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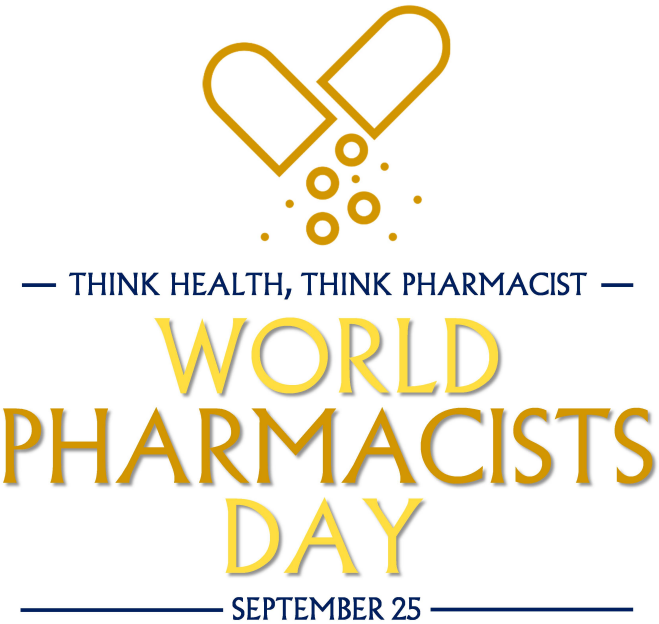

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CONTENTS / SADRŽAJ

World Pharmacists Day (WPD) is celebrated annually on September 25 with the aim of raising awareness of the importance of pharmacists and their great contribution to improving people's health. Each year, the WPD theme highlights various aspects of the pharmaceutical profession and the essential role of pharmacists in educating patients on the proper use of medications. This year's theme for WDP is "Think Health, Think Pharmacist".

Svetski dan farmaceuta (SDF) se obeležava 25. septembra svake godine, u cilju podizanja svesti o značaju farmaceuta i njihovom velikom doprinosu poboljšanju zdravlja ljudi. Tema SDF svake godine ističe različite aspekte farmaceutske profesije i značajnu ulogu farmaceuta u edukaciji pacijenata o pravilnoj upotrebi lekova. Tema ovogodišnjeg SDF je „Misliš na zdravlje, misli na farmaceuta".

ORIGINAL ARTICLES

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Diagnosis of bone metastasis in patients with non-small cell lung cancer by combined detection of CEA, CYFRA21-1, and ALP

Dijagnoza metastaza u kostima kod obolelih od nesitnoćelijskog karcinoma pluća kombinovanom detekcijom CEA, CYFRA21-1 i ALP

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Abstract

Background/Aim. Despite significant advances in the diagnosis and treatment of non-small cell lung cancer (NSCLC), the overall prognosis remains poor, especially when bone metastasis occurs with disease progression. The aim of this study was to examine the diagnostic value of combined detection of carcinoembryonic antigen (CEA), cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), and alkaline phosphatase (ALP) for bone metastasis in NSCLC patients. **Methods.** In total, 148 patients with NSCLC were included in the study. They were selected from patients hospitalized for treatment between April 2020 and March 2022. Out of the total number of patients, 68 were assigned to the metastasis group and 80 to the non-metastasis group. Their blood samples were collected to measure CEA, CYFRA21-1, and ALP levels in the serum. Multivariate logistic regression analysis was conducted to determine the factors contributing to bone metastasis. Receiver operating characteristic (ROC) curves were plotted to analyze the diagnostic value. **Results.** Bone metastasis occurred in 68 (45.94%) patients. The baseline data exhibited no significant intergroup differences ($p > 0.05$). The metastasis group had significantly raised serum CEA, CYFRA21-1, and ALP levels compared to those of the non-metastasis group ($p < 0.05$). The increases in serum CEA [odds ratio (OR): 1.062, 95% confidence interval (CI): 1.031–1.094], CYFRA21-1 (OR: 1.155, 95% CI: 1.061–1.258), and ALP (OR: 1.027, 95% CI: 1.008–1.047) were risk factors for bone metastasis (OR > 1, $p < 0.05$). The areas under the ROC curves of CEA, CYFRA21-1, ALP, and their combination were all greater than 0.600, suggesting high diagnostic values. **Conclusion.** CEA, CYFRA21-1, and ALP levels in the serum can predict bone metastasis in NSCLC patients, and the predictive value of their combination is higher than that of any single indicator.

Key words:
biomarkers, tumor; bone neoplasms; carcinoma, non-small-cell lung; diagnosis; neoplasm metastasis; treatment outcome.

Apstrakt

Uvod/Cilj. Uprkos značajnom napretku u dijagnostici i lečenju nesitnoćelijskog karcinoma pluća (*non-small cell lung cancer* – NSCLC), ukupna prognoza ostaje loša, posebno kada se sa progresijom bolesti javljaju metastaze u kostima. Cilj rada bio je da se ispita dijagnostička vrednost kombinovane detekcije karcinoembrionalnog antigena (*carcinoembryonic antigen* – CEA), fragmenta citokeratina 19 (*cytokeratin 19 fragment antigen 21-1* – CYFRA21-1) i alkalne fosfataze (*alkaline phosphatase* – ALP) za predikciju metastaza u kostima kod obolelih od NSCLC. **Metode.** U studiju je ukupno bilo uključeno 148 obolelih od NSCLC. Oni su odabrani među bolesnicima koji su bili hospitalizovani radi lečenja između aprila 2020. i marta 2022. godine. Od ukupnog broja bolesnika, njih 68 svrstano je u grupu sa metastazama, a 80 u grupu bez metastaza. Prikupljeni su uzorci krvi bolesnika da bi se izmerili nivoi CEA, CYFRA21-1 i ALP u serumu. Multivarijantna logistička regresija korišćena je da bi se utvrdili faktori koji doprinose nastanku metastaza u kostima. Za analizu dijagnostičke vrednosti korišćene su *receiver operating characteristic* (ROC) krive. **Rezultati.** Metastaze kostiju javile su se kod 68 (45,94%) bolesnika. Osnovni podaci bolesnika nisu pokazali značajne razlike između grupa ($p > 0,05$). Grupa bolesnika sa metastazama imala je značajno povišene nivoe CEA, CYFRA21-1 i ALP u serumu, u poređenju sa grupom bez metastaza ($p < 0,05$). Povišeni nivoi CEA [*odds ratio* (OR): 1,062, 95% *confidence interval* (CI): 1,031–1,094], CYFRA21-1 (OR: 1,155, 95% CI: 1,061–1,258) i ALP (OR: 1,027, 95% CI: 1,008–1,047) u serumu bili su faktori rizika za metastaze kostiju (OR > 1, $p < 0,05$). Površine ispod ROC kriva za CEA, CYFRA21-1, ALP i njihove kombinacije bile su veće od 0,600, što ukazuje na visoke dijagnostičke vrednosti. **Zaključak.** Nivoi CEA, CYFRA21-1 i ALP u serumu mogu predvideti metastaze kostiju kod obolelih od NSCLC, pri čemu je prediktivna vrednost njihove kombinacije veća od bilo kog pojedinačnog markera.

