higher *vs.* low	0.54 (0.22–1.36)	0.192
Employment		
Employed *vs.* unemployed	2.17 (0.9–5.19)	0.083
Retired *vs.* unemployed	2.34 (1.03–5.36)	0.043*
Residence		
Urban *vs.* rural	0.75 (0.32–1.77)	0.515
Living arrangement		
Family *vs.* alone	0.32 (0.1–1.06)	0.061
Marital status		
Single *vs.* married	1.45 (0.57–3.69)	0.433
Other *vs.* married	1.35 (0.5–3.63)	0.549

*p < 0.05 was considered statistically significant

Table 7. Multivariate logistic regression analysis of factors associated with self-initiated treatment of others (STO) with antibiotics

Variable	OR (95% CI)	p-value
Sex		
Female *vs.* male	0.57 (0.29–1.09)	0.088
Living arrangement		
Family *vs.* alone	0.36 (0.11–1.22)	0.102
Employment status		
Employed *vs.* unemployed	1.82 (0.75–4.43)	0.185
Retired *vs.* unemployed	2.13 (0.92–4.93)	0.078

DISCUSSION

This study showed a high prevalence of SM (43%) and STO (16.6%) with antibiotics. To our knowledge, this is the first study demonstrating a significant positive association between these behaviors, suggesting they tend to co-occur. These findings should be considered in future public health interventions aimed at addressing both simultaneously. Although age, education, employment, and living arrangements were associated with SM and STO in univariate analyses, none remained significant in multivariate models, indicating a complex, multifactorial background.

The high prevalence of SM among primary healthcare users in Nikšić may be explained by limited awareness of risks (e.g., adverse effects, interactions, AMR), time constraints, perceived knowledge of symptoms and antibiotics, prior positive experience, mild symptom perception, as well as insufficient control of over-the-counter antibiotic sales in Montenegro.

Interestingly, the 43% SM rate in our study is comparable to the global pooled prevalence reported in a recent meta-analysis [11] and earlier findings from EU countries such as Bulgaria (43%) and Greece (44.6%) [15, 16]. In Montenegro, a study from Podgorica reported that 61% of respondents used antibiotics in the previous year, but only 44.1% had a prescription [17]. Similar patterns are reported in Serbia (47.2% lifetime SM) [25] and Albania (53%) [26], highlighting a regional public health concern requiring urgent action.

This study is also the first to show a statistically significant positive association between SM and STO, suggesting a behavioral continuum where individuals who self-medicate are more likely to administer antibiotics to others. In our study, STO refers to self-initiated administration of antibiotics to both adults and children. However, due to the lack of comparable studies, direct comparison is limited. However, a systematic review reported that about 24% of parents in Italy self-medicated their children with antibiotics [27]. It may be assumed that children are often exposed to antibiotic SM in our setting, but this should be examined in future studies.

Socio-demographic trends suggest higher SM among middle-aged, employed, and more educated individuals. Similar findings were reported in China [28]. Our results can be explained by time constraints, perceived knowledge about symptoms and antibiotics, and easier access to information. The availability of antibiotics without prescription in our country may further reinforce these behaviors [17]. However, these associations were not confirmed in multivariate analysis, supporting the view that SM is multifactorial [19, 29].

STO may be influenced by close social relationships and caregiving roles. Previous research in Montenegro showed that 24.6% of parents self-medicate their children. Specific maternal factors that independently increased the probability of SM were higher education, working in a health profession, and smoking [25]. Retired individuals were associated with STO in univariate analysis, possibly reflecting caregiving roles and prior experience. In the USA, 65% of older adults reported keeping leftover antibiotics [30], increasing the risk of their inappropriate use and misuse. This topic should also be further investigated in our setting.

Although not significant in multivariate analysis, these patterns suggest underlying behavioral mechanisms shaped by contextual factors, which require further investigation in larger samples.

This study has several limitations. Causality cannot be established due to its cross-sectional design, and the sample – limited to primary healthcare patients – reduces generalizability. Some subgroups were small, potentially affecting statistical power. The questionnaire did not capture detailed information on antibiotic types, sources, or reasons for SM and STO. Finally, self-reported data may be subject to bias.

CONCLUSION

Self-medication and STO with antibiotics are highly prevalent in the investigated population. A significant positive association was found between these two behaviors for the first time, indicating that individuals who practice SM are also more likely to treat others with antibiotics on their own initiative. These findings should be considered in the development of future public health interventions aimed at simultaneously addressing and reducing these negative behavioral patterns.

Socio-demographic factors such as age, education, employment, and living arrangements were associated with these behaviors in univariate analyses, but none remained significant in multivariate analysis, suggesting a complex and multifactorial pattern that should be further investigated in a larger sample by exploring behavioral, cultural, and systemic drivers.

Conflict of interest: None declared.

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Преваленција и социодемографски фактори повезани са самолечењем антибиотицима у општини Никшић, Црна Гора

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САЖЕТАК

Увод/Циљ Самолечење (СЛ) и самоиницијативно лечење других (СЛД) антибиотицима доприносе антимикробној резистенцији и представљају значајан јавноздравствени проблем. Циљ овог истраживања био је да се процене њихова учесталост и повезаност са социодемографским факторима на нивоу примарне здравствене заштите у Никшићу, Црна Гора.

Методе Спроведена је студија пресека на узорку од 356 одраслих испитаника који су посетили доктора медицине или специјалисту породичне медицине у Дому здравља Никшић у периоду од 1. марта до 31. маја 2024. године. Подаци су прикупљени путем интервјуа коришћењем структурираног упитника и анализирани у програму *SPSS 25*. Повезаности су испитиване хи-квадрат и Фишеровим тестом, док је Спирманова корелација коришћена за процену јачине односа. Логистичка регресија је примењена за испитивање фактора повезаних са СЛ и СЛД.

Резултати Учесталост СЛ износила је 43%, док је 16,6% испитаника пријавило СЛД. Утврђена је значајна позитивна повезаност између ова два обрасца понашања ($p < 0,001$). СЛ је било чешће међу особама старости 35–49 година, запосленима и онима са вишим образовањем, док је СЛД било чешће код особа које живе у породичном домаћинству или заједници. У униваријантној анализи, узраст 50–64 године, више образовање и запосленост били су повезани са СЛ, а пензионерски статус са СЛД; међутим, ниједан фактор није остао значајан у мултиваријантној анализи.

Закључак СЛ и СЛД су чести и значајно повезани обрасци понашања у испитиваној популацији у Никшићу и могу представљати важне јавноздравствене изазове, укључујући настанак и ширење антимикробне резистенције, неповољне здравствене исходе и непотребне трошкове. Одсуство независних социодемографских фактора повезаних са овим понашањима указује на сложену, мултифакторску основну структуру, што захтева даља истраживања.

Кључне речи: антибиотици; самолечење; социодемографски фактори; Црна Гора

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Comprehensive treatment of *dens invaginatus* type IIIB

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SUMMARY

Introduction *Dens invaginatus* (DI) is a rare developmental anomaly characterized by invagination of the enamel organ into the dental papilla, creating complex internal anatomy. Type III DI, according to Oehlers' classification, presents diagnostic and therapeutic challenges, especially in teeth with incomplete root development. Early diagnosis and proper management are crucial to prevent pulpal and periodontal complications, preserve tooth vitality, function and aesthetics. The aim of this report is to describe the long-term interdisciplinary management of a rare DI type IIIB, highlighting diagnostic challenges, treatment strategies, successful tooth preservation, and importance of long-time controls.

Case outline A maxillary lateral permanent incisor with DI type IIIB in a nine-year-old boy presented with peri-invagination periodontitis while maintaining pulp vitality and incomplete root formation. A staged, minimally invasive approach was adopted, initially focusing on periodontal surgery and regenerative therapy to control infection while preserving pulp vitality and allowing continued root development. After more than two years of successful vitality maintenance, irreversible pulpitis developed, requiring complex orthograde root canal treatment followed by apical surgery. Long-term follow-up exceeding nine years demonstrated complete periapical healing, stable periodontal conditions, functional integrity, and retention of the tooth in the dental arch.

Conclusion Early diagnosis of DI is essential, as type III cases pose major challenges to pulp preservation and root canal treatment and are often considered for extraction. This case shows that long-term tooth preservation is achievable despite complex anatomy using an individualized, interdisciplinary approach.

Keywords: *dens invaginatus*; immature permanent tooth; interdisciplinary management

INTRODUCTION

Dens invaginatus (DI) is a developmental anomaly characterized by invagination of the enamel organ into the dental papilla prior to calcification [1]. The extent of invagination and pulp chamber anatomy varies considerably, with Oehlers' classification – types I to III – being the most widely used [2].

According to this classification, type I is a minor, enamel-lined invagination restricted to the crown and not extending beyond the cementoenamel junction [2]. Type II extends into the root but remains confined within it as a blind sac. Type III is the most severe form, in which the invagination extends through the root, creating a pseudo-foramen that opens either apically (type IIIB) or laterally (type IIIA) into the periodontal ligament. In type III cases, the apical portion of the invagination is often lined with cementum [2].

Prevalence ranges from 0.25–10% in full-mouth surveys [1] and up to 26.1% in specific populations [3]. The maxillary lateral permanent incisor is most commonly affected, accounting for 86% of cases [4]. Although its etiology is not fully understood, genetic factors are considered likely [5].

The abnormal anatomy predisposes affected teeth to caries, pulpal and periodontal inflammation, posing significant challenges for endodontic treatment, particularly in immature teeth with incomplete root formation [1, 2]. Management focuses on preserving pulp vitality to allow continued root development or maintaining symptom-free, adequately treated non-vital teeth, with extraction reserved for untreatable or severely compromised cases [4, 6].

The aim of this report is to present the long-term interdisciplinary management of a rare DI type IIIB (Oehlers), initially diagnosed with peri-invagination periodontitis and a vital pulp, emphasizing diagnostic challenges, treatment strategies, and the successful preservation of function and aesthetics.

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CASE OUTLINE

Clinical and radiographic description

A nine-year-old boy was referred to the Department of Pediatrics and Preventive Dentistry of the University Medical Centre Ljubljana due to the signs of inflammation in the area of erupting maxillary right lateral permanent incisor (the tooth 12). There was no history of trauma or dental caries. His parents reported a mild pain in this area, accompanied by oedema, which ceased nine days ago after antibiotic therapy (500 mg amoxicillin with 125 mg clavulanic acid, twice per day for five days). The antibiotic regimen was prescribed by the referring physician prior to the patient's presentation at our department. The choice of this broad-spectrum antibiotic is consistent with the management of acute odontogenic infections in pediatric patients, particularly when a mixed aerobic-anaerobic flora is suspected.

Clinical examination revealed healthy mixed dentition and healthy oral soft tissue, except red gingiva on the buccal side, associated with the tooth 12, with remnants of inactive sinus tract (Figure 1a). The crown of tooth 12 was yellowish and exhibited an unusual morphology. Enlarged palatal cingula, a disto-palatal groove, and a deep mesio-palatal radicular groove on each side of the cingula were noticed; however, invagination entrance was hardly visible. The patient's oral hygiene was good, with tooth surfaces free of microbial plaque, but abundant supragingival and subgingival calculus was present. There was no swelling of the surrounding mucosa, and no tenderness to palpation. The tooth was not sensitive to percussion nor pathologically mobile. Gentle periodontal probing revealed loss of attachment on the distobuccal aspect, with a probing pocket depth of 5 mm on the not fully erupted tooth crown. The dental pulp vitality of tooth 12 was confirmed using the electric pulp test and cold test. The periapical radiograph revealed a cylindrical root that was slightly widened apically. The coronal half of the invagination canal was very narrow and lined with a thin layer of enamel, while in the apical half, the invagination canal expanded dramatically and opened widely into the periradicular tissues, giving a bell-shaped appearance. The dental pulp was compressed to the sides, encircling the bell-shaped invagination. A wide-open pulpo-periodontal communication was observed. Periapically, a diffuse bony radiolucency was present. Based on these findings, tooth 12 was diagnosed as DI type IIIB according to Oehlers' classification [2] (Figure 1b).

Treatment summary

The primary source of infection was determined to be periodontal (peri-invagination periodontitis), as direct communication between the invagination and the periodontal ligament facilitated bacterial contamination from the oral environment. Periodontal surgery was performed to treat peri-invagination periodontitis and prevent apical bacterial spread, aiming to preserve pulp vitality, in accordance with the principles of periodontal regenerative therapy. Following removal of hard and soft deposits using an ultrasonic scaler, a full-thickness mucoperiosteal flap was elevated. Granulation tissue and significant cortical bone loss were observed (Figure 1c, d); however, the apical region was left untouched to avoid pulp devitalization. The bone defect was irrigated with saline, and the exposed root surface was treated with 24% EDTA gel (PrefGel, Straumann, Basel, Switzerland) for two minutes, rinsed with saline and dried. Enamel matrix derivative gel (Emdogain, Straumann) was applied to stimulate new bone and connective tissue formation. The flap was sutured with resorbable sutures (Safil Quick 4/0, B. Braun Melsungen AG, Melsungen, Germany), and crown fissures were sealed with glass-ionomer cement – GIC (Fuji Triage Pink, GC Dental Products, Luzern, Switzerland). A week later, the crown was additionally sealed with resin (Helioseal F, Ivoclar Vivadent, Schaan, Liechtenstein). Initial healing was uneventful. The tooth remained vital and asymptomatic at one-, three-, and six-month follow-ups.

The initial decision to perform periodontal surgery but no endodontic treatment was based on the fact that the tooth was vital and primary pathology was peri-invagination periodontitis, not an endodontic infection. The goals were to eliminate the periodontal inflammatory focus, promote bone regeneration, and preserve pulp vitality to allow continuing root development in this immature tooth.

At 12 months, the tooth was vital and asymptomatic with continued root formation confirmed on periapical image. Nevertheless, a new palatal sinus tract appeared, prompting additional surgery. A palatal mucoperiosteal flap was raised, granulation tissue excised, and the area irrigated. At 18-, 24- (Figure 1e), and 30-month (Figure 1g) evaluations, the tooth remained vital and symptom-free. Periapical radiographs showed continued root formation and attenuation of periapical radiolucency (Figures 1f and h), although a sinus tract intermittently reappeared at the mucogingival junction.

At 33 months, despite vitality and lack of symptoms, the invagination canal was treated under the operating microscope (OPMI PICO, Carl Zeiss Meditec AG, Jena, Germany), with magnification up to 20×, coaxial halogen illumination, and a 250 mm working distance. The resin composite was removed from the palatal surface (Figure 2a and b), and the invagination entrance in the enlarged palatal cingulum was exposed and enlarged (Figure 2c). The canal was instrumented with hand files up to ISO 40, irrigated with 2.5% NaOCl and 17% EDTA gel (Straumann), and medicated with calcium hydroxide paste (Calasept, Nordiska Dental AB, Helsingborg, Sweden). The entrance was sealed with GIC (GC Dental Products).

At one- and three-month follow-ups, the tooth remained vital, but the sinus tract persisted. The persistence of the sinus tract after initial surgery was due to the inability to completely seal the invagination and eliminate the bacterial reservoir, which eventually led to pulp involvement. Five months later, the patient presented with cold sensitivity and episodes of spontaneous pain. Vitality testing indicated irreversible pulpitis. Cone beam computed

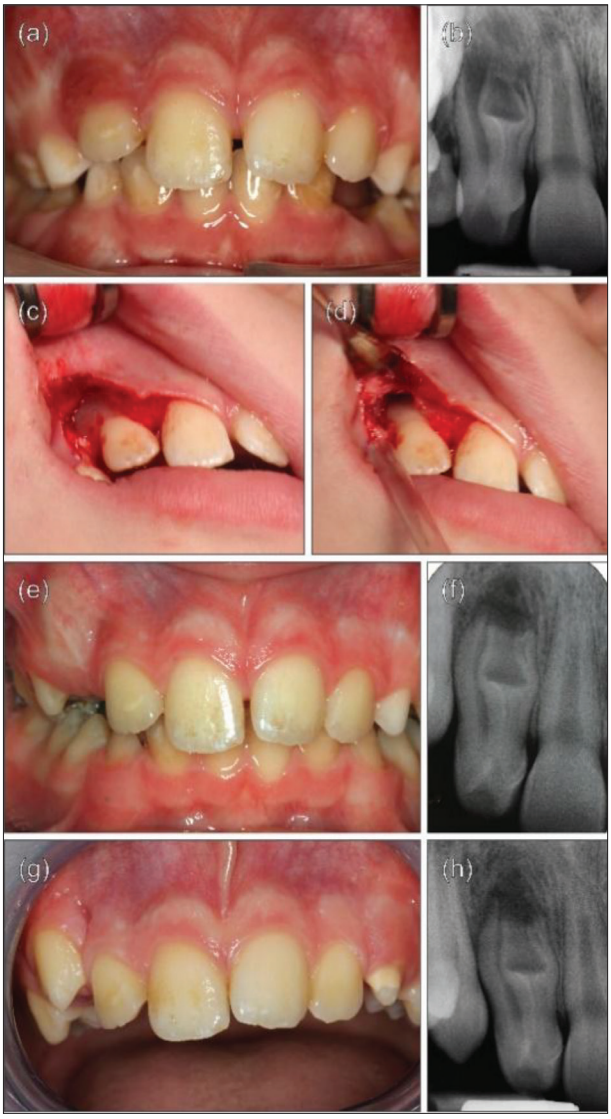

Figure 1. Preoperative clinical examination (a) and radiograph (b) showing an erupting maxillary right lateral incisor with type III *dens invaginatus*; a large bony defect identified during flap surgery was carefully debrided (c, d); clinical and radiographic views of the vital and asymptomatic maxillary lateral incisor at the 24-month (e, f); 30-month (g, h) follow-up examinations

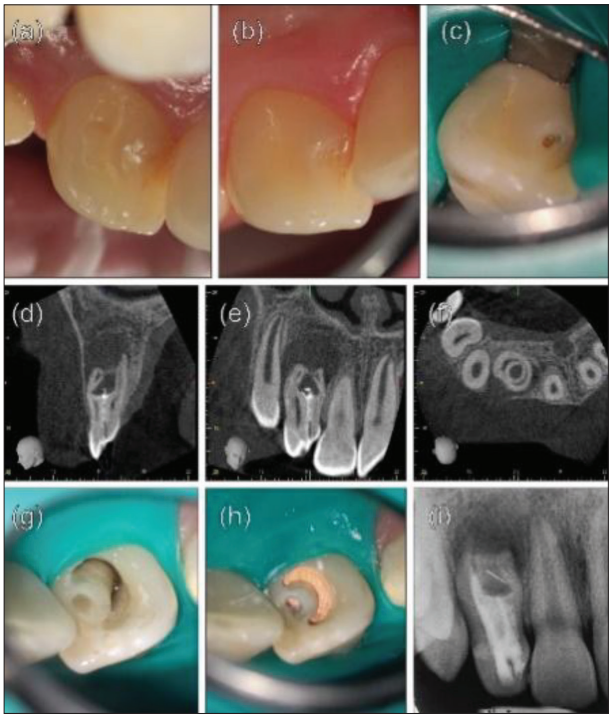

Figure 2. Tooth 12 with a sealant placed on the palatal surface (a); after removal of the resin composite and preparation using a small carbide bur and a scaler, the entrance of the invagination became evident (b); the invagination entrance was then slightly enlarged (c); cone beam computed tomography images of the maxillary right lateral incisor showing the invaginated developmental canal on sagittal (d); frontal (e); and axial (f) cross-sectional views; entry of the invagination canal with a surrounding semicircular root canal orifice (g); both canal systems filled with gutta-percha (h); a periapical radiograph shows the root canal obturation (i): the distal canal is filled up to the apical constriction, whereas the palatal and buccal canals are filled only up to the "bending point;" a fractured ultrasonic file is visible in the apical portion of the invagination

tomography analysis (Figure 2d, e, and f) was followed by root canal treatment (RCT) under rubber dam and magnification. After local anesthesia (Scandonest 2% L, Septodont, Saint-Maur-des-Fossés, France), semi-circular access cavities to the pulp and invagination were made (Figure 2g). Cleaning included ultrasonic tips, XP-Endo rotary file (FKG Dentaire, La Chaux-de-Fonds, Switzerland), and irrigation with 5% NaOCl. To address the complex internal anatomy in DI type III, 5% sodium hypochlorite was used. This choice was necessary because necrotic pulp tissue and microbial biofilm reside in narrow, irregular spaces that are very difficult, and sometimes impossible, to access mechanically. Therefore, a higher concentration of NaOCl provides enhanced tissue-dissolving and antimicrobial efficacy. In addition, 17% EDTA (Straumann) was used to remove the smear layer, thereby allowing deeper penetration of the irrigants. Calcium hydroxide was placed and sealed with GIC (GC Dental Products) and Cavit W (3M Deutschland GmbH, Neuss, Germany).