Ključne reči:
tumorski markeri; kosti, neoplazme; pluća, nesitnoćelijski karcinom; dijagnoza; neoplazme, metastaze; lečenje, ishod.

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Introduction

Classified as lung cancer with the highest incidence rate, non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases [1]. Despite significant advances in the diagnosis and treatment of NSCLC in recent years, the overall prognosis remains poor, especially when bone metastasis occurs with disease progression, which not only remarkably reduces the quality of life but also seriously affects the survival rate of patients. Furthermore, bone metastasis increases pain and fracture risks, and it is likely to cause serious complications such as spinal cord compression and hypercalcemia [2]. Therefore, early diagnosis and effective monitoring of bone metastasis are essential for patients with NSCLC to perfect treatment options and improve prognosis. The frequently applied imaging techniques include magnetic resonance imaging (MRI), X-ray, positron emission tomography-computed tomography (PET-CT), and bone scan [3]. A bone scan can sensitively detect bone metastases by measuring the uptake of radioisotope-labeled compounds into the bones of the whole body. MRI can more clearly display the disease condition within the bone marrow.

Due to imaging limitations, the detection of serum tumor markers has become important for diagnosing and monitoring bone metastasis. For instance, carcinoembryonic antigen (CEA) is a glycoprotein secreted by tumor cells and some normal cells in the liver, pancreas, and gastrointestinal tract, which was widely studied and initially applied as a marker for colorectal cancer. The significant elevation of CEA has been observed not only in colorectal cancer but also in lung cancers, mammary gland, pancreas, and stomach [4]. Notably, elevated CEA levels in NSCLC patients have been associated with higher metastatic burden, including bone metastasis [5].

Cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) plays a pivotal role as an important tumor marker besides CEA in clinical diagnosis and monitoring. In spite of a significant expression under squamous cell carcinoma, CYFRA21-1 also exhibits high diagnostic sensitivity and specificity in NSCLC patients, especially those with bone metastasis [6]. With unique biological characteristics, CYFRA21-1 is irreplaceable in the early diagnosis, treatment response assessment, and condition monitoring of NSCLC. It may outperform CEA in detecting bone metastatic involvement due to a higher correlation with tumor burden in the skeletal system [7].

Alkaline phosphatase (ALP), an enzyme closely related to bone metabolism, also presents an evidently raised level in NSCLC patients with bone metastasis, which can reflect the process of bone formation and destruction; hence, it possesses crucial reference value in the diagnosis of bone metastasis. The detection of ALP not only helps capture early signals of bone metastasis but also provides dynamic monitoring information during treatment [8]. Serum ALP level is significantly higher in NSCLC patients with bone metastasis compared to those without, suggesting its utility for early detection and monitoring [9].

CEA, CYFRA21-1, and ALP are each valuable in identifying some patients diagnosed with cancer, but single-marker testing usually fails to achieve sufficient diagnostic accuracy and sensitivity. In clinical practice, therefore, it is often necessary to combine the detection results of multiple markers to improve the overall diagnostic accuracy and reliability. However, the detection of a single marker cannot often meet clinical needs.

The aim of this study was to investigate the combined detection of CEA, CYFRA21-1, and ALP in serum to evaluate its diagnostic value and clinical application prospects for diagnosing bone metastasis in NSCLC patients. With this, we hope to achieve earlier and more accurate diagnosis, and provide patients with more timely and effective treatment strategies.

Methods

Subjects

A total of 148 NSCLC patients admitted to the Department of Laboratory Medicine, Xinchang Hospital of Traditional Chinese Medicine, Shaoxing, China, from April 2020 to March 2022 were selected for this study. Of them, according to the presence or absence of bone metastasis, 68 were assigned to the metastasis group and 80 to the non-metastasis group. Inclusion criteria were as follows: patients with NSCLC diagnosed pathologically [10], those with complete clinical data, and those who were informed of and consented to this study. Exclusion criteria included patients with primary bone tumors or osteoporosis.

The study was approved by the Ethics Committee of Xinchang Hospital of Traditional Chinese Medicine, China, (from April 20, 2020).

Detection methods and tools

To ensure detection accuracy and reliability, all procedures of sample collection and processing were performed under standard laboratory conditions using Cobas® 8000 modular analyzer (Roche, Basel, Switzerland) and AU5800 analyzer (Beckman Coulter, Brea, USA), as well as kits from Roche Diagnostics Shanghai Ltd. (Shanghai, China) and Beckman Coulter Laboratory Systems Suzhou Co., Ltd. (Suzhou, China). Briefly, fasting venous blood was collected in a volume of 5 mL and allowed to stand for 30 min to facilitate serum separation, followed by centrifugation at 3,000 revolutions *per* minute (rpm) for 10 min. Then, the acquired supernatant (serum) was utilized for determining the levels of serum CEA [normal range (NR): 0–5 ng/mL], CYFRA21-1 (NR: 0–3.3 μg/L), and ALP (NR: 45–125 U/L) using the abovementioned analyzers and kits.

Diagnostic criteria for bone metastasis

All patients underwent a whole-body bone scan to initially detect the presence or absence of signs of bone metas-

tasis. If the scan results suggested possible bone metastases, further evaluation by more than two physicians was required. CT, MRI, and other imaging examinations were also required to confirm the diagnosis results and accurately locate bone metastases.

Statistical analysis

Statistical analysis was performed using SPSS 26.0 software. The count data were presented with numbers or percentages, while measurement data were expressed as mean ± standard deviation. Intergroup comparisons were accomplished through the *t*-test and the Chi-square test. The value of $p < 0.05$ indicated a difference of statistical significance.

Results

Bone metastasis occurred in 68 (45.94%) out of the 148 patients.

The baseline data manifested no statistically significant intergroup differences ($p > 0.05$) (Table 1).

The metastasis group, compared to the non-metastasis group, presented significantly raised concentrations of serum CEA, CYFRA21-1, and ALP, and the differences were statistically significant ($p < 0.05$) (Table 2).

Multivariate logistic regression analysis was carried out with serum CEA, CYFRA21-1, and ALP as independent variables, and bone metastasis present or absent in NSCLC patients as a dependent variable (0 = metastasis, 1 = non-metastasis). The results revealed that the increases in serum CEA [odds ratio (OR): 1.062, 95% confidence interval (CI): 1.031–1.094], CYFRA21-1 (OR: 1.155, 95% CI: 1.061–1.258), and ALP (OR: 1.027, 95% CI: 1.008–1.047) were risk factors for bone metastasis (OR > 1, $p < 0.05$) (Table 3).

To explore the diagnostic value of serum biomarkers in relation to NSCLC histological subtypes, patients were stratified into adenocarcinoma and squamous cell carcinoma subgroups. For each subtype, the levels of CEA, CYFRA21-1, and ALP were compared between the metastasis and non-metastasis groups. In the squamous cell carcinoma subgroup, serum CYFRA21-1 level was significantly higher in patients with bone metastasis compared to those without metastasis ($p < 0.05$). No significant differences were observed in CEA or ALP levels between metastasis and non-metastasis groups ($p > 0.05$). In contrast, within the adenocarcinoma subgroup, CEA, CYFRA21-1, and ALP levels were all significantly elevated in the metastasis group ($p < 0.05$) (Table 4).

Table 1

Clinical data of patients

Parameters	Metastasis group (n = 68)	Non-metastasis group (n = 80)	χ^2/t	*p*
Age, years	65.32 ± 7.45	66.18 ± 7.12	0.716	0.474
Gender				
male	41 (60.29)	46 (57.50)	0.118	0.730
female	27 (39.71)	34 (42.50)		
Diabetes mellitus				
yes	26 (38.24)	32 (40.00)	0.048	0.826
no	42 (61.76)	48 (60.00)		
Hypertension				
yes	32 (47.06)	37 (46.25)	0.009	0.921
no	36 (52.94)	43 (53.75)		
Smoking				
yes	29 (42.65)	33 (41.25)	0.029	0.863
no	39 (57.35)	47 (58.75)		
Pathological type				
adenocarcinoma	43 (63.24)	49 (61.25)	0.061	0.804
squamous cell carcinoma	25 (36.76)	31 (38.75)		
Site of lesion				
left	28 (41.18)	32 (40.00)	0.021	0.884
right	40 (58.82)	48 (60.00)		

n – number.
All values are given as numbers (percentages) or mean ± standard deviation.

Table 2

CEA, CYFRA21-1, and ALP in the serum

Parameters	Metastasis group (n = 68)	Non-metastasis group (n = 80)	*t*	*p*
CEA (ng/mL)	12.43 ± 2.42	6.23 ± 1.53	18.903	0.001
CYFRA21-1 (μg/L)	6.49 ± 2.78	4.14 ± 0.21	7.540	0.001
ALP (U/L)	115.32 ± 21.56	106.24 ± 15.48	2.972	0.003

CEA – carcinoembryonic antigen; CYFRA21-1 – cytokeratin 19 fragment antigen 21-1; ALP – alkaline phosphatase; n – number.
All values are given as mean ± standard deviation.

Table 3

Associations of serum CEA, CYFRA21-1, and ALP with bone metastasis in NSCLC patients assessed by multivariate logistic regression analysis

Variables	*β*	SE	Wald	*p*	OR	95% CI
CEA	0.060	0.015	15.803	0.001	1.062	1.031–1.094
CYFRA21-1	0.144	0.044	10.980	0.001	1.155	1.061–1.258
ALP	0.027	0.010	7.881	0.005	1.027	1.008–1.047

NSCLC – non-small cell lung cancer; SE – standard error; OR – odds ratio; CI – confidence interval. For other abbreviations, see Table 2.

Table 4

Serum biomarker levels in patients stratified by histological subtype

Histological subtype	Metastasis group	Non-metastasis group	*t*	*p*
Squamous cell carcinoma				
CEA (ng/mL)	11.06 ± 2.29	6.03 ± 1.21	1.923	0.061
CYFRA21-1 (μg/L)	7.18 ± 2.81	5.31 ± 1.97	3.004	0.004
ALP (U/L)	112.40 ± 19.85	107.65 ± 14.29	1.142	0.258
Adenocarcinoma				
CEA (ng/mL)	13.11 ± 2.33	6.42 ± 1.61	16.267	< 0.001
CYFRA21-1 (μg/L)	6.06 ± 2.74	3.58 ± 0.87	6.471	< 0.001
ALP (U/L)	118.55 ± 21.71	104.33 ± 13.98	3.973	< 0.001

For abbreviations, see Table 2.

All values are given as mean ± standard deviation.