Two weeks later, canals were obturated with TotalFill BC Sealer (Brasseler USA, Savannah, GA, USA) using single-cone and warm vertical gutta-percha (Figure 2h). A temporary seal was placed, and postoperative radiograph was taken (Figure 2i). Final resin composite restorations were placed at the next visit.

Periapical surgery was performed one month later due to persistent sinus tract. A 1 mm apicoectomy was done, canals were prepared with ultrasonic tips (ISO 25), and retrograde filling was completed using ProRoot MTA White (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA). Radiographs confirmed obturation (Figure 3a). After one week, healing was satisfactory and the patient remained asymptomatic.

Follow-up

At the follow-up visits six, 12, 18 months (Figure 3c, d) and nine years and five months after orthograde and retrograde treatment, the tooth 12 was asymptomatic, and with no sinus tract. Objective criteria confirming treatment success were as follows: (1) absence of clinical symptoms (pain, swelling, sinus tract), (2) normal periodontal

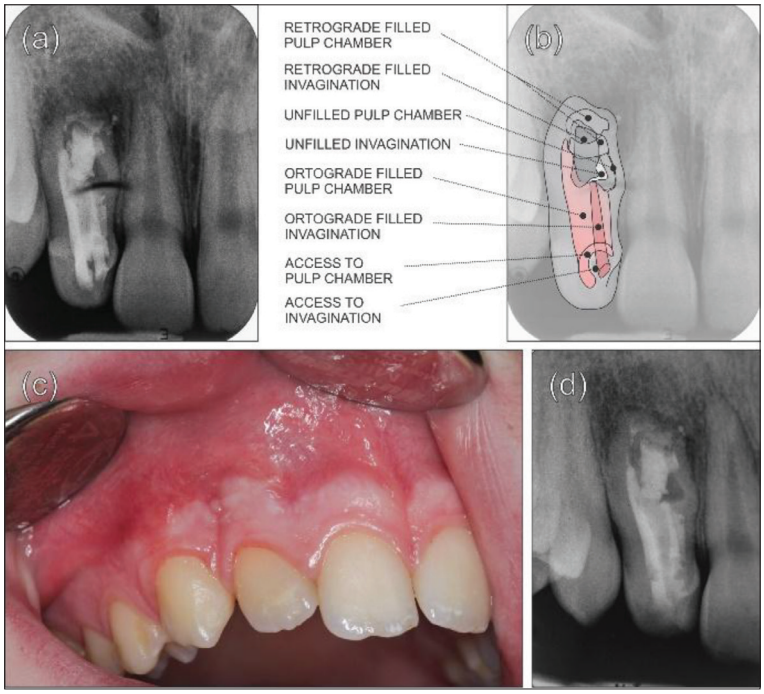

Figure 3. Periapical radiograph obtained after periapical surgery (a) with a schematic representation of the retrograde and orthograde obturation (b); clinical and radiographic views of the maxillary lateral incisor at the 18-month follow-up after periapical surgery (c, d)

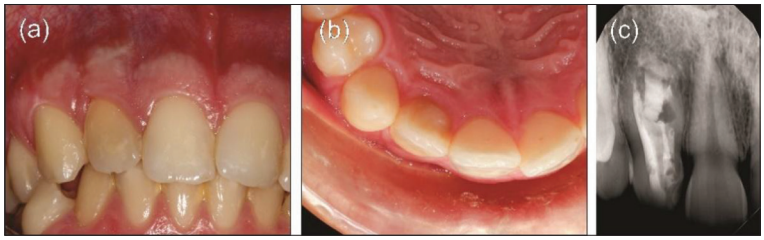

Figure 4. Clinical (a, b) and radiographic (c) views of the maxillary lateral incisor at the follow-up examination nine years and five months after periapical surgery; the tooth is functionally and aesthetically well positioned in the dental arch

probing depths and absence of pathological mobility, (3) normal response to vitality testing of adjacent teeth, and (4) radiographic evidence of complete periapical healing with a continuous lamina dura and a visible periodontal ligament space.

At the last follow-up, clinical examination revealed healthy gingiva around teeth 11, 12 and 13 (Figure 4a, b). The crown of tooth 12 was slightly yellowish in color. No swelling or tenderness was elicited upon palpation of the surrounding mucosa. None of the teeth was tender to percussion testing and demonstrated normal mobility. Periodontal probing demonstrated normal periodontal depths of tooth 12. Both adjacent teeth (teeth 11 and 13) responded normally to dental pulp vitality tests. The periapical radiograph, taken nine years and five months after the final treatment, showed completely healed bone adjacent to the root of tooth 12, with a visible lamina dura and a normal periodontal ligament space above the root apex (Figure 4b).

Ethics: The principles of the Declaration of Helsinki were respected in this case report. Written informed consent was obtained from the patient's parents for the publication of this case report and any accompanying images. All identifying details have been removed or anonymized to ensure patient privacy.

DISCUSSION

This case demonstrates a staged, conservative approach to type III DI, prioritizing pulp vitality and long-term tooth retention despite complex anatomy. Initial treatment targeted peri-invagination periodontitis caused by bacterial contamination. Following coronal sealing, periodontal regeneration with enamel matrix derivatives was performed without membranes or grafts to avoid interference with craniofacial growth. Pulp vitality was maintained for 2.5 years, allowing root development, although irreversible pulpitis later required complex endodontic and surgical management. In this respect, it is important to distinguish between infection and pulpitis, the presence of microorganisms and the host's response, respectively. In this case, bacterial infection spread from the peri-invagination periodontitis, triggering an inflammatory reaction that progressed to irreversible pulpitis.

DI is often underdiagnosed due to its subtle presentation, typically limited to a small invagination entrance [7], and is frequently discovered incidentally on radiographs [8]. Despite its inconspicuous appearance, DI can compromise pulpal and periodontal health. The invagination may contain dental papilla or periodontal tissue remnants, creating a bacterial niche. The pulp may be separated from the invagination by a thin enamel or dentin layer [1] or communicate directly with it [4]. Direct communication increases the risk of early pulp infection, whereas pulpitis from caries progression occurs later [4]. Pulp necrosis may develop within a few years and sometimes precedes apical closure [1].

Management strategies for DI range from prophylactic sealing and restorative measures to endodontic treatment of the invagination, pulp amputation, conventional or surgical RCT or extraction [7]. However, outcomes remain unpredictable. Even after prophylactic intervention, pulp inflammation may occur. Ridell et al. [6] reported inflammation in 11.3% of type I and 100% of type II DI cases. Pulp survival depends on canal morphology, apical development, and avoidance of iatrogenic irritation [7].

Type III DI presents particular therapeutic challenges. Although pulp vitality preservation after obturation of the invagination has been reported [9], communication between the invagination and periodontal ligament facilitates continuous inflammatory insult [10]. Consequently, more

than half of reported type III cases progress to pulp necrosis over time [11]. In the present case, irreversible pulpitis developed 2.5 years after periodontal surgery.

The sinus tract that developed during the vitality maintenance period originated from the peri-invagination periodontitis, not from pulp necrosis. Because the invagination communicated directly with the periodontal ligament, bacterial products and inflammatory exudate drained through the periodontal tissues, forming a sinus tract at the mucogingival junction. Pulp vitality was maintained because the pulp remained separate from the invagination and remained uninfected.

In this case, the invagination canal was wide and irregular, directly communicating with the periodontal ligament and partly inaccessible to both orthograde and retrograde approaches, as previously described in type III DI [12]. Furthermore, apical dilatation can cause inflammatory complications and eruption disturbances [13], but in this case eruption and alignment proceeded uneventfully.

Despite severe anatomical irregularities, the tooth was successfully preserved. Effective infection control, meticulous endodontic management, and adjunctive surgery achieved long-term stability exceeding nine years, demonstrating that extraction is not inevitable even in complex DI cases.

In conclusion, early diagnosis and timely management of DI are essential, ideally before the onset of pulpal or periodontal inflammation. Type III DI presents a particular challenge in preventing pulp infection, maintaining vitality, and performing RCT, and due to its complex anatomy is often considered for extraction. However, this case demonstrates that long-term tooth preservation can be achieved even in the presence of highly complex root morphology and internal anatomy through an individualized, interdisciplinary treatment approach.

Conflict of interest: None declared.

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Терапија *dens invaginatus* типа III*B*

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САЖЕТАК

Увод *Dens invaginatus* (*DI*) је ретка развојна аномалија коју карактерише инвагинација глеђног органа у зубну папилу, што ствара сложену унутрашњу анатомију. *DI* типа III, према Олерсовој класификацији, представља дијагностичке и терапијске изазове, посебно код зуба са незавршеним растом корена. Рана дијагноза и правилно лечење кључни су за спречавање пулпних и пародонталних компликација, очување виталности, функције и естетике зуба. Циљ овог приказа случаја је да опише дугорочно интердисциплинарно лечење ретког облика *DI* типа III*B*, истичући дијагностичке изазове, стратегије лечења и успешно очување зуба.

Приказ болесника Код деветогодишњег дечака дијагностикован је стални максиларни латерални секутић са *DI* типом III*B* и периинвагинационим пародонтитисом, при чему је виталност пулпе била очувана, а формирање корена непотпуно. Примењен је етапни, минимално инвазивни приступ, првобитно фокусиран на пародонталну хирургију и регенеративну терапију како би се контролисала инфекција, уз очување виталности пулпе и омогућавање континуираног раста корена. Након више од две године успешног одржавања виталности, развио се иреверзибилни пулпитис, што је захтевало сложено ортоградно лечење коренског канала, а затим и апикално хируршко лечење. Дугорочно праћење, дуже од девет година, показало је потпуно периапикално зарастање, стабилно пародонтално стање, функционални интегритет и задржавање зуба у зубном луку.

Закључак Рана дијагноза *DI* је неопходна, јер случајеви типа III представљају велике изазове за очување пулпе и лечење коренских канала и често се разматрају за екстракцију. Овај случај показује да је дугорочно очување зуба могуће упркос сложеној анатомији коришћењем индивидуализованог, интердисциплинарног приступа.

Кључне речи: *dens invaginatus*; зуб са незавршеним растом корена; интердисциплинарно лечење

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Sublingual immunotherapy – a good choice for all forms of birch pollen allergy

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SUMMARY

Introduction High prevalence of hypersensitivity to birch pollen significantly reduces the quality of life of affected individuals. Control of the simultaneous manifestation of allergic rhinitis and/or asthma and birch-apple allergy syndrome is particularly challenging. Opinions regarding the effects of allergen immunotherapy on the control of birch-apple allergy syndrome are divided.

Case outline We present a female patient with poorly controlled allergic rhinitis and asthma due to hypersensitivity to birch pollen and allergy to apples, in whom sublingual immunotherapy (SLIT) with an oral lyophilizate of birch pollen was administered. Nine months of SLIT, added to pharmacological therapy, led to good control of allergic rhinitis and asthma and to the development of apple tolerance.

Conclusion The favorable outcome of SLIT in controlling respiratory allergies and inducing apple tolerance suggests that it may be a beneficial therapeutic option for the management of all clinical manifestations of birch pollen allergy.

Keywords: allergic rhinitis; allergic asthma; pollen-food allergy syndrome; allergen immunotherapy

INTRODUCTION

Birch is the largest producer of allergenic tree pollen in Europe, with a sensitization prevalence ranging between 7% and 57% of the population. Most sensitized individuals have allergic rhinitis and/or asthma, and more than half also develop pollen-food allergy syndrome (PFAS), an immunoglobulin E (IgE)-mediated allergic reaction to foods from fruits and vegetables associated with sensitization to inhalant allergens, most commonly pollen [1, 2]. PFAS is based on IgE cross-reactivity to structurally similar, homologous allergens from different protein families present in both foods and pollen. The clinical presentation of PFAS is most often mild (itching and mild swelling of the lips, mouth, and throat), and only rarely includes symptoms of anaphylaxis. Pathogenesis-related protein 10 (PR-10) proteins, profilins and lipid transfer proteins are recognized plant panallergens involved in pollen-food cross-reactivity. Allergens from the PR-10 family and profilins are usually responsible for mild clinical manifestations, whereas allergens from other protein families are associated with more severe clinical forms of PFAS. More than 70% of individuals sensitized to birch pollen who have allergic rhinitis and/or asthma develop PFAS due to apple allergy (birch-apple allergy syndrome) [3, 4]. The quality of life of affected patients is further reduced, and their management represents a significant therapeutic challenge [5, 6, 7]. Avoidance of raw fruit and symptomatic therapy are recommended, while opinions regarding the use of allergen immunotherapy remain divided [7, 8].

We present a patient with concomitant seasonal allergic rhinitis, asthma and PFAS associated with apple allergy, in whom treatment with sublingual immunotherapy (SLIT) using an oral lyophilizate of birch pollen had a favorable effect on the control of all clinical manifestations of birch pollen allergy.

CASE REPORT

We present a 25-year-old female patient with seasonal allergic rhinitis and asthma due to hypersensitivity to birch pollen and PFAS, manifested as allergy to apples. The disease began suddenly in the spring of 2009, at the age of nine, with symptoms typical of allergic rhinitis and mild asthma, followed later that year by allergy to fresh apple (pronounced itching, burning sensation in the oral cavity and difficulty swallowing). Based on medical history, clinical examination, spirometry, skin prick testing, and measurement of serum specific IgE (sIgE) to standard inhalant and food allergens, allergic rhinitis and asthma due to hypersensitivity to birch pollen associated with allergy to apples were diagnosed one year later. With regular allergist follow-ups and pharmacological therapy, in accordance with recommendations for moderate-to-severe allergic rhinitis and mild allergic asthma, the patient maintained good control of respiratory symptoms until the end of 2021. In late April 2022 and early April 2023, despite regular therapy, the patient was hospitalized due to a sudden worsening of asthma and severe nasal obstruction. Control of

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Table 1. Results of allergological tests and laboratory findings

Features	Before SLIT	One month after SLIT	12 months after SLIT
Skin prick test to birch pollen (wheal diameter in mm)	8	7	8
Skin prick to prick test to apples (wheal diameter in mm)	9	9	8
Oral apple provocation test (visual analogue score)	8	0	3
sIgE Bet v1 (kUA/L)	31.08	26.90	25.89
sIgE Mal d1 (kUA/L)	8.09	7.53	7.11
sIgG4 Bet v1 (mgA/L)	0.09	0.84	0.75
sIgG4 Mal d1 (mgA/L)	0.06	0.62	0.31

SLIT – sublingual immunotherapy; sIgE – specific immunoglobulin E; sIgG4 – specific immunoglobulin G4; Bet v1 – *Betula verrucosa* 1 major allergen; Mal d1 – *Malus domestica* 1 major allergen

Table 2. Control of allergic rhinitis and asthma before, during and after SLIT

Features	Season 2022 before SLIT	Season 2023 before SLIT	Season 2024 during SLIT	Season 2025 after SLIT
AR VAS	8.66	8.33	2.66	2.73
ATDMS AR	5.16	6.49	1.38	1.32
ATDMS A	2.08	2.55	0.66	0.52
Number of severe AE	1	1	0	0
ACT	16	15	21	22

AR – allergic rhinitis; VAS – visual analogue scale; ATDMS – average total daily medication score; A – asthma; AE – asthma exacerbation; ACT – asthma control test; SLIT – sublingual immunotherapy

respiratory symptoms was achieved with parenteral methylprednisolone administered in tapering doses. PFAS was successfully controlled by avoidance of fresh fruit. In the family history, the patient's brother, father, and uncle have confirmed diagnoses of allergic asthma. Due to a sudden worsening of breathing during two consecutive tree pollen seasons, the patient presented for examination at the outpatient clinic of the Clinic for Allergy and Immunology, University Clinical Center of Serbia, Belgrade**,** in late May 2023. Investigations conducted in accordance with guidelines for allergic rhinitis, asthma, and PFAS confirmed the diagnoses of moderate-to-severe poorly controlled seasonal allergic rhinitis, mild partially controlled asthma due to hypersensitivity to birch pollen, and PFAS with allergy to apples. The diagnosis of allergic rhinitis and asthma was based on medical history and clinical presentation during the March–May pollen season, in correlation with a positive skin prick test exclusively to birch pollen (papule diameter 8 mm; saline solution 0 mm; histamine solution 5 mm) and a high serum concentration of sIgE to birch pollen (30.29 kUA/L, class 4) (ImmunoCAP system, Thermo Fisher Scientific, Uppsala, Sweden). The diagnosis of birch-apple allergy syndrome was established based on the patient's history (itching and burning in the throat and oral cavity and difficulty swallowing immediately after apple consumption), a positive prick-to-prick skin test with apple (9 mm; saline solution 0 mm; histamine solution 6 mm), and elevated serum sIgE to apples (7.80 kUA/L, class 3). Due to poor control of respiratory allergies and long-term avoidance of fresh fruit, component-resolved diagnostics (ImmunoCAP system, Thermo Fisher Scientific) was performed with the aim of introducing allergen immunotherapy. Primary sensitization to *Betula verrucosa* 1 major allergen (Bet v1), the major allergen of birch pollen, and cross-reactivity to *Malus domestica* 1 major allergen (Mal d1), the major apple allergen (PR-10 type), were demonstrated (Table 1). Sensitization to the minor allergens of birch pollen and apple, Bet v2 and Mal d4, was not detected (sIgE Bet v2 0.11 kUA/L, sIgE Mal d4 0.16 kUA/L). Based on these findings, SLIT with an oral lyophilizate of a standardized allergen extract from birch pollen was initiated. A pre-seasonal and co-seasonal protocol was applied for nine months (from early September 2023 to the end of May 2024) in addition to pharmacological therapy. Before initiation of SLIT, a double open oral food challenge with a fresh Granny Smith apple was performed with a cumulative dose of 200 g (one medium apple). The test was positive (visual analogue score 8). Symptoms resolved after administration of two tablets of desloratadine. The efficacy of SLIT was assessed based on clinical parameters (Table 2) and laboratory criteria (oral provocation test with fresh apple and serum concentrations of sIgE and sIgG4 to the major allergens of birch pollen Bet v1 and apple Mal d1) (Table 1). During the tree pollen season while receiving SLIT, the patient achieved good control of rhinitis and asthma, and one month after discontinuation she also demonstrated good tolerance to apples, for the first time in more than ten years (Table 1). The favorable outcome of SLIT was accompanied by high serum concentrations of specific IgG4 to the major birch pollen allergen Bet v1 (0.84 mgA/L) and to the cross-reactive major apple allergen Mal d1 (0.62 mgA/L). The patient decided to discontinue SLIT after the first treatment season. During additional follow-up one year after discontinuation of SLIT, the patient maintained well-controlled mild allergic rhinitis and mild asthma (Table 2), but apple tolerance was not maintained. The oral provocation test with a Granny Smith apple was again positive, with a lower score of oropharyngeal symptoms, accompanied by reduced levels of sIgG4 to the major allergens of birch pollen and apple (Table 1).

Informed consent: Written informed consent was obtained from the patient for this case report publication, including the medical history and laboratory analyses.

Ethics approval: The publication of this case report was approved by the Ethics Committee of the University Clinical Center of Serbia.

DISCUSSION

The high prevalence of birch-apple allergy syndrome is part of the global "allergy epidemic" and results from the frequent sensitization to birch pollen and its strong cross-reactivity with apple. In these patients, seasonal allergic

rhinitis and/or asthma are often difficult to control with standard pharmacological therapy [9, 10]. This difficulty is attributable to cross-reactivity between birch pollen and homologous tree pollens [11], as well as to the global increase in airborne pollen concentrations [12]. These factors were also the main reasons for the poor control of allergic rhinitis and asthma in our patient. Good control of allergic rhinitis and asthma until 2021 was associated with relatively low concentrations of birch pollen in the environment, which did not exceed 100 pollen grains/m^3 of air. However, in the following years very high concentrations were recorded (298–593 pollen grains/m^3 of air) during April and May, which coincided with poor control of respiratory allergies. Despite persistently high birch pollen concentrations, SLIT resulted in good control of allergic rhinitis and asthma, not only during treatment but also after its discontinuation. The favorable clinical effect of SLIT was accompanied by an increase in serum concentrations of specific IgG4 and a decrease in specific IgE directed against the major birch pollen allergen, as reported by other authors as well [11, 13, 14]. Nine months of SLIT resulted in good tolerance of fresh apples, accompanied by increased concentrations of sIgG4 and decreased concentrations of sIgE to the major allergens of apple and birch pollen in serum. The significant role of sIgG4 in blocking IgE-dependent cross-reactivity between the major allergens of birch pollen and apple has also been reported by other authors [3, 4, 15]. The blocking role of sIgG4 is directly related to the clinical effects of SLIT in inducing tolerance to apples in birch-apple allergy syndrome [16, 17]. Identification of the carriers of primary and cross-reactive IgE responses between birch pollen and apple is of great importance for the effectiveness of SLIT in our patient [8, 15, 17]. Although the major birch pollen allergen is the most common carrier of primary sensitization and cross-reactive IgE response to apples, this is not always the case in clinical practice. In approximately 10% of patients, the primary (and only) carrier is the minor allergen Bet v2 or another minor birch pollen allergen [17, 18]. In such patients, SLIT unfortunately does not lead to tolerance to apples. A considerable number of studies, in which SLIT efficacy was not demonstrated, lack evidence on the carriers of cross-reactive IgE responses with apples [19]. The favorable outcome of SLIT in our patient was also influenced by the daily administration of an oral lyophilizate containing a high concentration of the major birch pollen allergen and by the absence of sIgE to minor allergens of birch pollen and apples. The beneficial effect of SLIT in establishing tolerance to apples was not sustained to the same extent as the control of respiratory allergies. Six months after discontinuation of SLIT, the patient reported oral itching associated with apple consumption for the first time. During further follow-up, itching occurred occasionally, after consumption of only certain apple varieties, and resolved spontaneously. One year after discontinuation of SLIT, the repeated oral provocation test with apple was again positive. Although the score was low, it negatively affected the patient's choice of fruit. The gradual loss of tolerance to apples was accompanied by a heterogeneous decrease in serum concentrations of sIgG4 to the major allergens of birch pollen and apple, as also reported by other authors [17, 19]. Several explanations for the failure of SLIT in controlling birch-apple allergy syndrome have been described in the literature. The most common reason is insufficient knowledge of the allergens responsible for cross-reactive IgE responses between birch pollen and apple [3, 8], structural variations of the major apple allergen among different apple varieties [17], and insufficient maintenance dose or short duration of treatment [8, 9]. Recent studies indicate that SLIT-induced sIgG4 directed against the major birch pollen allergen blocks all IgE-binding epitopes on Bet v1 but not on Mal d1. A high degree of cross-reactivity of sIgG4 directed against Bet v1 toward Mal d1 requires a high degree of structural homology between the major allergens of birch pollen and apple (above 80%), which in reality does not exceed 56% [16, 20]. This is assumed to be the main reason for the limited efficacy of SLIT in controlling birch-apple allergy syndrome. It has been established that sIgG4 directed against the major apple allergen binds different epitopes from those recognized by sIgG4 directed against the major birch pollen allergen. For effective blocking of IgE-binding epitopes on the major apple allergen – and consequently for successful SLIT-induced tolerance to apples – a high concentration and high affinity of sIgG4 directed toward different epitopes of the major apple allergen are required. An increase in their serum concentration is usually detected after four months, while maximal concentrations are achieved only after long-term SLIT administration lasting at least three years [21]. The efficacy of SLIT in our patient is consistent with these findings [21, 22, 23]. Tolerance to apples was gradually lost due to premature discontinuation of SLIT, which represents the main limitation of this case report. Nevertheless, the favorable outcome observed in our patient suggests that, under certain conditions, SLIT may represent a good therapeutic option for the control of all clinical manifestations of birch pollen allergy.

Conflict of interest: None declared.

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Сублингвална имунотерапија – добар избор за све форме алергије на полен брезе

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САЖЕТАК

Увод Висока учесталост преосетљивости на полен брезе значајно умањује квалитет живота оболелих. Нарочито је отежана контрола истовременог испољавања алергијског ринитиса и/или астме и алергијског синдрома бреза–јабука. Мишљења о ефектима алергенске имунотерапије на контролу алергијског синдрома бреза–јабука су подељена.

Приказ болесника Приказујемо болесницу са лоше контролисаним алергијским ринитисом и астмом услед преосетљивости на полен брезе и алергије на јабуке, код које је примењена сублингвална имунотерапија оралним лиофилизатом полена брезе. Деветомесечна примена сублингвалне имунотерапије, придодата фармаколошкој терапији, довела је до добре контроле алергијског ринитиса и астме, као и до успостављања толеранције на јабуку.

Закључак Повољан исход сублингвалне имунотерапије у контроли респираторних алергија и постизању толеранције на јабуку указује на то да би овај вид терапије могао бити повољан избор за контролу свих клиничких облика алергије на полен брезе.

Кључне речи: алергијски ринитис; алергијска астма; синдром полен–храна; алергенска имунотерапија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Malignant chondroid syringoma with calvarial invasion and intracranial extension

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SUMMARY
Introduction Malignant chondroid syringoma (MCS) is an exceptionally rare malignant adnexal tumor with aggressive biological behavior. This report describes a rare case of frontotemporal MCS with calvarial invasion and intracranial extension.
Case Outline A 65-year-old male presented with a recurrent ulcerated cutaneous tumor in the left frontotemporal region, with four previous surgical excisions that had been histopathologically diagnosed as malignant basal cell carcinoma. Preoperative computed tomography and magnetic resonance imaging revealed calvarial osteolysis with intracranial tumor extension. Radical excision with craniectomy, dural resection, and duraplasty was performed, followed by reconstruction using a local transposition flap and a secondary free split-thickness (Thiersch) skin graft. Histopathological and immunohistochemical analyses confirmed MCS with bone and perineural invasion and tumor involvement of the deep surgical margin (R1). Adjuvant conformal radiotherapy was administered. No recurrence or metastasis was observed during a nine-month follow-up period.
Conclusion MCS may clinically mimic other cutaneous malignancies, which can lead to delayed diagnosis. Wide surgical excision and long-term follow-up are essential for adequate disease control.
Keywords: sweat gland neoplasms; skin neoplasms; neoplasm invasiveness

INTRODUCTION

Malignant chondroid syringoma (MCS) is an exceptionally rare malignant tumor of the eccrine sweat glands, first described by Hirsch and Helwig [1] in 1961 as a "mixed tumor of the skin" with chondroid stroma. Benign chondroid syringoma accounts for less than 0.1% of primary adnexal tumors and typically presents as a slow-growing, painless subcutaneous nodule in the head and neck region of middle-aged men [2, 3]. Its malignant counterpart is even rarer. By the early 1980s, only isolated cases had been reported, with Gupta et al. [4] publishing a collective review in 1982. Subsequent reports confirmed its aggressive behavior and metastatic potential [5]. Approximately 51 cases have been documented worldwide [3, 6]. Unlike the benign type, MCS more commonly affects the extremities and trunk and shows a female predominance [3]. It is a potentially aggressive tumor with a tendency for local recurrence after inadequate excision and has the capacity for distant metastasis [7].

We present a rare case of MCS in the left frontotemporal region of a 65-year-old male, with calvarial invasion and intracranial extension.

CASE REPORT

A 65-year-old male patient was admitted to the University Clinic for Maxillofacial Surgery in Skopje due to a recurrent cutaneous tumor in the left frontotemporal region. The patient reported four previous surgical excisions in the same region in another hospital in 2018, 2019, 2021, and 2022, with repeated histomorphologic diagnoses of basal cell carcinoma, without immunohistochemical confirmation of the

Figure 1. Clinical appearance of malignant chondroid syringoma

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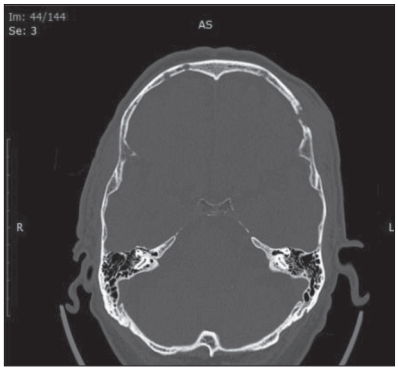

Figure 2. Preoperative computed tomography

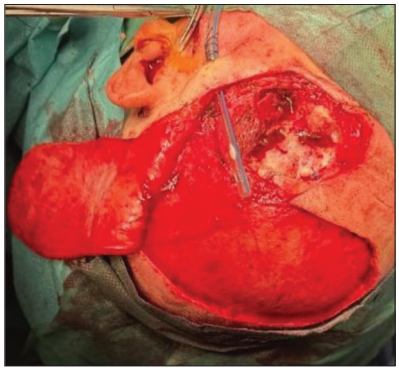

Figure 3. Surgical procedure: tumor excision, craniectomy, duraplasty and flap design

Figure 4. Postoperative appearance

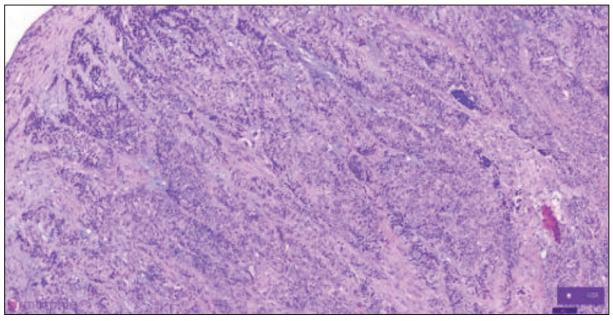

Figure 5. Digital micrography – Hematoxylin & Eosin (× 40)

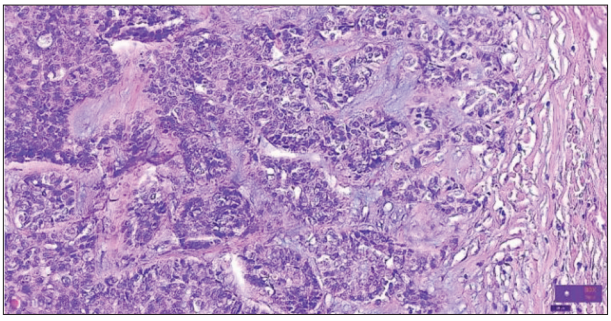

Figure 6. Digital micrography – Hematoxylin & Eosin (× 80)

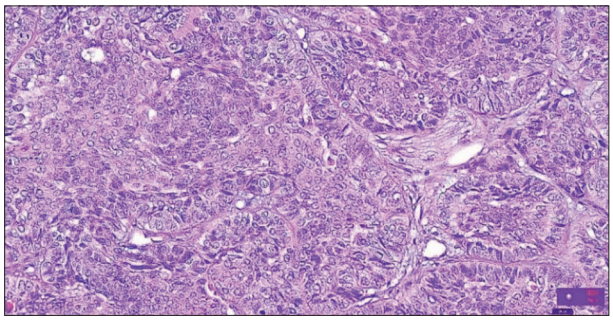

Figure 7. Digital micrography – Hematoxylin & Eosin (× 80)

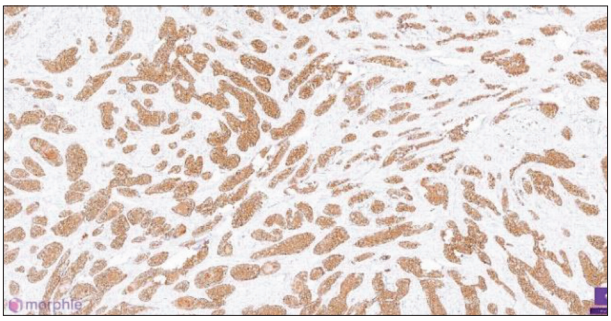

Figure 8. Digital micrography – high molecular weight cytokeratin (× 20)

tumor cell origin. These pathohistological reports were issued by a non-reference pathology laboratory in a regional center. Clinically, the lesion presented as a nodular mass with central ulceration and a peripheral telangiectatic vessels, elevated above the base, firm and painless on palpation, with scar tissue from previous surgeries (Figure 1). An incisional biopsy was performed. Preoperative computed tomography (CT) and magnetic resonance imaging (MRI) of the head and neck revealed bone invasion and osteolysis in the left frontotemporal region, with intracranial tumor extension (Figure 2). Due to confirmed intracranial extension, multidisciplinary surgical treatment was indicated.

The patient underwent surgery at the Neurosurgery Clinic in Skopje, performed by a team consisting of a neurosurgeon, plastic surgeon, and maxillofacial surgeon under general endotracheal anesthesia. Radical tumor excision, craniectomy, and excision of the dura mater followed by duraplasty were performed (Figure 3). The primary surgical defect was reconstructed using a local transposition skin flap, while the secondary defect was reconstructed with a free split-thickness skin graft according to Thiersch from the anterior thigh (Figure 4).

The surgical specimen was submitted to the Institute of pathology at the Medical Faculty in Skopje, which is the central reference pathohistological laboratory in the country. Macroscopic examination of the surgical specimen revealed lobulated tumor tissue with a maximum dimension of 3.5 cm. Microscopically, tissue sections demonstrated skin covered by a thin epidermis showing central ulceration. At this level, a malignant neoplasm composed of round to oval tumor cells arranged in nests, cribriform, and trabecular structures was observed (Figure 5). The peripheral cells of the nests had a cuboidal appearance (Figure 6). The tumor cells exhibited moderate cellular and nuclear atypia. The stroma was fibro-collagenous, focally thickened, with areas showing a chondroid appearance (Figure 7). A moderate lymphocytic inflammatory

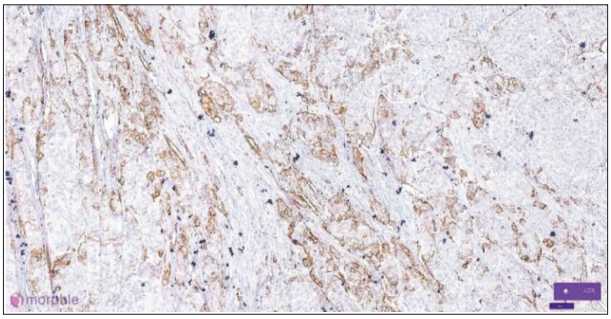

Figure 9. Digital micrography – smooth muscle actin (× 40)

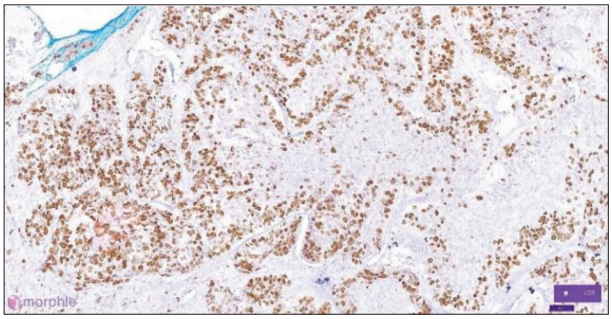

Figure 10. Digital micrography – Ki-67 (× 40)

infiltrate was present within the stroma. The tumor diffusely infiltrated into deeper tissues, involving subcutaneous adipose tissue and underlying striated muscle. Tumor involvement was present at the deep resection margin, while peripheral resection margins were free of tumor. Lymphovascular invasion was not identified; however, perineural invasion was present.

Additionally, tumor infiltration into fragments of bone tissue was confirmed. Immunohistochemical analysis demonstrated expression of high-molecular-weight cytokeratin in tumor cells (Figure 8), as well as focal weak expression of smooth muscle actin (Figure 9). In contrast, there was no expression of epithelial membrane antigen (EMA), epithelial-specific antigen, cytokeratin 7, cytokeratin 8/18, cytokeratin 20, c-kit (CD117), CD34, or carcinoembryonic antigen (CEA). The proliferative index Ki-67 was high, approximately 40–50% (Figure 10). The histomorphologic and immunophenotypic findings were consistent with MCS. According to the Union for International Cancer Control classification, the tumor was staged as pT4a, N0, M0, G2, L0, V0, R1, corresponding to stage IVA. Following surgical treatment, due to high-risk pathological features – including tumor infiltration of the deep resection margin (R1) and confirmed perineural invasion (Pn1) – adjuvant conformal radiotherapy was administered, with a total tumor dose of 60 Gy delivered in 30 daily fractions of 2 Gy each. At a follow-up nine months after surgery, there was no evidence of local, locoregional, or distant recurrence.