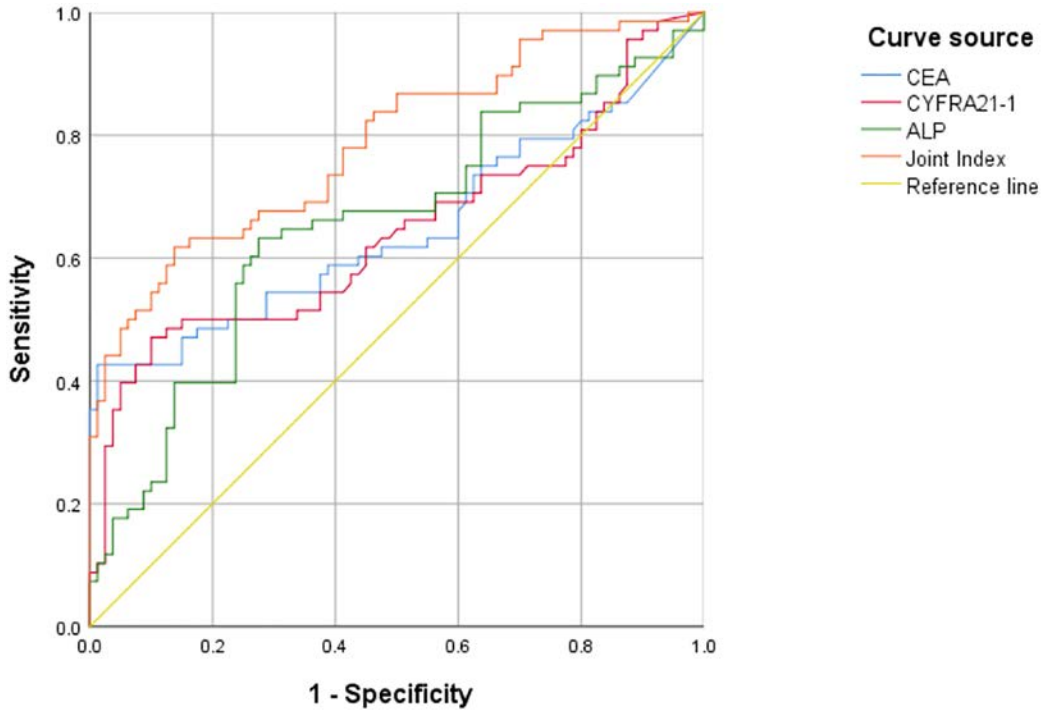

Fig. 1 – ROC curves of CEA, CYFRA21-1, and ALP in the serum and their combination for diagnosing bone metastasis in NSCLC patients. The yellow reference line represents the 45-degree line, which indicates no predictive ability. Factors with ROC curves that are farther away from this reference line have better predictive performance. ROC – receiver operating characteristic. For other abbreviations, see Tables 2 and 3.

Serum CEA, CYFRA21-1, ALP, and a combination of the three were set as test variables, and bone metastasis present or absent in NSCLC patients was determined as a state variable (0 = metastasis, 1 = non-metastasis) to generate receiver operating characteristic (ROC) curves. The results showed that CEA, CYFRA21-1, ALP, and their combination all had an area under the curve (AUC) greater than 0.600, suggesting that these indicators possess high diagnostic value for bone metastasis in NSCLC patients (Figure 1, Table 5).

Table 5

Significance of combined detection of CEA, CYFRA21-1, and ALP for diagnosing bone metastasis in NSCLC patients

Factor	AUC	95% CI	Specificity	Sensitivity	Youden index	Cut-off value
CEA	0.648	0.553–0.742	0.712	0.515	0.227	9.5 ng/mL
CYFRA21-1	0.641	0.548–0.735	0.562	0.574	0.136	5.3 μg/L
ALP	0.654	0.564–0.744	0.900	0.221	0.121	110.0 U/L
Combination	0.787	0.719–0.861	0.537	0.868	0.405	-

AUC – area under the curve; CI – confidence interval.
For other abbreviations, see Tables 2 and 3.

Discussion

As a complex and severe disease, lung carcinoma poses a great challenge to public health worldwide. According to statistics, pulmonary carcinoma emerges as a major contributor to carcinoma-associated death globally, with millions of deaths every year. From the perspective of the medical field, pulmonary carcinoma is classified primarily into two types (small cell lung carcinoma and NSCLC) according to its pathological characteristics, with NSCLC as the most ubiquitous type [11], accounting for nearly 85%. Bone metastasis is a ubiquitous and serious complication of NSCLC, which usually occurs at the progressive stage when cancer cells metastasize from the primary site to the bone. Bones are rich in blood supply and have an excellent bone marrow microenvironment; therefore, cancer cells can easily colonize and continue to grow in the bones [12]. Bone metastasis frequently occurs in the spine, pelvis, ribs, and long bones of the extremities of NSCLC patients, causing a variety of clinical problems ranging from hypercalcemia, pathological fractures, pain, to bone-spinal cord compression. The symptoms of bone metastasis are highly similar to those of primary bone disease, which complicates early diagnosis and accurate identification. Patients usually report nonspecific bone pain first, which may be intermittent or persistent, and which may gradually aggravate at night. As the disease progresses, the risk of pathological fractures will increase, especially at the bone under heavy load. When invading the spine, cancer cells are more likely to cause neurological dysfunction due to spinal cord compression, including paresthesia, dyskinesia, or even paralysis [13]. Imaging diagnosis using MRI, X-ray, CT, and bone scan plays an important role in detecting bone metastasis. X-rays can detect large-scale bone damage, but they are less sensitive to early small-scale lesions. Bone scans can detect bone lesions at multiple sites of the body, but are less specific [14]. Therefore, other ways of accurately identifying and diagnosing bone metastasis need to be considered in NSCLC patients.