Ethics: Written informed consent was obtained from the patient for publication of this case report and any accompanying images. All identifying details have been removed or anonymized to ensure patient privacy.

DISCUSSION

MCS, also known as malignant cutaneous mixed tumor (CMT), represents an exceptionally rare malignant tumor of the skin adnexa [8]. Four mechanisms of malignant transformation in cutaneous mixed tumors have been described in the literature [9]: *De novo* malignant development, malignant transformation of a long-standing benign CMT that begins to grow rapidly, secondary skin infiltration by a malignant mixed tumor originating from another organ such as the salivary glands, and in extremely rare cases development from a pre-existing apocrine or eccrine adnexal tumor, such as spiradenoma. Clinically, MCS presents as a firm, painless dermal or subcutaneous nodule with slow growth, often mimicking benign lesions [6]. However, rapid enlargement, ulceration, and deep invasion may occur [7]. Lesions exceeding 3 cm in size are typically indicative of malignancy, although benign lesions over 10 cm have also been documented [8, 10, 11]. Head and neck involvement is uncommon for the malignant variant, with only a limited number of reported cases [8]. Our case is notable for aggressive behavior with calvarial and dural invasion, a rare finding documented only sporadically [12]. Recent reports of chondroid syringoma with bone erosion support careful imaging and complete excision when deep invasion is suspected [13]. Osseous metaplasia in benign chondroid syringoma may mimic bone involvement and should be distinguished from true bone invasion in malignant cases [14]. Due to nonspecific clinical features, MCS is frequently misdiagnosed. The differential diagnosis of MCS includes a sebaceous cyst, dermoid cyst, neurofibroma, pilomatrixoma, amelanotic nevus, and basal cell carcinoma [15]. Excisional biopsy with histopathological and immunohistochemical analysis remains the diagnostic gold standard [6, 10]. Similar to previously reported cases [12], our lesion was repeatedly misinterpreted as basal cell carcinoma, likely contributing to delayed diagnosis. Histologically, our case demonstrates nests and trabeculae of atypical epithelial cells within a sparse chondromyxoid stroma [8]. Perineural invasion, present in our case, has also been documented in malignant forms [6]. Immunohistochemically, MCS typically shows biphasic expression. The epithelial component is positive for cytokeratins (including CK5/6), EMA, CEA, and p63. Areas of mesenchymal chondroid differentiation demonstrate S-100 and vimentin positivity [16]. In our case, positivity for CK5/6 and partial smooth muscle actin expression, along with negativity for EMA, CEA, cytokeratin 7, and other markers, and a high Ki-67 index (40–50%), support adnexal origin and the diagnosis of MCS, despite absence of all typical glandular markers. Fine-needle aspiration biopsy may aid in suspected cases [17]. Imaging modalities, including CT and MRI, are essential for assessing local extension and metastases [6]. Recurrence rates reach 50%, and distant metastases occur in up to 60% of cases [3, 18]. Recent reports of pulmonary metastasis further emphasize the metastatic potential of

MCS [19]. Wide surgical excision with tumor-free margins remains the cornerstone of treatment, often complemented by adjuvant radiotherapy, with or without chemotherapy [20]. Nine months after treatment, the patient remained disease-free. Given the high recurrence potential and reports of late metastases even decades after excision, long-term follow-up is mandatory [8].

MCS is a rare and aggressive adnexal tumor that frequently results in delayed or incorrect diagnosis due to its clinical and histomorphologic variability. Our case, characterized by calvarial bone invasion and intracranial extension, highlights its potential for significant local destruction and underscores the necessity of early radical surgical excision. The high local recurrence and regional metastasis rates (50–60%) justify the use of adjuvant radiotherapy in patients with positive surgical margins or high-risk pathological features. Long-term and careful follow-up is essential, as recurrences have been documented even two decades after primary treatment.

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Малигни хондроидни сирингом са инвазијом калварије и интракранијалним ширењем

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САЖЕТАК

Увод Малигни хондроидни сирингом представља изузетно редак малигни аднексални тумор са агресивним биолошким понашањем. Циљ овог рада је да се прикаже редак случај фронтотемпоралног малигног хондроидног сирингома са инвазијом калварије и интракранијалним ширењем.

Приказ болесника Мушкарац стар 65 година јавио се због рецидивирајуће улцерисане туморске лезије у левој фронтотемпоралној регији, уз податак о четири претходне хируршке ексцизије које су хистопатолошки дијагностиковане као малигни базоцелуларни карцином. Преоперативна компјутеризована томографија и магнетна резонанца показале су остеолизу калварије са интракранијалним ширењем тумора. Изведена је радикална ексцизија тумора са краниектомијом, ресекцијом дуре и дурапластиком, након чега је урађена реконструкција локалним транспозиционим кожним режњем и секундарним слободним кожним трансплантатом делимичне дебљине (по Тиршу). Хистопатолошка и имунохистохемијска анализа потврдиле су малигни хондроидни сирингом са инвазијом кости и перинеуралном инвазијом, уз присуство тумора на дубокој ресекционој маргини (P1). Постоперативно је примењена адјувантна конформална радиотерапија. Током деветомесечног периода праћења није регистрован рецидив, као ни метастатска болест.

Закључак Малигни хондроидни сирингом може клинички имитирати друге кожне малигнитете, што може довести до одложене дијагнозе. Широка хируршка ексцизија и дуготрајно праћење болесника кључни су за адекватну контролу болести.

Кључне речи: неоплазме знојних жлезда; неоплазме коже; инвазивност тумора

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Hybrid endovascular treatment of a ruptured abdominal aortic aneurysm in a patient with small B-cell Non-Hodgkin lymphoma

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SUMMARY

Introduction Small lymphocytic lymphoma (SLL) / chronic lymphocytic leukemia (CLL) is an indolent lymphoproliferative disorder characterized by accumulation of mature but dysfunctional B lymphocytes. Aortic complications have been occasionally reported in lymphoma, most often in aggressive subtypes, while they are extremely rare in SLL/CLL. The aim of this report was to present a case of recently developed ruptured abdominal aortic aneurysm in a patient with SLL and its successful hybrid endovascular management.

Case outline A 58-year-old male with SLL/CLL presented with lower back and right flank pain. Initial computed tomography scan (CT) demonstrated marked progression of lymphadenopathy and interval enlargement of the infrarenal aortic diameter compared to prior imaging, remaining below the aneurysmal threshold. Due to persistent symptoms and elevated inflammatory markers, further imaging was performed. Magnetic resonance imaging of the lumbosacral spine and subsequent CT angiography revealed a newly developed saccular aneurysm of the infrarenal abdominal aorta with retroperitoneal hematoma and surrounding lymph node conglomerates. The patient underwent urgent endovascular repair using an aorto-uni-iliac stent graft with contralateral iliac occlusion, combined with femoro-femoral crossover bypass. Postprocedural imaging confirmed successful exclusion of the lesion without endoleak.

Conclusion This case highlights a rare vascular complication of SLL/CLL and suggests a possible role of lymphomatous infiltration and inflammation in aortic wall weakening. Hybrid endovascular treatment represents an effective therapeutic option.

Keywords: chronic lymphocytic leukemia; small lymphocytic lymphoma; abdominal aortic aneurysm; endovascular procedure

INTRODUCTION

Small lymphocytic lymphoma (SLL) / chronic lymphocytic leukemia (CLL) represents an indolent (slow-growing) lymphoproliferative disorder characterized by the accumulation of morphologically mature but immunologically dysfunctional B lymphocytes in lymph nodes, bone marrow, and blood [1, 2]. These two entities are considered different clinical manifestations of the same disease. In SLL, malignant cells are predominantly found in lymph nodes, while in CLL they primarily involve the peripheral blood and bone marrow. Together, CLL/SLL belongs to the group of non-Hodgkin lymphomas.

Given the normal distribution of lymph nodes along the aorta, paraaortic nodal involvement may occur in CLL due to lymphomatous infiltration, which may even extend to involve the adjacent aortic wall, presenting as periaortic lymphoma. Such infiltration may lead to structural weakening of the aortic wall and aneurysm formation [3, 4]. Aortic involvement presenting as aneurysm, rupture, or dissection has been reported in the literature, most often in association with high-grade lymphomas, typically diffuse large B-cell lymphoma, whereas reports of similar aortic complications associated with indolent B-cell lymphomas, such as SLL/CLL, are extremely rare. Reports describing endovascular management of lymphoma-related aortic complications are even less common.

The aim of this report was to describe the successful hybrid endovascular management of a ruptured abdominal aortic aneurysm (AAA) in a patient with SLL and to discuss possible pathogenetic links between lymphoproliferative disease and aortic wall weakening.

CASE REPORT

A 58-year-old man presented with a four-day history of pain in the lower back and right flank, radiating downward. He denied fever or urinary symptoms. The patient has a history of SLL/CLL, diagnosed in 2021, and underwent six cycles of treatment Gazyva–Chlorambucil from June 2023 to July 2024. Follow-up with a hematologist has been irregular despite

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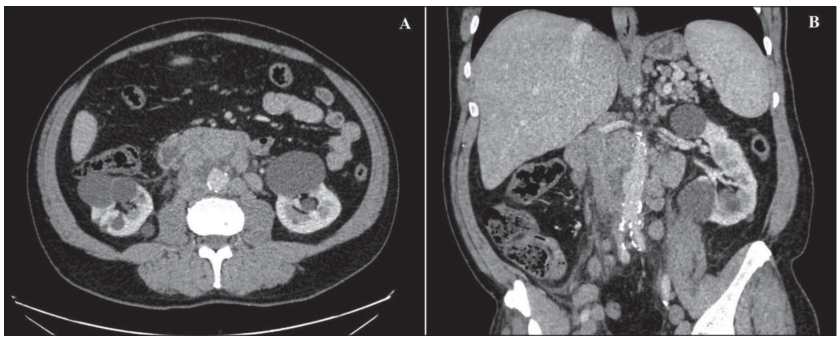

Figure 1. Initial contrast-enhanced computed tomography examination of the abdomen: A – axial image; B – coronal image; a conglomerate of enlarged lymph nodes is observed in the periaortic and retroperitoneal region, with a small amount of locoregional free fluid

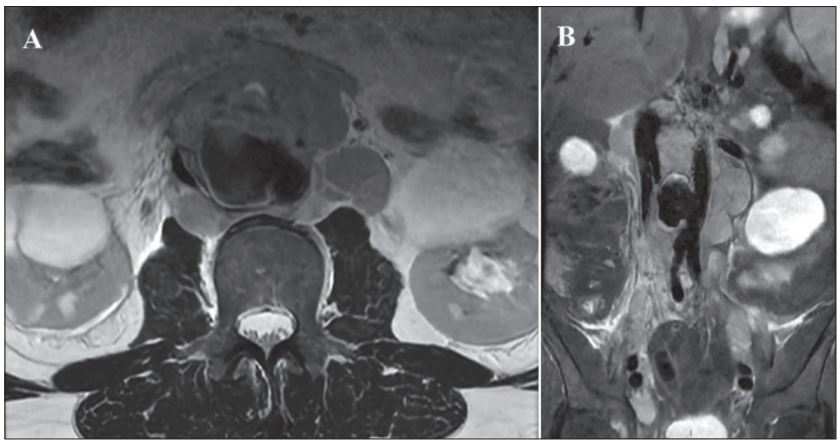

Figure 2. Magnetic resonance imaging of the lumbosacral spine: A – axial T2-weighted image; B – coronal T2-weighted image; a saccular aneurysmal dilatation of the infrarenal abdominal aorta is seen, with locoregional lymphadenopathy

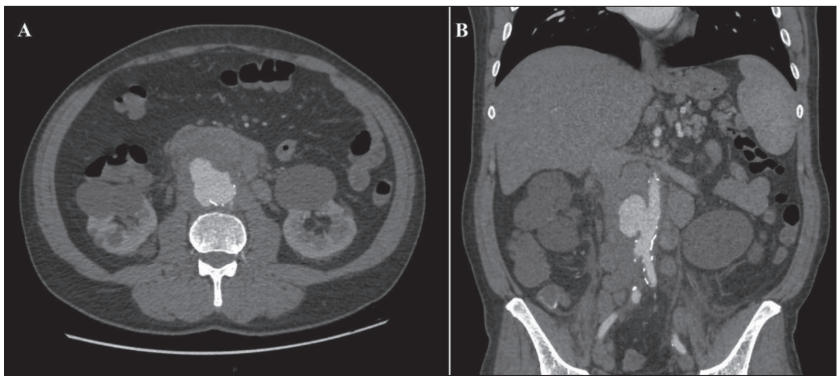

Figure 3. Computed tomography angiography of the abdominal vessels: A – axial image; B – coronal image; a saccular aneurysmal dilatation of the infrarenal abdominal aorta is clearly delineated, with locoregional lymphadenopathy and a small amount of retroperitoneal free fluid

recommendations. He denied B symptoms. His medical history was notable for chronic arterial hypertension, myocardial infarction in 2021, and renal colic on the right side, also in 2021. On physical examination, the patient was hemodynamically stable and cardiopulmonary compensated, with mild hypertension (145/85 mmHg) and normal heart rate. Enlarged regional lymph nodes were palpable: cervical and axillary bilaterally, measuring up to 2 cm, and inguinal bilaterally, measuring up to 2.5 cm. The liver was enlarged, while the spleen was not palpable. Laboratory tests revealed leukocytosis with lymphocytosis (white blood cells 33.07 × 10^9/L, lymphocytes 17.40 × 10^9/L), without anemia or thrombocytopenia, and normal renal and liver function. Marked elevation of inflammatory markers was also noted (C-reactive protein 197.5 mg/L).

The initial computed tomography (CT) scan, compared with a study from 18 months earlier, showed enlargement of the liver and spleen. There was a marked increase in intra-abdominal (both intra- and retroperitoneal), pelvic, and inguinal lymphadenopathy compared with the previous scan. The largest lymph nodes were located retroperitoneally and formed conglomerates measuring approximately 6.5 × 5 cm, with free fluid and stranding of the surrounding fat consistent with inflammation. Additionally, the infrarenal segment of the abdominal aorta, although non-aneurysmal dilated, showed an increase in luminal diameter from 18 mm on the prior study to 23 mm on the current examination (Figure 1).

Due to progression on CT imaging and abnormal laboratory findings, hospitalization was indicated, and parenteral antibiotic therapy was initiated, along with analgesic, symptomatic, and supportive treatment. After one week of severe back pain and elevated inflammatory markers despite ongoing therapy, spondylodiscitis was suspected, and an magnetic resonance imaging of the lumbosacral spine was performed. The examination revealed a highly suspicious focal saccular aneurysmal dilatation along the right contour of the infrarenal abdominal aorta, measuring approximately 41 × 30 mm, accompanied by locoregional conglomerates of lymph nodes. No changes were observed in the vertebral bodies suggestive of viable tissue or active infiltration by the underlying disease (Figure 2).

CT angiography of the aortoiliac segment, performed the following day, revealed a saccular aneurysm along the right wall of the infrarenal abdominal aorta, measuring approximately 4.2 × 2.7 cm in diameter, accompanied by a retroperitoneal hematoma and conglomerates of enlarged lymph nodes surrounding the lesion. Free fluid was also observed in the retroperitoneal space (Figure 3).

After consultation with the multidisciplinary team, the patient was considered eligible for an endovascular procedure – endovascular aortic stent graft (EVAR), employing an aortic uni-graft configuration (Medtronic Endurant II AUI with right iliac extension, Medtronic, Minneapolis, MN, USA) with contralateral (left) common iliac artery occlusion (plug), in combination with a hybrid surgical femoro-femoral crossover bypass (Figure 4). Given the

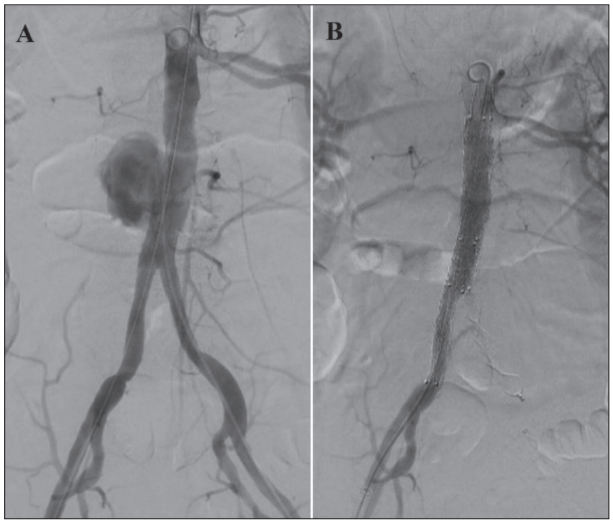

Figure 4. Abdominal aortic angiogram (digital subtraction angiography): A – before treatment: an aneurysmal sac is visible; B – after treatment: complete exclusion of the aneurysm from the circulation, with preserved flow through the right common and external iliac arteries, while the left common and external iliac arteries are occluded

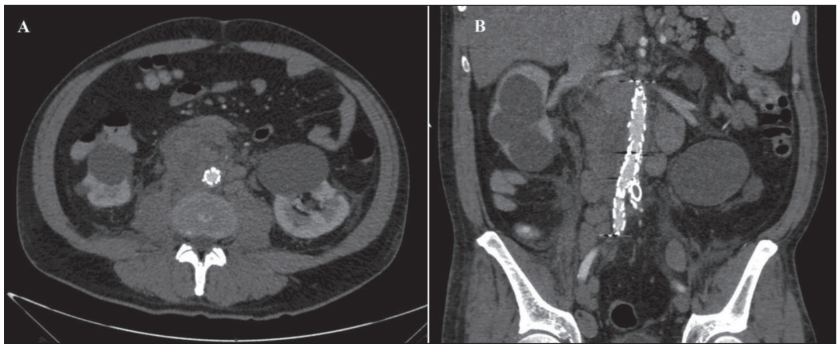

Figure 5. Follow-up computed tomography angiography: no evidence of endoleak, adjacent lymph node conglomerates with periaortic hematoma, and postprocedural occlusion of the left common iliac artery

presence of lymphadenopathy, excisional biopsy of a right inguinal lymph node, performed by a vascular surgeon, demonstrated no evidence of disease transformation, confirming the persistence of SLL.

Follow-up CT performed 24 hours after the procedure demonstrated persistent periaortic hematoma and lymph node conglomerates, with no evidence of contrast extravasation. After the procedurally, the left common and external iliac arteries were occluded, while flow was maintained in the right common and external iliac arteries (Figure 5), as well as in the femoro-femoral crossover bypass and the left femoral artery. A second follow-up CT scan performed one month later showed no significant changes – persistent periaortic hematoma and adjacent lymph node conglomerates, and no evidence of endoleak.

Ethics: Ethical approval for this study was obtained from the institutional Ethics Committee (No. 6 00-43/9, date: April 2, 2026).

DISCUSSION

AAA is defined as a localized dilatation of the abdominal aorta, typically diagnosed on imaging when the maximum aortic diameter measures ≥ 30 mm [5]. Several pathophysiological mechanisms have been described in the literature as playing a role in the development of AAA. These mechanisms include aortic wall inflammation, elastin degradation, oxidative stress, phenotypic changes and dysfunction of smooth muscle cells, and breakdown of the extracellular matrix [6]. Chronic inflammation and immune cell activation play a central role in the development of aneurysms, although the mechanisms governing their recruitment and activation remain incompletely understood [7]. In SLL/CLL, there is a marked accumulation of clonal B lymphocytes in the blood, bone marrow, and lymphoid tissues, reflecting both increased proliferation and impaired apoptosis. B cells play an active role in the pathogenesis of AAA as they accumulate in the adventitia, promote inflammatory responses and immunoglobulin deposition, interact with macrophages, and enhance the expression of matrix metalloproteinase-9, an enzyme involved in extracellular matrix degradation [7]. Interventions targeting B cells, such as anti-CD20 or B-cell activating factor receptor blockade, reduce AAA development and inflammation, demonstrating that B cell activity directly drives disease progression [8]. Although mechanisms linking B cells to AAA development have been described, and SLL/CLL is characterized by elevated counts of mature B lymphocytes, no study to date has directly investigated the relationship between these two conditions. Moreover, retroperitoneal lymphoma can form periaortic infiltrates, which may mechanically compromise the aortic wall, trigger localized inflammatory responses, and lead to aneurysm formation.

In literature, individual cases of patients with coexisting AAA and more aggressive forms of lymphoma are reported, whereas our patient has an indolent form of the disease. Some authors have reported that distinguishing periaortic lymphoma from a ruptured AAA is challenging due to overlapping clinical features and imaging findings, particularly when the aneurysm and tumor are in close proximity [9, 10]. Because both conditions may present with mass lesions around the abdominal aorta extending into the retroperitoneum, differentiating them based solely on imaging studies is often difficult. Abdominal pain in AAA associated with periaortic malignant lymphoma may result from infiltration of lymphoma cells into the aortic wall, causing rapid aneurysmal expansion, and can persist even after treatment of the AAA [11]. Cases linking SLL/CLL to aortic wall damage are exceedingly rare, as reported in a case of thoracic aortic dissection associated with this type of lymphoma, which raises the possibility that even

the indolent form of the disease may lead to severe vascular complications [3].

The development of AAA is shaped not only by underlying pathophysiological mechanisms but also by demographic, lifestyle, and clinical risk factors. The main recognized risk factors for AAA are advanced age, male sex, history of smoking, coronary heart disease, hypertension, peripheral artery disease, previous myocardial infarction, and a family history of AAA [12, 13]. Our patient is a 58-year-old male with a history of hypertension and prior myocardial infarction, and a smoking habit, representing multiple established risk factors for AAA.

After multidisciplinary team review, an endovascular approach was chosen, as patients with lymphoma are generally not managed with open surgery due to the high risk of postprocedural complications. Retroperitoneal dissection could result in bleeding from enlarged lymph nodes that would be difficult to control, and access to the aneurysm neck would also be impeded by their bulk. The literature also suggests that, in such patients, EVAR is preferred over conventional aortic replacement [11]. According to standard protocol for aorto-uni-iliac EVAR, occlusion of the contralateral common iliac artery is performed to prevent endoleak type II, specifically by eliminating potential retrograde flow through internal iliac artery collaterals. This approach is considered routine in this procedure, as it significantly reduces the risk of endoleak. Additionally, following the endovascular procedure, a femoro-femoral bypass was created by a vascular surgeon as a standard adjunct after deployment of an aorto-uni-iliac stent graft, in accordance with the guidelines of the European Society for Vascular Surgery [14].

Endovascular treatment of AAA is minimally invasive and considered the preferred approach for patients with relevant comorbidities, as illustrated in our patient with SLL/CLL.

Conflict of interest: None declared.

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Хибридни ендоваскуларни третман руптуриране анеуризме абдоминалне аорте код болесника са Б-ситноћелијским нон-Хоџкиновим лимфомом

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САЖЕТАК

Увод Б-ситноћелијски нон-Хоџкинов лимфом (СЛЛ) / хронична лимфоцитна леукемија (ХЛЛ) представљају индолентни лимфопролиферативни поремећај који карактерише накупљање зрелих, али дисфункционалних Б лимфоцита. У литератури се повремено наводе аортне компликације код лимфома, претежно код агресивнијих подтипова, док су код СЛЛ/ХЛЛ изузетно ретке. Циљ овог рада је да се прикаже руптура новонастале анеуризме абдоминалне аорте код болесника са СЛЛ и њено успешно лечење хибридном ендоваскуларном техником.

Приказ болесника Болесник стар 58 година са претходно дијагностикованим СЛЛ/ХЛЛ јавио се због бола у доњем делу леђа и десној лумбалној ложи. Иницијални преглед компјутеризованом томографијом (КТ) показао је значајну прогресију лимфаденопатије у односу на претходни преглед, као и повећање дијаметра инфрареналног сегмента абдоминалне аорте, али без остварених критеријума за анеуризматско проширење. Због перзистирајућег бола и повишених параметара инфламације начињени су додатни радиолошки прегледи. Магнетнорезонантни преглед лумбосакралне кичме и накнадно урађена КТ ангиографија открили су новоформирано сакуларно анеуризматско проширење инфрареналног сегмента абдоминалне аорте са ретроперитонеалним хематомом и локорегионалним ретроперитонеалним конгломератима лимфних чворова. Болесник је хитно збринут хибридним ендоваскуларним третманом, односно пласирањем аортоуниилијачног стент-графта са контралатералном оклузијом илијачне артерије, у комбинацији са феморофеморалним бајпасом. Контролна КТ ангиографија потврдила је успешно искључење анеуризме из циркулације, без знакова ендолика.

Закључак Овај случај истиче ретку васкуларну компликацију код болесника са СЛЛ/ХЛЛ и указује на то да лимфомска инфилтрација и инфламација могу допринети слабљењу зида аорте. Хибридно ендоваскуларно лечење представља ефикасну терапијску опцију.

Кључне речи: хронична лимфоцитна леукемија; Б-ситноћелијски нон-Хоџкинов лимфом; анеуризма абдоминалне аорте; ендоваскуларна процедура

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Subcutaneous onlay laparoscopic approach (SCOLA) in the treatment of epigastric hernia in an obese patient: a feasible minimally invasive option – a case report and literature review

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SUMMARY

Introduction Primary ventral hernias are a common surgical entity, with epigastric hernias representing a specific subtype. The evolution of minimally invasive surgery has led to the development of various techniques, including the subcutaneous onlay laparoscopic approach (SCOLA). This report aims to present a case of an epigastric hernia in an obese patient successfully treated with the SCOLA technique and to review the relevant literature.

Case outline A 31-year-old female patient with a body mass index of 36.7 kg/m^2 presented with a symptomatic epigastric hernia. The diagnosis was confirmed by clinical examination and ultrasound, which revealed a 4.3 cm fascial defect containing preperitoneal fat. The patient underwent elective SCOLA repair. The procedure involved creating a subcutaneous working space above the anterior rectus sheath, hernia sac reduction, primary closure of the fascial defect, and placement of a TiO_2 mesh fixed to the anterior rectus sheath. The surgical procedure was completed successfully without intraoperative complications. The patient was discharged on the first postoperative day. On day 14, the skin sutures were removed, revealing a well-healed wound. Follow-up examinations at three, six, and 12 months revealed no evidence of recurrence or mesh-related complications.

Conclusion The SCOLA technique represents a safe and effective minimally invasive option for the treatment of primary epigastric hernias, particularly in obese patients. It combines the benefits of mesh reinforcement with a completely extraperitoneal and extrafascial position, avoiding intra-abdominal mesh placement and its associated risks.

Keywords: epigastric hernia; SCOLA; minimally invasive surgery; ventral hernia; obesity

INTRODUCTION

Minimally invasive surgical approaches to ventral hernia repair continue to present a challenge for many surgeons, despite their well-documented advantages over open surgery. The first minimally invasive technique for ventral hernia repair was described by LeBlanc and Booth in the early 1990s, marking a significant milestone in the field [1]. Since that time, there has been a continuous effort to establish minimally invasive approaches as the standard of care, leading to the development of numerous techniques. These range from intraperitoneal onlay mesh (IPOM) procedures to various preperitoneal and extraperitoneal approaches, each with specific indications and technical nuances [2].

In recent years, Claus and Malcher introduced a completely novel concept: the subcutaneous onlay laparoscopic approach (SCOLA) [3]. Originally described for the treatment of ventral hernias associated with rectus abdominis diastasis (RAD), this technique has since been applied to a broader spectrum of conditions. Contemporary literature documents its use in primary ventral hernias, including umbilical and epigastric hernias, both with and without concomitant diastasis [4, 5].

A primary ventral hernia is defined as a defect in the anterior abdominal wall that occurs in the absence of prior trauma or surgical intervention. These hernias are characterized and classified according to their localization and size [6]. Clinically, patients most commonly present with a visible bulge on the anterior abdominal wall, accompanied by varying degrees of discomfort, pain, and functional limitations that correlate with the size of the hernial defect. Diagnostic options include clinical examination, ultrasound imaging, and computed tomography, which together provide comprehensive anatomical information essential for surgical planning [6, 7].

Surgical intervention remains the only curative treatment modality. It is now widely accepted that minimally invasive approaches have become superior to open surgery in terms of postoperative recovery, wound complications, and hospital stay. However, the choice of operative technique depends significantly on the surgeon's experience, patient-specific

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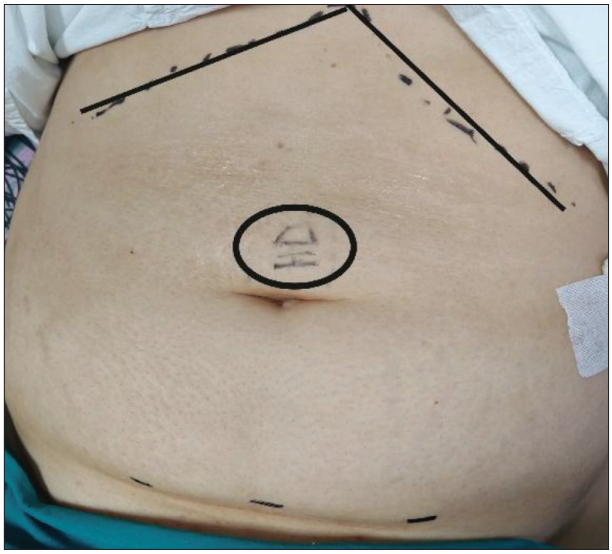

Figure 1. Preoperative marking of anatomical landmarks and planned port positions on the anterior abdominal wall

characteristics, and the availability of appropriate equipment [7, 8, 9].

The aim of this report is to present a case of a minimally invasive approach to primary ventral hernia repair in an obese patient, utilizing the SCOLA technique, and to provide a detailed description of the operative method.

CASE REPORT

A 31-year-old female patient with a body mass index of 36.7 kg/m² was admitted to the Department of Minimally Invasive Surgery for elective surgical treatment of an epigastric hernia. The diagnosis had been established prior to hospitalization through clinical examination and ultrasound imaging of the anterior abdominal wall. Ultrasound examination confirmed a hernial defect located approximately 4 cm above the umbilicus, with hernial contents corresponding in density to adipose tissue. The diameter of the fascial defect was measured at 4.3 cm.

Preoperatively, anatomical landmarks were marked on the patient's anterior abdominal wall, including the costal margins, the position of the hernial defect, and the planned port insertion sites. Additionally, the anesthesiology team administered a bilateral rectus sheath block to achieve prolonged postoperative analgesia (Figure 1).

Following standard preparation of the operative field and under general endotracheal anesthesia, a 12 mm skin incision was made in the midline, at the level of a line connecting the anterior superior iliac spines. Dissection was carried down to the anterior sheath of the rectus abdominis muscle. A 12 mm working port was inserted, through which a laparoscope was introduced. Using the laparoscope, the subcutaneous space was developed laterally on both sides. Subsequently, two additional 5 mm working ports were inserted at the pre-marked positions.

Using an ultrasonic dissecting device (Harmonic scalpel), the working space was further developed along the anterior rectus sheath, progressing cranially toward the hernial defect. The defect was clearly identified, and the hernia sac was opened, revealing preperitoneal adipose tissue as its contents (Figure 2). Dissection was continued proximally toward the xiphoid process to ensure adequate space for mesh placement.

After achieving sufficient working space, the fascial defect was primarily closed using 2-0 Prolene sutures. An oval TiO_2-coated mesh, measuring 15 × 12 cm, was then introduced and positioned over the closed defect. The mesh was secured to the anterior rectus sheath with interrupted 2-0 Prolene sutures. Additionally, the umbilicus was fixed to the anterior rectus sheath using a 2-0 V-Loc™ suture to restore normal anatomical contour (Figure 3).

The subcutaneous space was desufflated, and all skin incisions were closed with interrupted sutures. The patient was discharged on the first postoperative day. Skin sutures were removed on postoperative day 14, revealing a well-healed surgical site (Figure 4). Follow-up examinations were performed at three, six, and 12 months postoperatively. No recurrence or postoperative complications were observed during this period.

Written consent was obtained from the patient for the use of medical documentation for publication.

DISCUSSION

The SCOLA technique represents a recent addition to the spectrum of minimally invasive anterior abdominal wall

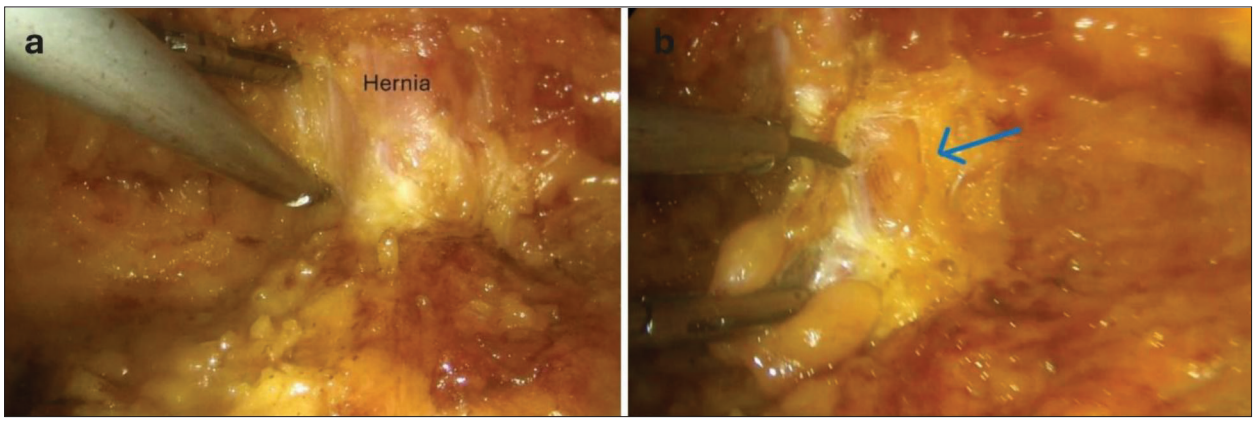

Figure 2. a, b: Intraoperative view: the hernial defect is identified, and the hernia sac is opened, revealing preperitoneal adipose tissue

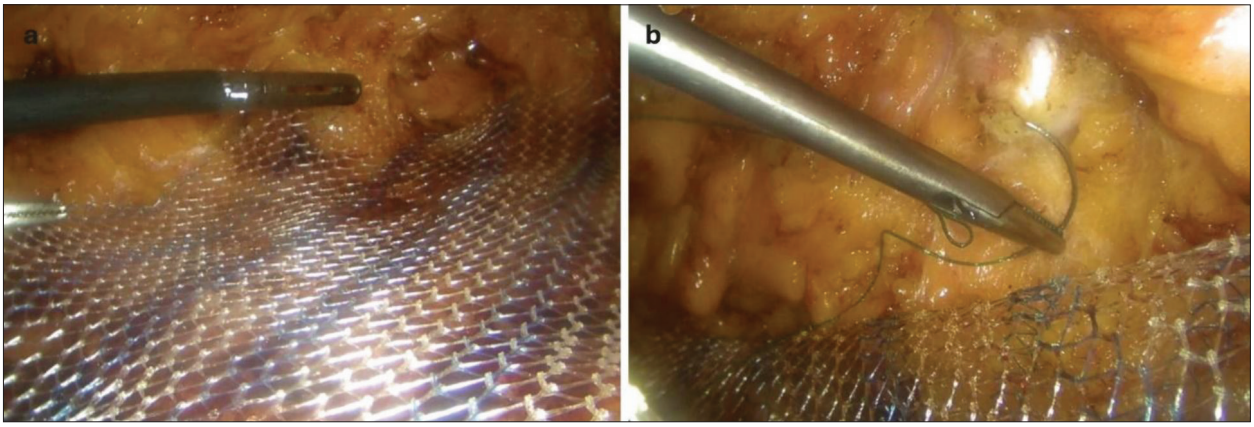

Figure 3. Intraoperative view: the TiO_2-coated mesh is positioned and fixed to the anterior rectus sheath with interrupted sutures following primary defect closure

Figure 4. Postoperative appearance at 14 days following suture removal, showing well-healed incisions and restored umbilical contour

hernia surgery. Initially described by Claus et al. in 2018 for the treatment of ventral hernias associated with rectus diastasis, its application has progressively expanded [3]. Our case demonstrates the successful application of this technique in an obese patient with a primary epigastric hernia, contributing to the growing body of evidence supporting its broader utility [4, 5, 8].

Obesity presents a particular challenge in ventral hernia repair. This patient population is at increased risk for wound complications, recurrence, seroma formation, and surgical site infections following both open and laparoscopic procedures [7]. In our patient, with a BMI of 36.7 kg/m^2, the choice of surgical technique was carefully considered. Traditional IPOM repair, while effective, requires entry into the peritoneal cavity and placement of an intraabdominal mesh, which carries risks of mesh migration, visceral adhesions, and other complications [2, 9]. Recent comparative studies have demonstrated that the material cost of IPOM plus is significantly higher than that of SCOLA, which is an important consideration in contemporary healthcare systems [3, 4]. The SCOLA technique elegantly circumvents these issues by maintaining the mesh in a completely extraperitoneal and extrafascial position.

From a technical standpoint, our approach followed the principles outlined by Claus and Malcher [3], with certain modifications tailored to our patient's anatomy. Primary closure of the fascial defect prior to mesh placement, a maneuver emphasized by several authors, was performed to restore the functional integrity of the abdominal wall and to reduce the likelihood of seroma formation [4, 7]. Mehta et al. [5], in their prospective study of 33 patients undergoing SCOLA, reported no recurrences during follow-up periods ranging from 4 to 18 months, supporting the efficacy of this approach.