Inflammation and immune response are key players in the occurrence and development of tumors, suggesting that the detection of indicators related to inflammation and immune response may contribute to earlier or more accurate diagnosis of bone metastasis [15]. In addition, biochemical markers of bone metabolism and tumor markers have been widely applied in the diagnosis and monitoring of diversified tumors, which provide key information about bone health as well as the tumor and its activity. The concentration of tumor markers can reflect the presence and progression of cancer, and these biomolecules are significantly important for cancer in terms of early diagnosis, therapeutic effect assessment, and relapse monitoring [16]. CEA and CYFRA21-1 are common tumor markers associated with lung cancer. CEA is a protein produced during embryonic development, and its level is normally very low in the blood of adults. Still, it can be significantly elevated in several carcinoma types (e.g., mammary adenocarcinoma, colorectal cancer, and pulmonary carcinoma) [17]. Therefore, CEA is considered a broad-spectrum tumor marker, and its level detected by immunochemistry can reflect the presence and progression of some tumors. Monitoring CEA helps assess efficacy and evaluate the prognosis of NSCLC [18]. In this study, there was a significant correlation between CEA and the presence or absence of bone metastasis ($p < 0.05$), and CEA was found to be a risk factor independently affecting bone metastasis, that is, the higher the CEA level was, the higher the bone metastasis potential in NSCLC patients would be (AUC = 0.648). However, CEA is a nonspecific cancer marker, and its elevation may also exist in some benign diseases and other non-cancerous conditions. Therefore, the detection of CEA alone is insufficient for cancer diagnosis, and it is generally necessary to combine it with other indicators for comprehensive evaluation. Being a soluble cytokeratin 19 fragment, CYFRA21-1 usually presents a high level, indicating a severe condition or active tumor, which is of essential reference value for clinicians in developing individualized treatment plans. Since it is widely applied for lung cancer, CYFRA21-1 has become one of the crucial diagnostic tools in clinical practice. CYFRA21-1 has also been discovered with critical effects on lung cancer regarding metastasis and prognosis [19]. In this study, the blood CYFRA21-1 level rose significantly in NSCLC subjects manifesting bone metastasis, which was determined through multivariate logistic regression analysis as a factor independently influencing bone metastasis (AUC = 0.641) ($p < 0.05$). Nevertheless, CYFRA21-1 is not a specific marker, and its elevated level may also be associated with other diseases like breast cancer, bladder carcinoma, and colorectal carcinoma [20]. Therefore, CYFRA21-1 is less specific and sensitive in diagnosing bone metastasis. In contrast, bone metabolic biochemical markers, possibly including blood calcium and phosphatase, can more directly and specifically reflect bone metabolic activities, which are often used to evaluate bone health status and diagnose bone diseases [21]. ALP, the most frequently employed biochemical indicator of bone metabolism, is a kind of enzyme system widely present in tissues of the human body, which is

mainly synthesized in the liver and bone tissues and catalyzes the hydrolysis reaction of phosphate compounds. The ALP level rises significantly when bone formation increases or bone resorption decreases, and its close correlation with NSCLC prognosis has been confirmed [22]. In this study, the sensitivity, specificity, and AUC of ALP for diagnosing bone metastasis in NSCLC patients were 22.1%, 90.0%, and 0.654, respectively, which could be used for combined de-tection.

ALP level can be an early marker for bone metastasis in NSCLC patients, and an abnormal ALP level may indicate a high potential for bone metastasis, especially in patients with less elevated CEA and CYFRA21-1 levels. These two indicators possess high predictive value for bone metastasis in NSCLC [23]. As a marker of bone destruction and formation, ALP exhibits unique diagnostic value for bone metastasis. As a result, the combined detection of CEA, CYFRA21-1, and ALP may provide a more accurate and effective clinical basis for early diagnosis of NSCLC patients with bone metastasis. In this study, CEA, CYFRA21-1, and ALP levels in the serum of NSCLC patients were detected and analyzed. Compared with patients without bone metastasis, those suffering from bone metastasis demonstrated increased content of these three markers. Specifically, elevated CEA usually indicated an increased tumor size or distant metastasis, elevated CYFRA21-1 further indicated enhanced activity and spread capacity of tumor cells, and elevated ALP directly reflected abnormal bone metabolism. The results of ROC curve analysis showed that a sensitivity of 86.8% and an AUC of 0.787 were obtained for the combined detection. Both values were higher than those of any marker alone, suggesting that this combined detection offers greater clinical value for diagnosing bone metastasis in NSCLC patients.

Nevertheless, this study has limitations. Firstly, the retrospective design may introduce selection bias, potentially limiting the generalizability of the results. Secondly, this study did not analyze the relationship between serum biomarker levels and the extent or anatomical distribution of bone metastases (e.g., solitary vs. multiple lesions; spinal vs. pelvic or appendicular sites) due to incomplete or inconsistent imaging records in the retrospective dataset. These stratifications should be carefully explored in future prospective studies with standardized imaging and staging protocols.

Conclusion

The detection integrating CEA, CYFRA21-1, and ALP is an effective means of diagnosing NSCLC patients for bone metastasis early, which can efficiently reduce missed diagnoses and misdiagnoses in bone metastasis screening. It not only expands the application of biomarkers in tumor diagnosis but also provides clinicians with more comprehensive and accurate information in NSCLC treatment and management. With the application and popularization of this detection strategy, the early screening results of NSCLC may be significantly improved, thereby prolonging both disease-free survival and overall survival, as well as raising the quality of life. As more in-depth research is conducted and high-precision detection technology develops in the future, testing incorporating CEA, CYFRA21-1, and ALP will have broader prospects in tumor diagnosis and treatment.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Chen P, Liu Y, Wen Y, Zhou C.* Non-small cell lung cancer in China. Cancer Commun (Lond) 2022; 42(10): 937–70.
2. *Bessa CM, Silva LMD, Zamboni MM, Costa GJ, Bergmann A, Thuler LCS,* et al. Bone metastasis after stage IIIA non-small cell lung cancer: risks and prognosis. J Bras Pneumol 2022; 48(5): e20220211.
3. *Lim CH, Ahn TR, Moon SH, Cho YS, Choi JY, Kim BT,* et al. PET/CT features discriminate risk of metastasis among single-bone FDG lesions detected in newly diagnosed non-small-cell lung cancer patients. Eur Radiol 2019; 29(4): 1903–11.
4. *Imura Y, Yamamoto S, Wakamatsu T, Tanaka T, Tamiya H, Sugimura K,* et al. Clinical features and prognostic factors in patients with esophageal cancer with bone metastasis. Oncol Lett 2020; 19(1): 717–24.
5. *Li C, Liu L, You R, Li Y, Pu H, Lei M,* et al. Trajectory patterns and cumulative burden of CEA during follow-up with non-small cell lung cancer outcomes: A retrospective longitudinal cohort study. Br J Cancer 2024; 130(11): 1803–8.
6. *Li Q, Sang S.* Diagnostic Value and Clinical Significance of Combined Detection of Serum Markers CYFRA21-1, SCC Ag, NSE, CEA and ProGRP in Non-Small Cell Lung Carcinoma. Clin Lab 2020; 66(11): 2189.
7. *Mohamed E, García Martínez DJ, Hosseini MS, Yoong SQ, Fletcher D, Hart S,* et al. Identification of biomarkers for the early detection of non-small cell lung cancer: a systematic review and meta-analysis. Carcinogenesis 2024; 45(1–2): 1–22.
8. *Chai X, Yinwang E, Wang Z, Wang Z, Xue Y, Li B,* et al. Predictive and Prognostic Biomarkers for Lung Cancer Bone Metastasis and Their Therapeutic Value. Front Oncol 2021; 11: 692788.
9. *Yang T, Cheng J, Fu S, Sun T, Yang K, You J,* et al. Pretreatment levels of serum alkaline phosphatase are associated with the prognosis of patients with non-small cell lung cancer receiving immune checkpoint inhibitors. Oncol Lett 2023; 25(4): 154.
10. *Dietel M, Bubendorf L, Dingemans AM, Dooms C, Elmberger G, García RC,* et al. Diagnostic procedures for non-small-cell lung cancer (NSCLC): recommendations of the European Expert Group. Thorax 2016; 71(2): 177–84.
11. *Alexander M, Kim SY, Cheng H.* Update 2020: Management of Non-Small Cell Lung Cancer. Lung 2020; 198(6): 897–907.
12. *Del Conte A, De Carlo E, Bertoli E, Stanzione B, Revelant A, Bertola M,* et al. Bone Metastasis and Immune Checkpoint Inhibitors in Non-Small Cell Lung Cancer (NSCLC): Microenvironment and Possible Clinical Implications. Int J Mol Sci 2022; 23(12): 6832.
13. *Li Y, Xu C, Yu Q.* Risk factor analysis of bone metastasis in patients with non-small cell lung cancer. Am J Transl Res 2022; 14(9): 6696–702.
14. *Ikeda T, Kitajima K, Tsuchitani T, Takahashi Y, Hama Y, Kotura N.* Effectiveness of quantitative bone SPECT/CT for bone metastasis diagnosis. Hell J Nucl Med 2022; 25(3): 253–9.