The choice of mesh material represents another critical consideration. We utilized a TiO_2-coated mesh, which has demonstrated favorable tissue integration properties and reduced inflammatory response in experimental studies. In the context of SCOLA repair, where the mesh is placed directly beneath the subcutaneous tissue, such properties are particularly advantageous. The fixation technique, utilizing interrupted transfascial sutures, ensures stable mesh positioning while minimizing the risk of migration. Additionally, we performed umbilicopexy to restore the natural umbilical contour, an often overlooked but aesthetically important step that contributes to patient satisfaction [10].

When comparing SCOLA to other minimally invasive approaches, several distinct advantages emerge. Unlike IPOM, it avoids peritoneal entry, thereby reducing the risk of organ injury and adhesion formation. Deshpande et al. [4] conducted a prospective observational study comparing IPOM plus with SCOLA for medium ventral hernias (2–4 cm) and found that postoperative pain was significantly lower in the SCOLA group on postoperative day 1, at discharge, and at first follow-up. This finding aligns with our experience, as our patient required minimal postoperative analgesia and was discharged on the first postoperative day. Compared to TAPP or eTEP, SCOLA does not require dissection of the retrorectus space, which can be technically demanding and time-consuming, particularly in obese patients [6, 10]. Furthermore, in patients with concomitant rectus diastasis, SCOLA offers the unique advantage of addressing both pathological conditions through a single approach [1, 8, 11].

The diagnostic pathway in our patient followed established recommendations. Ultrasound examination proved sufficient for accurate diagnosis and measurement of the fascial defect, consistent with the experience of other authors who advocate for ultrasound as a first-line imaging modality in suspected ventral hernias [6]. While computed tomography provides additional anatomical detail and is invaluable in complex or recurrent cases, our experience suggests that in straightforward primary hernias with clear clinical presentation, ultrasound alone may be adequate for surgical planning.

Postoperative recovery in our patient was rapid, with discharge on the first postoperative day and return to normal activities within two weeks. This favorable outcome aligns with published series reporting reduced postoperative pain, shorter hospital stays, and earlier return to work following SCOLA repair compared to open and intraperitoneal techniques [4, 8].

The absence of recurrence at 12-month follow-up is encouraging, although longer-term surveillance is necessary. Current literature reports low recurrence rates following SCOLA repair, with most series documenting follow-up periods of 12–24 months [4, 5, 8]. Nevertheless, the principle of mesh reinforcement of a primarily closed defect, which forms the basis of this technique, is well-established in hernia surgery and provides a sound biomechanical rationale for its efficacy.

Several limitations of this report should be acknowledged. As a single case report, our findings cannot be generalized without confirmation in larger series. Additionally, the follow-up period, while adequate for assessing early outcomes, is insufficient to draw definitive conclusions about long-term recurrence rates. Seroma formation, reported in 10–30% of SCOLA cases in larger series [3, 4, 5], was not observed in our patient, possibly due to meticulous surgical technique. However, the primary value of this report lies in its detailed technical description and demonstration of feasibility in a challenging patient population.

The SCOLA technique represents a safe, feasible, and effective minimally invasive option for the treatment of primary epigastric hernias, particularly in obese patients. It combines the benefits of mesh reinforcement with the advantages of an extraperitoneal approach, avoiding intra-abdominal mesh placement and its associated risks. Our experience demonstrates that with appropriate patient selection and meticulous surgical technique, good outcomes can be achieved.

Ethical compliance statement: We confirm that we have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines.

Ethical standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Conflict of interest: None declared.

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Супкутани онлеј лапароскопски приступ (*SCOLA*) у лечењу епигастричне хернијe код гојазног болесника: изводљива минимално инвазивна опција – приказ болесника и преглед литературе

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САЖЕТАК

Увод Примарне вентралне хернијe представљају чест хируршки ентитет, при чему епигастричне хернијe чине специфичан подтип. Развој минимално инвазивне хирургије довео је до појаве различитих техника, укључујући и поткожни онлеј лапароскопски приступ (*SCOLA*). Циљ овог рада је да прикаже случај епигастричне хернијe код гојазне болеснице успешно лечене *SCOLA* техником, уз опис оперативне технике и преглед литературе.

Приказ болесника Болесница старости 31 годину, са индексом телесне масе 36,7 kg/m^2, јавила се са симптоматском епигастричном хернијом. Дијагноза је потврђена клиничким и ултразвучним прегледом, којим је откривен фасцијални дефект од 4,3 *cm* са садржајем који је чинило преперитонеално масно ткиво. Болесници је урађена елективна *SCOLA* репарација. Процедура је подразумевала стварање поткожног радног простора изнад горње фасције правог мишића (*musculus rectus abdominis*), репонирање хернијалне кесе, примарно затварање фасцијалног дефекта и постављање TiO_2 мрежице фиксиране за предњу фасцију правог мишића. Хируршка процедура је успешно завршена без интраоперативних компликација. Болесница је отпуштена првог постоперативног дана. Четрнаестог дана након операције уклоњени су кожни шавови, при чему је рана уредно зарасла. На контролним прегледима након три, шест и 12 месеци није било знакова рецидива нити компликација повезаних са мрежицом.

Закључак *SCOLA* техника представља сигурну и ефикасну минимално инвазивну опцију за лечење примарних епигастричних хернија, посебно код гојазних болесника. Она комбинује предности ојачања мрежицом са потпуно екстраперитонеалним и екстрафасцијалним приступом, чиме се избегава интраабдоминално постављање мрежице и пратећи ризици.

Кључне речи: епигастрична хернија; *SCOLA*; минимално инвазивна хирургија; вентрална хернија; гојазност

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Electroconvulsive therapy in a young adult patient with treatment-resistant depression and electroencephalogram epileptiform activity

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SUMMARY

Introduction Electroconvulsive therapy (ECT) causes a seizure which is different from a convulsion in epilepsy. Despite this, ECT remains a challenge in patients with comorbid epileptiform activity. Our aim was to indicate the importance and safety of the ECT in a young adult with treatment-resistant depression (TRD) and electroencephalogram (EEG) epileptiform activity.

Case report A 21-year-old female patient was scheduled for ECT due to TRD and suicidal behavior. During the preparation for ECT, EEG epileptiform activity was noticed, and upon the anticonvulsant medication administration, the patient received the acute ECT, combined with quetiapine XR 600 mg/d, duloxetine 60 mg/d and pregabalin 450 mg/d, since the disease was life threatening. We noted a significant increase in the stimulus dose, i.e., elevation in the convulsive threshold during ECT. The clinical outcome was without adverse events and with a reduction of the depression severity. The continuation of the maintenance ECT, psychopharmacotherapy and psychotherapy was planned.

Conclusion ECT is the most effective therapy for TRD, while the presence of EEG epileptiform activity is not a contraindication for ECT, with the advantage of the ECT Thymatron IV device (Somatics Inc., Venice, FL, USA), as a four-channel digital EEG machine, allowing us to record and analyze EEG.

Keywords: depression; ECT; EEG; young adults; suicidal behavior

INTRODUCTION

In the era of technological advancement, depression is one of the leading mental health problems among young adults. The prevalence of treatment-resistant depression (TRD) in young adults is as high as 40% and is associated with a higher risk of suicide [1, 2]. Young adults with depression often use deliberate self-harm which is an important risk factor for suicide attempts [3].

ECT (electroconvulsive therapy) reduces the severity of depression faster and more significantly compared to antidepressants [4, 5]. In adolescents younger than 18 years with depression and suicidal behavior (SB), the effectiveness of ECT is increasing with age, and with more than eight ECT sessions in a series (acute ECT) [6, 7].

It is important to keep in mind that ECT causes a seizure which is different from a convulsion in epilepsy, namely there is no abnormal connection and therefore does not lead to the appearance of spontaneous epileptic activity. Despite this, ECT remains a challenge in patients with epilepsy and comorbid epileptiform activity [8, 9].

Our aim was to indicate the importance and safety of ECT in a young adult patient with TRD and electroencephalogram (EEG) abnormal waveforms.

CASE REPORT

A 21-year-old female patient has been treated for depression since the age of 18, and the diagnosis of recurrent depressive disorder was established based on the International Classification of Diseases, tenth revision criteria at the age of 19. The course of treatment is characterized by frequent hospitalizations (six in total) due to maintenance of depression and SB (deliberate self-harm using razor blades – approximately 50 new and 100 old scars in the area of breasts, abdomen, upper legs, forearms; and deliberate self-poisoning using medications and alcohol) (Figure 1).

She has mostly been prescribed two or more psychiatric medications: antidepressants (duloxetine 30–60 mg/d, fluoxetine 20–40 mg/d, sertraline 50 mg/d, escitalopram 10–20 mg/d), antipsychotics (olanzapine 5–20 mg/d, cariprazine 3 mg/d, aripiprazole 15 mg/d, chlorpromazine 150 mg/d, clozapine – missing data, quetiapine 50–600 mg/d), mood stabilizers (lithium carbonate 600–1200 mg/d, valproate 500 mg/d), benzodiazepines and non-benzodiazepines (clonazepam 1 mg/d, pregabalin – missing data), psychostimulants (methylphenidate 18–36 mg/d).

Due to TRD and high suicide risk, the patient was referred to the Clinic of Psychiatry Military Medical Academy for acute ECT. She

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Figure 1. Deliberate self-harm in our patient with treatment-resistant depression

was admitted with the following therapy: duloxetine 60 mg/d, pregabalin 450 mg/d, and quetiapine XR 600 mg/d, and this therapy was continuously maintained during acute ECT. Among other medications, she had desmopressin 120 mg/d, prescribed by a urologist for nocturnal enuresis. Due to recent deliberate self-harm, she was surgically examined and antibiotic therapy (trimethoprim sulfamethoxazole) was prescribed for seven days. With a body mass index (BMI) of 33.1 kg/m^2, a hygienic-dietary regimen was implemented in accordance with the dietician instructions.

It should be noted that she is unemployed, childless, and that she lives with her sister. At the age of two, she was observed at the Institute for Maternal and Child Health Care in Belgrade, due to a head injury as the result of the fall. An EEG was performed, and the findings were normal. A computed tomography (CT) of the head showed discrete brain edema. During growing up, nocturnal enuresis, nail biting and thumb sucking were present. The patient's mother informed us that, during the school period, patient had episodes of staring at one spot or at another student, for couple of seconds during which she seemed "absent". Since she was 12 years old, due to nocturnal enuresis classified as primary enuresis, the urologist prescribed her desmopressin at a dose of 120 mg/d. She was subjected to verbal abuse and social isolation by her peers because of her physical appearance. Her mother punished her physically, with frequent verbal insults, while her father was absent and uninterested. She does not abuse psychoactive substances and alcohol. Heredity factor is present: father with alcohol use disorder, brother being treated for bipolar disorder, sister for anxiety-depressive disorder, two uncles committed suicide.

Preparation for ECT, description of ECT and ECT treatment

In accordance with the guidelines for ECT preparation [9] the following diagnostic procedures were performed: blood tests (complete blood count, metabolic panel, thyroid hormones) – test results were within the reference ranges; chest X-ray, electrocardiography, fundoscopy – unremarkable; magnetic resonance imaging of the brain – normal finding; EEG. She was examined by a cardiologist and an anesthesiologist prior to induction into general anesthesia.

The EEG showed a generalized spike-wave complex-like activity with an anterior maximum for about one second during hyperventilation and spontaneously at the end of

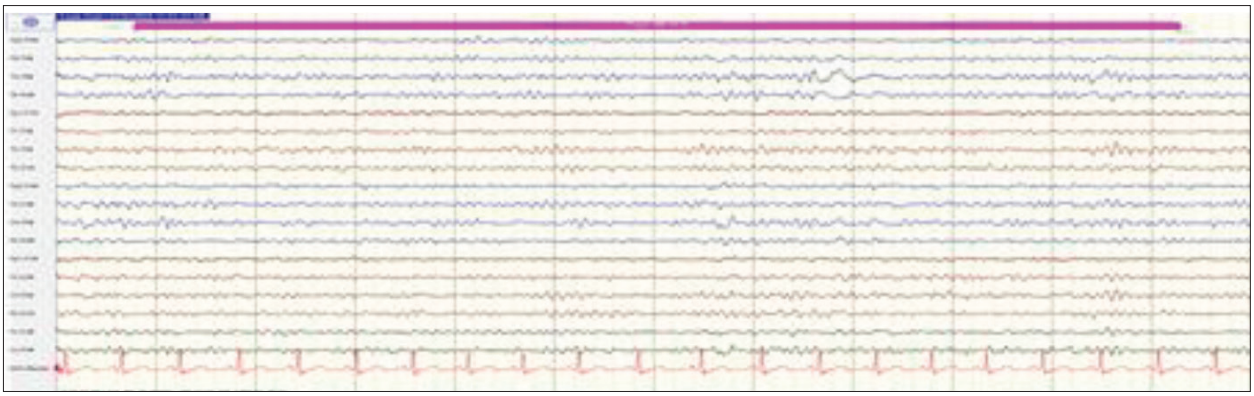

Figure 2. Abnormal electroencephalogram with epileptiform activity in our patient with treatment-resistant depression

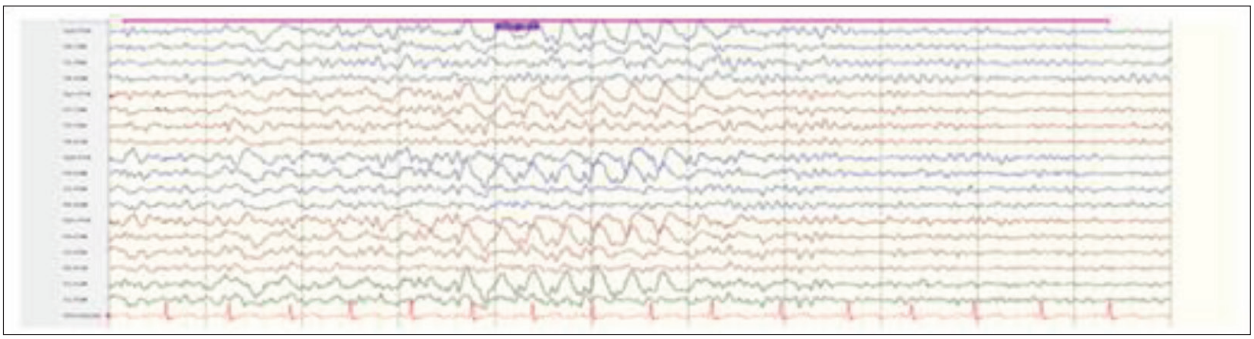

Figure 3. Abnormal electroencephalogram with epileptiform activity after sleep deprivation in our patient with treatment-resistant depression

ECT Tretman No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Days since ECT initiation	0	2	5	7	9	12	14	16	19	21	23	26	28	30	36	39	41	43	46	48
CD (mC)	50.4	50.6	75.0	75.1	74.8	100.2	99.1	150.4	151.0	175.7	200.9	200.2	199.5	199.5	199.6	199.3	200.1	201.2	200.8	201.1
SD (s)	5.6	5.6	4.2	4.2	4.2	5.6	5.6	5.6	5.6	6.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Frequency (Hz)	10	10	20	20	20	20	20	30	30	30	30	30	30	30	30	30	30	30	30	30
EMG	14	14	13	19	15	13	9	12	15	17	13	13	12	10	12	12	10	12	13	13
EEG	/	12	31	22	/	22	9	19	45	24	23	15	17	18	17	14	12	15	14	14
PSI (%)	/	19.4	92.8	64.9	/	<10	27.1	82.2	41.6	90.7	93.2	87.4	40.1	96.4	81	95.4	96.6	78.9	96.2	95.8

Figure 4. Acute electroconvulsive (ECT) therapy in our patient with treatment-resistant depression and abnormal electroencephalogram with epileptiform activity; CD – charge delivered; SD – stimulus duration; EMG – electromyography; EEG – electroencephalography; PSI – postictal suppression index

the recording, i.e., there are interictal borderline to specifically generalized epileptiform discharges (Figure 2).

The EEG after sleep deprivation indicated, that, during wakefulness and sleep, spike-wave complex series of 3 Hz lasting up to 5–6 seconds became more frequent with a maximum over the frontal regions and, more dominantly on the right side (Figure 3). Due to recorded EEG abnormalities, nocturnal bedwetting, and data about episodes of staring, the neurologist introduced anticonvulsant medication lamotrigine, with a gradual increase in the dose to 150 mg/d, desmopressin was removed from the therapy.

The following clinical scales were performed: Hamilton Depression Rating Scale 21 (HAM-D 21) score 32; childhood trauma questionnaire total score 62, emotional abuse score 21, physical abuse was 18; Mini-Mental State Examination score 30.

The patient signed an informed consent for ECT and general anesthesia. For ECT we utilized Thymatron System IV device (Somatics Inc., Venice, FL, USA), and brief pulses (0.5 ms), the electrodes were placed bi-temporal. Setting the stimulus dose was based on the "half-age" method [10]. Acute ECT was applied three times a week in general anesthesia. The patient received a total of 20 ECT sessions in acute ECT, with a recorded increase from the initial to final dose of the stimulus, 50.4 mC *vs.* 200.1 mC, respectively (Figure 4).

A more significant reduction in depressive symptomatology was recorded after fifth ECT session (HAM-D 21 score was 21), however therapy continued until 20th ECT session, i.e., until HAM-D 21 score was 11. There were no changes in cognitive functioning.

The combined administration of ECT and psychopharmacotherapy (quetiapine XR 600 mg/d, duloxetine 60 mg/d and pregabalin 450 mg/d), along with an anticonvulsant medicine, resulted in the termination of nocturnal enuresis and reduction in the severity of depression and SB. BMI at the end of hospital treatment was 29.4 kg/m^2, i.e., she lost 10 kg.

Ethics: The patient gave her informed consent and the study was approved by the Ethics Committee of the Military Medical Academy (Decision No. 19/2025).

DISCUSSION

The use of ECT in psychiatric patients with comorbid epilepsy may be considered a serious clinical challenge [9, 11, 12]. Accordingly, a study from 2006 reported that ECT is safe to use in patients with epilepsy and that no dose adjustments of anticonvulsant medications are necessary [13], and ECT can also be used for the treatment of refractory epilepsy and status epilepticus [14, 15]. There are studies stating the contradictory results, that after ECT there was a tendency to epilepsy [16, 17]. However, our experience so far, as in other researches, did not establish this [14, 15, 18]. The main disadvantage of the "half-age" method which we used is that it is not possible to know the individual seizure threshold, i.e., whether the stimulus dose is sufficiently above the convulsive threshold to ensure the effectiveness of ECT. In our patient, a significant increase in the stimulus dose, i.e., a raise in the convulsive threshold is evident.