15. *Göbel A, Dell'Endice S, Jaschke N, Pählig S, Shahid A, Hofbauer LC,* et al. The Role of Inflammation in Breast and Prostate Cancer Metastasis to Bone. Int J Mol Sci 2021; 22(10): 5078.
16. *Filella X, Rodríguez-García M, Fernández-Galán E.* Clinical usefulness of circulating tumor markers. Clin Chem Lab Med 2022; 61(5): 895–905.
17. *Uygur MM, Gümüş M.* The utility of serum tumor markers CEA and CA 15-3 for breast cancer prognosis and their association with clinicopathological parameters. Cancer Treat Res Commun 2021; 28: 100402.
18. *Bes-Scartezini F, Saad Junior R.* Prognostic assessment of tumor markers in lung carcinomas. Rev Assoc Med Bras (1992) 2022; 68(3): 313–7.
19. *Nakao M, Kinoshita R, Kuriyama M, Kiyotoshi H, Sugihara M, Takeda N,* et al. Serum CYFRA 21-1 Level as a Prognostic Marker for Extensive Disease Small Cell Lung Cancer. Anticancer Res 2024; 44(2): 845–51.
20. *Xue F, Meng Y, Jiang J.* Diagnostic Value of Dynamic Enhanced Magnetic Resonance Imaging Combined with Serum CA15-3, CYFRA21-1, and TFF1 for Breast Cancer. J Healthc Eng 2022; 2022: 7984591.
21. *Zou Y, Liu Z, Li H, Hou L, Pang J, Liu X,* et al. Evaluation of bone metabolism-associated biomarkers in Tibet, China. J Clin Lab Anal 2021; 35(12): e24068.
22. *Shalata W, Yakobson A, Steckbeck R, Jama AA, Abu Saleh O, Agbarya A.* Is Elevation of Alkaline Phosphatase a Predictive Factor of Response to Alectinib in NSCLC? Curr Oncol 2021; 29(1): 173–7.
23. *Li L, Xu Y, Wang Y, Zhang Q, Wang Y, Xu C.* The Diagnostic and Prognostic Value of the Combination of Tumor M2-Pyruvate Kinase, Carcinoembryonic Antigen, and Cytokeratin 19 Fragment in Non-Small Cell Lung Cancer. Technol Cancer Res Treat 2024; 23: 15330338241265983.

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Association of platelet-to-lymphocyte ratio with sepsis based on MIMIC-IV database

Povezanost odnosa trombocita i limfocita sa sepsom na osnovu baze podataka MIMIC-IV

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Abstract

Background/Aim. Sepsis is a potentially lethal condition that ranks among the most severe medical disorders globally and results in elevated mortality rates. The aim of this study was to examine the correlation between sepsis outcomes in patients and the platelet-to-lymphocyte ratio (PLR), including mortality and duration of intensive care unit (ICU) stay from admission, while considering relevant demographic and clinical factors. **Methods.** In this retrospective study, the Medical Information Mart for Intensive Care (MIMIC)-IV dataset information was used. Sepsis patients were de-identified, and their PLR values were calculated upon admission. Multivariate logistic regression models were used to assess the relationship between PLR and mortality, adjusting for confounding variables such as age, gender, comorbidities, and vital signs. Additionally, the association between PLR and the length of the ICU stay was analyzed using linear regression models. **Results.** This study included 4,624 sepsis patients. Higher PLR values were significantly correlated with decreased survival probabilities in the unadjusted model [Model 1 – odds ratio (OR): 0.890, 95% confidence interval (CI): 0.810–0.970, $p < 0.001$]. This association remained significant after adjusting for demographic factors (Model 2 – OR: 0.920, 95% CI: 0.850–0.995, $p < 0.001$), comorbidities and biochemical parameters (Model 3 – OR: 0.880, 95% CI: 0.800–0.960, $p = 0.0273$), and vital signs (Model 4 – OR: 0.860, 95% CI: 0.780–0.940, $p = 0.0301$). Furthermore, our analyses revealed a trend towards prolonged ICU stay with higher PLR values, although the association did not reach statistical significance. Survivors were younger (median age 63.37 vs. 70.84 years) and had lower Charlson Comorbidity Index (CCI) scores (median CCI 4.00 vs. 6.00, $p < 0.001$) compared to non-survivors. **Conclusion.** The outcomes indicate that higher PLR levels correlate with greater fatality rates in sepsis patients, underscoring its potential as a predictive biomarker. The observed trend towards prolonged ICU stay with higher PLR warrants further investigation. Model 4, which includes demographic factors, comorbidities, biochemical parameters, and vital signs, demonstrated the strongest association between PLR and mortality, suggesting it may be the most clinically useful model for predicting sepsis outcomes. Incorporating PLR into risk assessment and therapeutic decision-making frameworks may enhance sepsis treatment and improve patient outcomes.

Key words:
biomarkers; blood platelets; intensive care unit; lymphocytes; mortality; sepsis.

Apstrakt

Uvod/Cilj. Sepsa je potencijalno smrtonosno stanje koje se ubraja među najteže zdravstvene poremećaje širom sveta i rezultira povišenom stopom smrtnosti. Cilj rada bio je da se ispita korelacija između ishoda sepse kod bolesnika i odnosa trombocita i limfocita (TLO), uključujući mortalitet i dužinu boravka u jedinici intenzivne nege (JIN), uzimajući u obzir relevantne demografske i kliničke faktore. **Metode.** U ovoj retrospektivnoj studiji, korišćene su informacije iz baze podataka *Medical Information Mart for Intensive Care* (MIMIC)-IV. Bolesnici sa sepsom bili su anonimni, a njihove TLO vrednosti izračunate su po prijemu. Za procenu odnosa između TLO i moratliteta korišćeni su modeli multivarijantne logističke regresije, uz prilagođavanje za varijable koje mogu da utiču na rezultat kao što su starost, pol, komorbiditeti i vitalni znaci. Pored toga, analizirana je povezanost između TLO i dužine boravka na JIN korišćenjem modela linearne regresije. **Rezultati.** Ova studija obuhvatila je 4 624 bolesnika sa

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sepsom. Više vrednosti TLO značajno su korelisale sa smanjenom verovatnoćom preživljavanja u modelu bez prilagođavanja [Model 1 – *odds ratio* (OR): 0,890, 95% *confidence interval* (CI): 0,810–0,970, $p < 0,001$]. Ova povezanost ostala je značajna nakon prilagođavanja za demografske faktore (Model 2 – OR: 0,920, 95% CI: 0,850–0,995, $p < 0,001$), komorbiditete i biohemijske parametre (Model 3 – OR: 0,880, 95% CI: 0,800–0,960, $p = 0,0273$) i vitalne znake (Model 4 – OR: 0,860, 95% CI: 0,780–0,940, $p = 0,0301$). Štaviše, naše analize su pokazale trend ka produženom boravku u JIN kod viših vrednosti TLO, iako ta povezanost nije dostigla statističku značajnost. Preživeli su bili mlađi (medijana starosti 63,37 vs. 70,84 godine) i imali su niže *Charlson Comorbidity Index* (CCI) rezultate (medijana CCI 4,00 vs. 6,00, $p < 0,001$) u poređenju sa onima koji nisu preživeli. **Zaključak.** Rezultati ukazuju da viši nivoi TLO korelišu sa većim stopama smrtnosti kod bolesnika sa sepsom, što naglašava potencijal TLO kao prediktivnog biomarkera. Uočeni trend ka produženom boravku u JIN kod viših vrednosti TLO zahteva dalja istraživanja. Model 4, koji uključuje demografske faktore, komorbiditete, biohemijske parametre i vitalne znake, pokazao je najjaču vezu između TLO i mortaliteta, što sugeriše da bi mogao biti klinički najkorisniji model za predviđanje ishoda sepse. Uključivanje TLO u okvire za procenu rizika i donošenje odluka u vezi sa terapijom može poboljšati lečenje sepse i ishode stanja bolesnika.

Ključne reči:
biomarkeri; trombociti; intenzivna nega, odeljenja; limfociti; mortalitet; sepsa.

Introduction

Sepsis is characterized by a dysregulated host reaction to infection, representing a potentially lethal condition that ranks among the most severe medical disorders globally and results in elevated mortality rates among patients in intensive care units (ICUs) [1, 2]. Despite various biomarkers having been identified to influence the development and prognosis of sepsis, there is still a need to discover useful biomarkers to improve patient survival [3].

The platelet-to-lymphocyte ratio (PLR) is a widely accessible haematological metric linked to diverse inflammatory and immunological responses. In certain diseases, such as cardiovascular diseases and cancer, PLR has been considered an important prognostic indicator. PLR has been reported to have potential predictive value in sepsis, but current studies are limited, and most have small sample sizes [4, 5]. A recent systematic review and meta-analysis by Wang et al. [6] found that PLR levels were significantly higher in sepsis non-survivors than in survivors, indicating its potential as a prognostic marker. Additionally, Zheng et al. [7] reported that both low and high PLR levels are associated with higher in-hospital mortality, and specifically, the early decrease in PLR after admission correlates with increased mortality in sepsis patients. Existing research has analyzed the predictive value of PLR in sepsis using the Medical Information Mart for Intensive Care (MIMIC) – III database [8], but systematic studies utilizing larger databases (e.g., MIMIC-IV) are scarce. Given the wealth of clinical data provided by the MIMIC-IV database, including patients' laboratory test results and clinical courses, it presents a unique opportunity to further investigate the role of PLR in predicting mortality in sepsis.

The aim of this study was to conduct an in-depth analysis of the application of PLR in sepsis patients using the MIMIC-IV database, analyzing the relationship between PLR and mortality rates in sepsis patients, as well as to explore the associations between PLR and other clinical parameters to evaluate its potential as an effective biomarker for sepsis severity and prognosis.

Methods

Data sources

The MIMIC-IV database was utilized for this retrospective study. MIMIC-IV is an extensive repository encompassing detailed patient data from the ICU of premier tertiary care facilities in Boston, Massachusetts, the United States, spanning from 2008 to 2019 [9]. This database contains extensive patient data, including vital signs, care plan documents, severity evaluations, diagnoses, therapeutic interventions, and laboratory findings. All patient information in the dataset has been de-identified to safeguard privacy. The dataset's ethical usage was approved by the review boards at Beth Israel Deaconess Medical Center and the Massachusetts Institute of Technology, which exempted this study from the requirement for written consent due to the de-identified content of the data.