Despite the fact that ECT shows a response rate of 50% to 90% in young adults with TRD with suicidal ideation or psychotic symptoms, its use in practice remains low [2, 19]. Our patient was diagnosed with recurrent depression at the age of 19, and was referred for ECT at the age of 21. Explanations for this discrepancy can be partly explained by the small number of ECT centers in Serbia. Namely, ECT was only applied at the Military Medical Academy Clinic of Psychiatry in Belgrade, until 2016, when ECT center at the Clinic of Psychiatry, Clinical Center Kragujevac was open [18]. Another possible explanation is a common belief about the association of ECT with cognitive impairments. By using reliable methods of cognitive testing (computerized neuropsychological tests), it has been shown that the deficit of cognitive functions

improves during the treatment of depression with ECT [20, 21, 22], therefore, the effect of ECT is the same as in the adult population [2].

Due to the severity of the clinical presentation, it was not possible to discontinue the use of psychopharmacotherapy in our patient, which is in accordance with the results of the positive effects of ECT in combination with antidepressants and antipsychotics, while lamotrigine of all mood stabilizers raises the seizure threshold the least [23].

This presentation also aims to emphasize the importance of EEG in psychiatry, namely, our patient had an EEG performed immediately after a traumatic brain injury and as part of preparation for ECT. After traumatic brain injury, EEG epileptiform activities were determined in 16 % of patients [24], however EEG changes are not uniform, that is, they depend on the severity and location of traumatic brain injury and can gradually develop over time (if serial recording is performed), which was not done in our patient [25]. In patients with bipolar disorder, it has been shown that abnormal EEG with epileptiform activity is possible predictor of lithium resistance [26]. Also, EEG is important for treating TRD, considering that treatment-resistance can occur in patients with epilepsy, that is, EEG can contribute to more effective treatment strategies for TRD, which was the case in our patient [27, 28]. The development of artificial intelligence indicates that deep learning methods based on EEG help in the early detection of patients with depression *vs*. healthy subject, with accuracy from 90% to even 99.24% [29].

In conclusion, the increase in mental health problems in young adults in recent decades, especially depression, requires comprehensible guidelines for the TRD therapy in this population. When choosing a treatment, one should always consider the risk and benefit of the proposed treatment, and the fact is that ECT is still the most effective therapy for TRD. ECT, in addition to being effective, is also a safe therapy for TRD, and the presence of EEG abnormalities is not a contraindication for ECT, with the advantage of the ECT Thymatron System IV device (Somatics Inc.), as a four-channel digital EEG machine, allowingus to record and analyze EEG.

Conflict of interest: None declared.

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Електроконвулзивна терапија код младог одраслог болесника са терапорезистентном депресијом и електроенцефалографском епилептиформном активношћу

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САЖЕТАК

Увод Електроконвулзивна терапија (ЕКТ) изазива напад који се разликује од конвулзија код епилепсије. Упркос томе, ЕКТ остаје изазов код болесника са коморбидном епилептиформном активношћу. Циљ рада је био да се укаже на значај и безбедност ЕКТ у лечењу терапорезистентне депресије код младе одрасле особе код које су утврђене електроенцефалографске (ЕЕГ) абнормалности са епилептиформном активношћу.

Приказ болесника Приказујемо болесницу стару 21 годину код које је због терапорезистентне депресије и суицидалног понашања била планирана ЕКТ. Током припреме за ЕКТ установљене су ЕЕГ абнормалности са епилептиформном активношћу, а болесница је након укључивања антиконвулзива подвргнута акутној ЕКТ, у комбинацији са кветиапином *XR* 600 *mg/d*, дулоксетином 60 *mg/d* и прегабалином 450 *mg/d*, јер је болест била опасна по живот. Примећено је значајно повећање дозе стимулуса, односно пораст конвулзивног прага током ЕКТ. Клинички исход је био без нежељених догађаја, уз редукцију тежине депресије. Планиран је наставак лечења одржавајућом ЕКТ, психофармакотерапијом и психотерапијом.

Закључак ЕКТ је најефикаснија терапија за терапорезистентну депресију, а присуство ЕЕГ абнормалности није контраиндикација за ЕКТ, уз додатну предност апарата за ЕКТ *Thymatron IV* (*Somatics Inc.*, Венеција, ФЛ, САД), који као четвороканални дигитални ЕЕГ уређај омогућава снимање и анализу ЕЕГ.

Кључне речи: депресија; електроконвулзивна терапија; електроенцефалографија; млади одрасли; суицидално понашање

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REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

Atopic dermatitis – novel insights in the immunopathology of the disease

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SUMMARY
Atopic dermatitis is an inflammatory skin disease that is characterized by a chronic-relapsing course and a pronounced feeling of itching. Altered production of structural proteins, lipids and antimicrobial peptides results in epidermal impairment, leading to the early onset of disease, more severe lesions and numerous comorbidities. Atopic dermatitis has recently been recognized as a systemic type 2 inflammation and emerging novel therapeutic modalities reflect sophisticated molecular and immunopathological pathways. The aim of this article is to provide a thorough review of the existing knowledge regarding the most significant immunological aspects of the development and progression of atopic dermatitis.
Keywords: atopic eczema; epidermis; inflammation; Th2 cells

INTRODUCTION

Atopic dermatitis (AD) is a dermatosis characterized by pruritus and typical cutaneous lesions that vary through acute, subacute, and chronic phases [1–6]. It affects approximately 20% of children and 2–10% of adults [1, 2, 4]. Additionally, 40–60% of patients with atopic dermatitis also have another form of atopy, such as asthma, allergic rhinitis or food allergy [1, 2, 4, 6]. The pathogenesis of AD reflects a multifactorial condition, with genetics underpinning epidermal barrier impairment as an initial state, triggered by environmental insults (allergens, irritants, pollutants) and microbiome alteration, contributing to the sustained type 2 inflammation [1, 5]. This article seeks to provide a thorough review of the current knowledge with regard to key mechanisms involved in the development and progression of atopic dermatitis.

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EPIDERMAL BARRIER IN AD

A damaged skin barrier in some hereditary diseases is directly related to the disease phenotype, while in atopic dermatitis it represents an initial condition that triggers further inflammation and sensitization [5, 7–9]. Abnormalities in the production of structural proteins, lipids, and antimicrobial peptides result in skin barrier dysfunction, early onset of disease, more extensive lesions and a greater number of comorbidities [1, 2, 5, 10]. An impaired barrier culminates in increased permeability of the epidermis, heightened transepidermal water loss (TEWL), compromised natural moisturization of the skin, dysregulated skin surface pH, and penetration of exogenous agents: irritants, aeroallergens, contact sensitizers, and microbial products [5, 11–14]. Epidermal barrier deficiency manifests clinically as extremely dry skin, followed by intense pruritus, scratching and rubbing, creating 'itch-scratch' cycle that underlies secondary bacterial infections and contact sensitization [5, 14–17].

The cornerstone of the skin barrier is the integrity of the stratum corneum. The main components of corneocytes are keratin filaments organized by filaggrin (FLG), which form the basis of the extracellular lipid matrix [2]. Defective FLG or its reduction contributes to the reduced production of acidic metabolites and an increase in skin pH, reduced activity of enzymes involved in lipid metabolism (beta-glucocerebrosidase, acid sphingomyelinase), accumulation and impaired secretion of lamellar bodies, and disruption of the entire lipid organization of the stratum corneum [7, 18, 19]. Apart from FLG, other essential constituents are loricrin, involucrin, corneodesmosin, SPRR3/4 (small proline-rich proteins) and claudin-1 and -3, which are vital for epidermal integrity [19]. Additionally, diminished ceramide levels in the stratum corneum contribute to increased TEWL and epidermal damage. Significant accumulation of very short ceramide chains and reduced ceramide/cholesterol ratio are present in AD patients [20].

Environmental agents contribute to impaired barrier permeability, affecting its structure and function, while simultaneously triggering keratinocyte activation and alarmin

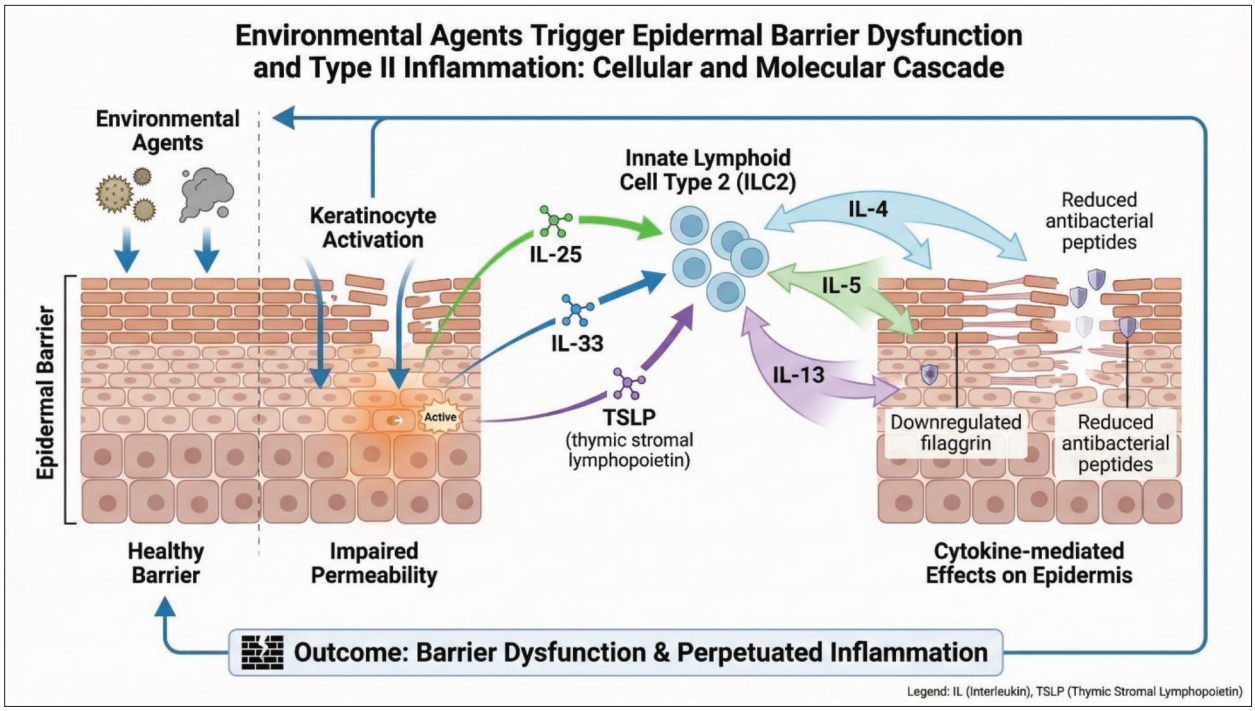

Figure 1. The ILC2 link between epidermal barrier damage and type 2 inflammation, following environmental insults; figure designed by figurelabs.ai

release – interleukin (IL)-25, IL-33 and thymic stromal lymphopoietin (TSLP), inducing involvement of type 2 innate lymphoid cells (ILC2s) with subsequent type 2 inflammation and IL-4, IL-5 and IL-13 secretion, reflecting the link between the epidermal barrier and inflammation [1, 9, 21, 22]. IL-4 and IL-13 in turn damage the epidermal barrier through altered filaggrin and loricrin expression, with reduced levels of antibacterial peptides, resulting in permanent epidermal dysfunction (Figure 1) [1, 9, 21, 22].

IMMUNE DYSFUNCTION DRIVES DISEASE

Keratinocytes and alarmin release

Thymic stromal lymphopoietin (TSLP), secreted by keratinocytes, plays a paramount role in altered immune response observed in allergic diseases and AD [2, 18]. TSLP, as an interleukin-7 cytokine, acts on dendritic cells to stimulate the naïve CD4+ T lymphocytes, inducing the production of IL-4, IL-5 and IL-13 [2, 21]. This induction of Th2 cytokines impacts other immune cells such as mast cells, basophils, innate lymphoid cells (ILCs), macrophages and epithelial cells [2]. Beyond TSLP, keratinocytes also release other alarmins such as IL-25 and IL-33 [2, 18]. IL-33, part of the IL-1 cytokine family, is produced mainly by epithelial cells in response to allergenic and microbial stimuli. It promotes a type 2 immune response by activating ILC2s, leading to the secretion of IL-5 and IL-13 [22]. IL-33 reduces filaggrin expression in keratinocytes, generates the itch sensation, and activates Th1 and Th2 cells. Elevated levels of IL-33 are observed in lesional AD skin, while serum IL-33 levels correlate with disease severity [22].

Langerhans cells and innate lymphoid cells type 2 are highly positioned in the immune hierarchy

Langerhans cells (LC) are pivotal in AD pathogenesis, alongside keratinocytes [23, 24]. LCs participate in the recruitment and polarization of various immune cells in AD [25]. GWAS studies linked CD207 gene mutations to a heightened risk of AD. LCs show increased activity and proliferation in an AD model [25]. LCs are basically antigen-presenting cells and are responsible for the activation of Th2 and B-lymphocytes. It has been experimentally shown that TSLP can induce AD precisely through the activation of LC. Activated LCs secrete CCL17 and CCL22 and thus further polarize the Th2 phenotype [25]. Additionally, LCs facilitate IgE production by mature B-cells and binding through low-affinity receptor FcεRII/CD23 and FcεRI, resulting in IL-16-mediated attraction of Th cells, inflammatory epidermal dendritic cell (IDEC) precursors, and eosinophils. IDECs in turn, bind IgE via FcεRI, polarizing naïve T-cells into Th1 characteristic of chronic AD [25]. LCs also secrete CCL5, enhancing eosinophil infiltration typical of AD [25].

The activation of T-cells, eosinophils, basophils, macrophages, mast cells, and innate lymphoid cells type 2 (ILC2), along with secretion of cytokines, culminates in localized inflammation in the atopic skin [23]. ILC2s are fundamental in shaping the immune response, maintaining tissue homeostasis, and driving inflammation [20]. Elevated levels of active ILC2s present in AD lesions are associated with increased levels of type 2 cytokines and inflammation, whereas decreased numbers of NK cells were noted in the blood of AD patients [20]. ILC2s secrete IL-4, IL-5, IL-9 and IL-13 upon activation by alarmins (TSLP, IL-25, IL-33)

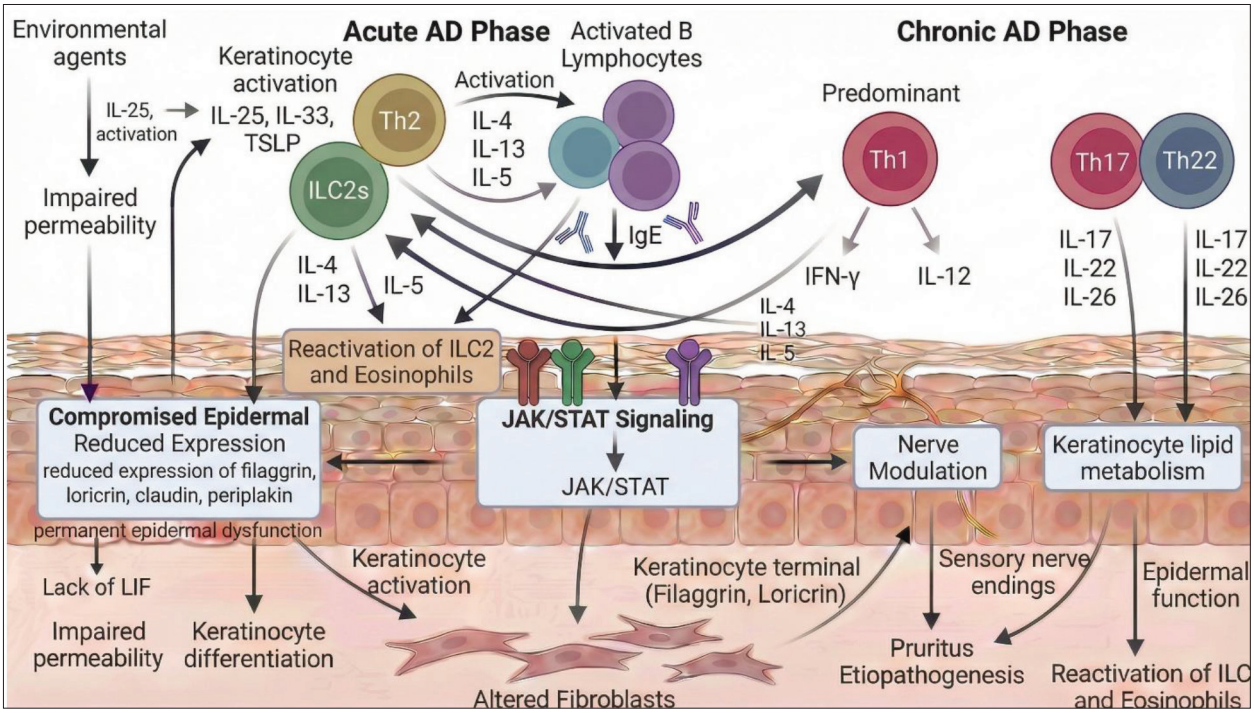

Figure 2. The immunopathogenesis of atopic dermatitis reflects the initial phase of keratinocyte activation, alarmin-stimulated ILC2s and the secretion of IL-4, IL-5 and IL-13, which further promote Th2-driven inflammation in acute atopic dermatitis; Th1 cells with gamma interferon and IL-12 release predominate in the chronic phase; activation of B cells is responsible for IgE secretion; figure designed by figurelabs.ai

or eicosanoids, even in the absence of antigenic stimulation, fostering a Th2 response [22, 26]. Regarding the main immune pathways, the interaction of OX40L/OX40 between stimulated dendritic cells and T cells, may represent a key mechanism in atopic inflammation [1, 9, 21, 22]. The OX40L-OX40 interaction between dendritic cells and T-cells also contributes to this response [22]. OX40 (CD134) expression is enhanced on T cells, following specific T cell receptor activation, OX40L-OX40 mediates the antigen presentation process and plays a significant role in allergic diseases and tumor pathologies. Recent studies indicate that inhibiting OX40-OX40L interactions suppresses T cell-driven inflammation in AD [22, 26].

The persistent "vicious cycle" of immune activation

The inflammatory "vicious cycle" and epidermal barrier damage are initiated by activated keratinocytes and Langerhans cells, which secrete IL-10, IL-12, IL-18, IL-23, IL-17, IL-22, TSLP. This cascade further triggers Th2-mediated inflammation and the secretion of cytokines IL-4, IL-13, IL-31 and IL-22, compromising the epidermal barrier by reducing the expression of filaggrin, loricrin, claudin, and periplakin [20, 22, 27]. Concurrently, type 2 cytokines (IL-4, IL-5, IL-13) reactivate ILC2 and eosinophils [23, 27].

The increased production of IgE in AD stimulates mast cells and basophils [5, 26]. IgE can be non-specific (intrinsic AD–iAD) and allergen-specific (extrinsic AD–eAD). Elevated levels of specific IgE increase the risk of developing atopic march [23]. IgE-mediated stimulation of mast cells triggers degranulation and release of histamine, IL-6, IL-8, PGD2, αTNF, IL-23, and IL-31 [27]. Specific IgE binds to and activates dendritic epidermal cells and epidermal Langerhans cells that secrete proinflammatory cytokines TSLP, CCL17, CCL18, CCL22, and IL-33, initiating T-cell sensitization and subsequent inflammation [20].

Numerous studies underscore the importance of Th2-mediated inflammation in allergic diseases [28]. Th2 inflammation is a key phenomenon in the onset of immune dysfunction leading to the acute phase of atopic dermatitis characterized by high levels of IL-4 and IL-13. These cytokines activate B lymphocytes to secrete IgE, adversely affecting filaggrin production in a feedback loop, while the release of IL-5 sustains eosinophilia in AD patients [19]. Type 2 inflammatory response is characteristic of antiparasitic and antitoxic effects, and allergic inflammation [28]. This type of immune response initially involves ILC2 and keratinocytes, along with dendritic cells, macrophages and Langerhans cells, extending to the activation of Th2, Tc, NK, B-cells, eosinophils, mast cells and basophilic granulocytes, leading to the secretion of IL-4, IL-5, IL-9, IL-13, IL-31 and IgE, the primary mediators of inflammation in AD (Figure 2) [28]. Clinical studies have already shown the efficacy of monoclonal antibodies directed against IL-4, IL-13, and IL-31, or their receptors, in the treatment of AD [1, 5, 22].

In chronic AD lesions, the Th1-mediated immune response predominates, featuring gamma-interferon and IL-12 [5, 26]. Over time, some patients also exhibit a Th17/Th22 response that significantly affects keratinocyte lipid metabolism and epidermal function [27, 29]. Dendritic cells and eosinophils produce IL-2 and IL-18 while activating Th1 lymphocytes and promoting secretion of

αTNF, IL-2, IL-12, αINF, favoring chronic inflammation in AD. Th1-released γIFN induces keratinocyte apoptosis, whereas IL-17 and IL-22 induce epidermal acanthosis clinically manifested by lichenoid lesions of chronic AD [22, 27]. Chronic AD lesions show elevated levels of IL-5 and IL-12, and decreased levels of IL-4 and IL-13 [27]. Furthermore, increased expression of VEGF originating from keratinocytes and mast cells is detected in AD, mediating angiogenesis in chronic inflammation [27]. Research has demonstrated that IL-17A induces Th2 inflammation, with IL-4 reversibly inhibiting IL-17A. Thus, elevated levels of IL-17A were detected in peripheral blood mononuclear cells in severe AD [30]. γδ T-cells, a primary source of IL-17A, participate in inflammation, tissue repair, pathogen elimination, immune regulation and tumor suppression [19]. AD patients exhibit elevated levels of circulating Vγ9Vδ2+ T-cells, which also produce IL-4 and IL-13, crucial in the pathogenesis of AD. Beyond allergic cutaneous inflammation, γδ T-cells also contribute to airway allergic inflammation by promoting IgE reactivity and Th2 inflammation [30]. According to previous studies, a subpopulation of lymphocytes, Th9 cells, and IL-9 may also have great potential in the development of allergic inflammation [31]. Elevated IL-9 levels in skin and blood observed in AD patients correlate with the SCORAD index, and findings suggest that IL-9 involved in the pathogenesis of AD originates from activated mast cells, not Th9 cells. However, studies found IL-9 levels correlate with Th9 cells and PU.1 transcript in peripheral blood mononuclear cells, as well as with VEGF expression in AD lesions [31].

Fibroblasts – the secret of AD inflammation

Recent studies highlighted the role of fibroblasts in the pathogenesis of AD. Altered fibroblasts in AD affect keratinocyte terminal differentiation markers, such as filaggrin and loricrin, and lack leukemia inhibitory factor (LIF), impairing epidermal differentiation [32]. In AD, particularly at the epidermal-dermal border, COL6A5+ COL18A1+ fibroblasts show an inflammatory phenotype (CCL2, CCL19, CCL26, IL-32). Abnormal fibroblast adhesion, disturbed synthesis and metabolism of collagen, and damaged epidermal barrier are observed [32]. Conversely, COL18A1 inhibits angiogenesis and disrupts dermal organization by binding to extracellular matrix components in AD. Furthermore, upregulated CCL19 and CCL2, interacting with CCR7, CCR1 and CCR2 on T-lymphocytes and dendritic cells, contribute to the infiltration of type 2 inflammatory response cells [32]. In addition, fibroblasts interact with Th2/Th22 and TRM (tissue-resident memory T cells) cells via CXCL12, promoting allergic inflammation in AD [32]. Experimental evidence has shown fibroblast-eosinophil interaction could play a significant role in the inflammatory cascade. In cell cultures, IL-37b inhibits IL-31/IL-33-induced expression of αTNF, IL-6, CXCL8, CCL2, and CCL5, promoting autophagic mechanisms through regulation of the AMPK-mTOR signaling pathway [32]. Namely, elevated levels of IL-33 and PRG4 inhibit NF-κB activation, a key regulatory inflammatory mechanism. Deletion of the Ikkb (inhibitor of nuclear factor kappa-B kinase subunit beta) gene leads to the development of AD manifestations, increased expression of CCL11, infiltration of eosinophils and Th2 inflammation [32].

The JAK/STAT signaling pathway as an integral part of AD inflammation

The JAK/STAT signaling pathway represents a central mechanism in modifying multiple immune processes in AD pathogenesis [26, 33, 34]. Previous research indicates that the JAK/STAT pathway enables IL-4, -5, -13, -31, and TSLP to exert effects, epidermal barrier regulation, nerve modulation and pruritus etiopathogenesis [26, 33, 34]. The JAK family includes four kinases associated with receptors: JAK1, JAK2, JAK3 (janus kinase 1/2/3) and TYK2 (tyrosine kinase 2), while STAT (signal transducer and activator of transcription) includes seven proteins: STAT1, STAT2, STAT3, STAT4, STAT5, STAT5a, STAT5b, STAT6 [26, 33, 34]. The proposed mechanism of the JAK/STAT signaling pathway includes phosphorylation of intracellular receptor domain, subsequently recruiting and inducing phosphorylated STAT dimerization that culminates in translocation into the nucleus and regulation of gene transcription by binding to DNA [26, 33, 34]. TSLP binds to the heterodimeric receptor TSLPR, containing the IL-7Rα receptor, and interacts with JAK1 and JAK2, with further STAT5 activation [34]. IL-4 binds to the IL-4R type I receptor, resulting in JAK1 and JAK3 phosphorylation, which activate and phosphorylate the IL-4Rα chain (common to IL-13Rα1 in type II IL-4 receptor) and STAT6. IL-4 and IL-13 bind to IL-4R type II leading to JAK1 and TYK2 phosphorylation, STAT3 and STAT6 activation, inducing reduced filaggrin expression, epidermal barrier dysfunction, and increased TSLP, IL-25, IL-33 production in keratinocytes [26, 33, 34]. IL-5 binding to the α subunit of its receptor, and IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) binding to the β subunit, activate and phosphorylate JAK1 and JAK2, as well as STAT, STAT3 and STAT5 [26, 33, 34]. IL-31 binds to its receptor complex's α subunit, containing oncostatin-M-receptor-β (OSMRβ). The IL-31Rα-OSMRβ complex activates JAK1 and JAK2, STAT3 and STAT5, and to a lesser extent, STAT1 [26, 33, 34].

During AD's chronic phase, Th1, Th17 and Th22 subpopulations and cytokines sustain local inflammation, the presence of proinflammatory cytokines, and epidermal hyperplasia [26, 33, 34]. The JAK-STAT signaling pathway indirectly influences differentiation and function of Th17 cells through the activation of STAT3, affecting the release of pro-Th17 cytokines: IL-6, IL-21, and IL-23, and the final production of IL-17. On the other hand, IL-22 after binding to its receptor leads to the phosphorylation of JAK1 and TYK2, and STAT3, STAT1 and STAT5 [26, 33, 34]. Elevated Th1 cytokine levels include γIFN, IL-12, and granulocyte colony-stimulating factor (G-SCF). IL-12 primarily stimulates naïve T-cell differentiation into the Th1 subpopulation. IL-12 receptor beta subunits binding activates JAK2 and TYK2, and STAT4, as well as STAT1, STAT3, and STAT5, to a lesser extent [26, 33, 34]. γIFN,

playing its pathophysiological role in epidermal dysfunction by reducing ceramide and long-chain fatty acids expression, upon its receptor binding, activates JAK1 and JAK2, and STAT1 [26, 33, 34]. JAK1's implication in epidermal barrier dysfunction is linked to JAK1-dependent exertion of IL-4, IL-5, IL-13, IL-22, TSLP, and γIFN [26, 33, 34]. Abundant JAK1 activation is associated with cutaneous overexpression of serine proteases and epidermal degradation, whereas STAT3, as a key transcription factor, impacts keratinocyte differentiation and thus skin integrity maintenance. Inhibition of JAK1/JAK2/JAK3/STAT3 axis benefits epidermal barrier functionality by enhancing filaggrin, loricrin and other factors responsible for epidermal homeostasis [26, 33, 34].

NOVEL DISCOVERIES AND EMERGING THERAPEUTIC TARGETS

Recent studies highlight the role of small molecules, receptors, ion channels, transcription factors and signaling pathways in AD development and treatment. Among these, phosphodiesterase-4 (PDE4) plays a crucial role in AD pathogenesis, with four subtypes, of which PDE4B particularly impacts the inflammatory response [26]. PDE4 is present in various cells, including basophils, mast cells, neutrophils, eosinophils, monocytes, macrophages, B and T lymphocytes, endothelial cells, and dermal fibroblasts [26, 35]. PDE4 primarily hydrolyzes cyclic adenosine monophosphate - cAMP, or cyclic guanosine monophosphate - cGMP [26, 36]. cAMP affects the regulation of the inflammatory and immune response, with elevated levels of cAMP suppressing lymphocytes and monocytes [26, 36]. AD patients exhibit increased adenylate cyclase levels, leading to accumulation of cAMP and compensatory PDE4 activation [35]. This stimulation of PDE4 results in inadequate cAMP metabolism, with elevated levels of prostaglandin E2, IL-6 and IL-10 in monocytes of AD patients, contributing to Th1/Th2 imbalance and Th2 cytokines prevalence [35, 36]. Inhibiting PDE4 prevents intracellular cAMP degradation, activation of PKA (protein kinase A) and Epac 1 / Epac 2 (exchange factor directly activated by cAMP 1/2), which lead to the inhibition of NF-κB mediation, promoting production of anti-inflammatory cytokines via CREB (cAMP-responsive element binding) protein interaction [26, 36]. PDE4 inhibition influences immune functions by regulating inflammatory cytokines, T cell activation, neutrophil degranulation, antigen presentation, epidermal integrity, and oxidative stress suppression [26].

AHR (aryl hydrocarbon receptor) and NRF2 (nuclear factor erythroid 2-related factor 2) are transcription factors of cytoprotective genes involved in detoxification mechanisms and antioxidant activity of enzymes [37]. AHR, located in the cytosol, forms complexes with several proteins [22, 26]. Upon ligand binding, AHR dissociates from other proteins, translocates to the nucleus, and binds to the DNA molecule, regulating target gene expression. These receptors exhibit pro-inflammatory and anti-inflammatory activity after exposure to various exogenous and endogenous factors. AHR is found in Treg, Th17, Th22, Tc, γδ T and ILCs [26]. AHR is involved in keratinocyte differentiation and proliferation mechanisms, inflammatory cytokine production, and T-cell immune regulation [22]. Studies confirmed AHR's critical role in FLG expression in human keratinocytes. Activated AHR binds to the EDC (epidermal differentiation complex) locus, regulates transcription factor OVOL1 and stimulates FLG, loricrin and involucrin expression [22]. Additionally, AHR regulates NRF2 activation, inducing expression of cytoprotective genes encoding detox and antioxidant enzymes [22, 38]. NRF2 activation interferes with Th2 cytokines via STAT6 dephosphorylation. NRF2 also suppresses proinflammatory cytokines (IL-6 and IL-1β) production by impairing NF-κB transcriptional activity [22]. Modulating AHR and NRF2 inducers achieves significant epidermal barrier regeneration and inflammation suppression in experimental AD models [22, 38, 39].

TLRs, as part of innate immunity, exhibit polymorphism in AD and pathological functions, affecting epidermal barrier repair delay, antimicrobial defense impairment, Th2 inflammation stimulation, Th1 response transition in AD chronicity, pruritus and UV radiation effects [40]. TLR activation is vital in microbial defense. Staphylococcal enterotoxin B increases TLR6 expression on monocytes in AD, while TLR2 stimulation on dendritic cells and monocytes leads to dysfunctional maturation and increased IL-17A production in AD [40]. Additionally, TLR2 activation interferes with IFNGR/JAK/STAT1 signaling pathway, reducing Th1 chemokines expression, while St. aureus ligands stimulate TLR2-mediated IL-10 secretion, directing Th1/Th17 response towards Th2 inflammation [40]. TLR agonists showed ameliorating effects on AD symptoms, barrier function and inflammation, while several experimental models underlined the need for careful modulation instead, considering diverse TLR functions in AD and inconsistent expression levels in various stages of the disease [40].

Research has focused on discovering new activation mechanisms for mast cells, crucial in allergic and atopic diseases, which could represent potential targets for novel therapies. MRGPRX2 (Mas-related G protein-coupled receptor-X2) has gained importance in AD pathogenesis research in recent years [41]. MRGPRX2 involves non-IgE-mediated mast cell degranulation [41]. Elevated levels of MRGPRX2 agonists, such as neuropeptide SP, human β-defensin, cortistatin 14, and TSLP, are present in AD [39]. MRGPRX2 is expressed by basophils and eosinophils, in addition to mast cells and sensory neurons. MRGPRX2 facilitates effector cell degranulation, leading to inflammation [41]. Antagonizing MRGPRX2 demonstrated significant improvement of AD phenotypic changes, preservation of involucrin expression and periostin downregulation, suggesting that MRGPRX2 may be a novel therapeutic target in AD [41].

In type 2 allergic diseases, including AD, there is an abnormally increased NLRP3 (NLR family pyrin domain containing 3) inflammasome activation [42]. NLRP3, a protein complex, consists of NLRP3 protein, ASC and

caspase-1. It can be activated via the NF-κB pathway, ion channels, and lysosome damage [42]. House dust mite allergens stimulate keratinocytes to form the NLRP3 inflammasome, secreting IL-1β and IL-18 and initiating inflammation [42]. NLRP3 inflammasome activation in keratinocytes increases production of ROS particles and NF-κB signaling pathway activation. Phosphorylation of p38, ERK and JNK in keratinocytes triggers the MAPK (mitogen-activated protein kinase) signaling pathway and in turn activates the inflammasome via MAPK/AP-1/NF-κB [42]. NLRP3/IPF4/IL-33 axis activation in keratinocytes is also identified in AD, contributing to disease pathogenesis [42]. Suppressing NLRP3 expression effectively reduces inflammation, highlighting NLRP3 inhibitors' therapeutic potential [42].

CONCLUSION

Given the accelerated implementation of new therapeutic options in clinical practice, such as biologics and small molecule therapies, we believe that an in-depth review of current knowledge in the field of immunopathogenesis of atopic dermatitis provides an essential overview of the basic principles that modern therapies are based on, while broadening horizons for future research. The coming years are expected to bring an expansion of the application of translational research in treatment and diagnostics, contributing to a more precise individual approach and stratification of patients with atopic dermatitis as well.

Ethics: The authors declare that the article was written in accordance with ethical standards of the Serbian Archives of Medicine as well as ethical standards of medical facilities for each author involved.

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Атопијски дерматитис – нови увиди у имунопатологију болести

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САЖЕТАК

Атопијски дерматитис је инфламаторна болест коже коју карактеришу хронично-рецидивајући ток и изражен осећај свраба. Нарушена продукција структурних протеина, липида и антимикробних пептида доводи до оштећења епидермиса и последичног раног настанка болести, израженијих лезија и бројних коморбидитета. Атопијски дерматитис је недавно препознат као системска инфламација типа 2, што уз нове терапијске модалитете одражава софистициране молекуларне и имунопатолошке путеве. Циљ овог чланка је да пружи свеобухватан преглед досадашњих сазнања о најзначајнијим имунолошким аспектима развоја и прогресије атопијског дерматитиса.

Кључне речи: атопијски екцем; епидермис; инфламација; *Th*2 ћелије

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарише. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, коментари по позиви, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant – Cross Check* (*iThenticate*). Радови код којих се докаже плагијаризам/ аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (https://www.nlm.nih.gov/mesh/meshhome.html)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (*°C*), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.

Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4* (*flv*). У првом кадру филма мора се навести: у

наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике" у СА се објављују фотографије, цртежи, схеме и графикони. Слике се означавају арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).

Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видео-приказа у е-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примери навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *https://www.nlm.*

nih.gov/bsd/uniform_requirements.html. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs/en/submission-letter/SubmissionLetterForm2023.pdf*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА И НАКНАДЕ ЗА ОБРАДУ И ОБЈАВЉИВАЊЕ ЧЛАНКА. Да би рад био разматран за објављивање у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 9 Статута Друштва) у години у којој рад предају на разматрање.

Следеће накнаде су обавезне како би рад био прегледан, обрађен и потенцијално објављен у *Српском архиву за целокупно лекарство*:

- накнада за преглед сваког примљеног рада домаћих аутора: 6.000 динара по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада домаћих аутора: 12.000 динара по раду;
- накнада за преглед сваког примљеног рада страних аутора: 75 евра (или 9000 динара) по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада страних аутора: 150 евра (или 18000 динара) по раду.

Накнаде се плаћају пре прегледања, односно пре објављивања рада. Радови за које нису плаћене накнаде неће бити прегледани, односно објављени.

Треба напоменути да уплата накнаде за преглед рада није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и накнаду за преглед чланка, као доказ о уплатама. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за преглед чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Онлајн систем за подношење радова водиће вас кроз поступак уноса података о чланку и отпремања ваших датотека. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и бројеве телефона.

АДРЕСА:
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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., ^{99}Tc, IL-6, O_2, CD8). If something is commonly written in italics, such as genes (e.g. *BRCA1*), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

CONFLICT OF INTEREST STATEMENT. The manuscript must be accompanied by a disclosure statement from all authors (contained within the Submission Letter) declaring any potential interest or stating that the authors have no conflict of interest. For additional information on different types of conflict of interest, please see World Association of Medical Editors (WAME, *www.wame.org*) policy statement on conflict of interest.

AUTHORSHIP. All individuals listed as authors should be qualified for authorship. Every author should have participated sufficiently in writing the article in order to take responsibility for the whole article and results presented in the text. Authorship is based only on: crucial contribution to the article conception, obtaining of results or analysis and interpretation of results; design of manuscript or its critical review of significant intellectual value; final revision of the manuscript being prepared for publication.

The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

PLAGIARISM. Since January 1, 2019 all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institution in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

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