For accessing the database, the research team completed the Collaborative Institutional Training Initiative course, achieving certification (ID: 60447838) and passing relevant examinations concerning "conflict of interest" and "data or specimen study only". This study was meticulously executed using STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) standards, thereby ensuring both methodological rigor and adherence to ethical guidelines. This structured approach underscores the commitment to ethical standards and scientific integrity in the handling and analysis of patient data. The MIMIC dataset is the primary source of the data used in this study, which has been previously approved for research use.

The study was approved by the Ethics Committee of Zhejiang Xinan International Hospital (approval No. 345/PLR/2022, from April 13, 2022).

Study population and definitions

Sepsis was defined according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), which identified individuals with a Sequential Organ

Failure Assessment (SOFA) score of ≥ 2 and suspected infections [10]. Exclusions for the study were as follows: patients under the age of 18, those with a SOFA score less than 2 upon ICU admission, or those with ICU stays shorter than 48 hrs. Initial ICU-acquired infections were defined according to the Centers for Disease Control and Prevention (CDC) standards [11] as any new infection occurring 48 hrs after ICU admission, evidenced by positive microbial culture and the initiation of a new antibiotic regimen [12].

Data collection

Only sepsis patients who satisfied the inclusion criteria and were admitted to the ICU for the first time were examined to prevent duplication. Extracted patient baseline parameters included age, gender, weight, duration of hospital and ICU stays, and death. Baseline ICU scoring standards collected at admission included SOFA, Acute Physiology Score III (APS III), and Charlson Comorbidity Index (CCI). Comorbidities at admission included shock, cardiovascular disease, surgical status, cancer, kidney diseases, and liver dysfunction. Intervention events covered pharmacological treatments (vasopressors, corticosteroids, heparin), blood transfusion, renal replacement therapy, mechanical ventilation, central venous catheters, and urinary catheters. Laboratory data were gathered at the time of ICU admission. The database's microbiological examination findings provided proof of illnesses acquired in the ICU.

Statistical analysis

Missing values were imputed using the random forest method to ensure data consistency and interpretability. Continuous factors were provided as median [interquartile range (IQR)]. Intergroup comparisons employed appropriate statistical methods, including the *t*-test, Wilcoxon rank-sum test, analysis of variance, or Kruskal-Wallis test. Linear correlations were evaluated between PLR and the duration of ICU stay. Extended model approaches were used for covariate adjustments as follows: Model 1 was a simple predictive model for survival rate based on PLR; Model 2 incorporated demographic indicators such as age, gender, and race; Model 3 added comorbidities and biochemical test results after 24 hrs of ICU admission; Model 4 integrated parameters of vital signs including heart rate, mean blood pressure, respiration rate, peripheral capillary oxygen saturation (SpO_2), and temperature. The goodness-of-fit tests were performed for all logistic regression models. In a two-tailed test, a *p*-value < 0.05 was determined statistically noteworthy. Utilizing R software, statistical analyses were conducted.

Results

This research involved a group of 4,624 patients who fulfilled the inclusion criteria. The demographic and clinical characteristics upon admission are delineated in Table 1. Of the total 4,624 patients, the survivor group comprised 1,819 patients, while the non-survivor group consisted of 2,805 patients. Within the entire cohort, male patients in the survivor group accounted for 22.34% (1,033/4,624) of the total sample, whereas male patients in the non-survivor group represented 34.08% (1,576/4,624). The proportion of males within the survivor group was 56.79% (1,033/1,819), and the proportion of males within the non-survivor group was 56.19% (1,576/2,805). The individuals who survived had an average age of 63.37 years (IQR: 52.37–74.35), significantly younger compared to those who did not survive, whose average age was 70.84 years (IQR: 64.02–80.87) ($p < 0.001$). Regarding comorbidities, patients who survived had a lower median CCI of 4.00 (IQR: 2.00–6.00) than those who did not, with a median of 6.00 (IQR: 4.00–8.00). At the time of ICU admission, the white blood cell count was notably higher in the survivor group with a median of 9.10 (IQR: 6.55–14.40) compared to 8.80 (IQR: 6.30–13.10) in the non-survivor group. Additionally, the median platelet count was comparable between the two groups, with 208.00 (IQR: 148.00–283.00) in the survivor group and 209.00 (IQR: 144.00–284.00) in the non-survivor group.

Table 1

Demographic and clinical characteristics of patients upon admission

Parameters	Survivors (n = 1,819)	Non-survivors (n = 2,805)	SMD	*p*
Demographics				
gender, male	1,033 (22.34)	1,576 (34.08)	-0.012	0.693
age, years	63.37 (52.37–74.35)	70.84 (60.42–80.87)	0.487	< 0.001
weight, kg	81.60 (67.80–98.00)	76.90 (64.00–93.20)	0.186	< 0.001
Comorbidities				
CCI	4.00 (2.00–6.00)	6.00 (4.00–8.00)	0.721	< 0.001
Laboratory results at admission				
WBCs, $\times 10^9$ (4.0–10.0)	9.10 (6.55–14.40)	8.80 (6.30–13.10)	0.042	0.002
platelets, $\times 10^9$ (150–400)	208.00 (148.00–283.00)	209.00 (144.00–284.00)	0.005	0.769
glucose, mg/dL (78–110)	205.08 (164.00–286.17)	207.00 (163.30–283.15)	0.007	0.884
Vital parameters at admission				
heart rate, bpm (60–100)	97.17 (90.47–104.13)	97.52 (89.68–104.14)	0.074	0.225
MBP, mmHg (70–105)	75.58 (72.47–79.23)	75.31 (71.84–78.99)	0.098	0.008
respiration rate, breaths/min (12–20)	21.00 (19.08–23.00)	21.02 (19.00–23.00)	0.001	0.683
SpO_2, % (95–100)	96.16 (95.00–97.04)	96.06 (95.00–97.04)	0.033	0.257
temperature, °C (36.0–37.5)	36.98 (36.74–37.18)	36.90 (36.65–37.13)	0.214	< 0.001

Table 1 (continued)

Parameters	Survivors (n = 1,819)	Non-survivors (n = 2,805)	SMD	*p*
Score				
SOFA	4.00 (3.00–5.00)	4.00 (3.00–6.00)	0.164	< 0.001
APSIII	50.00 (39.00–63.00)	62.00 (48.00–79.00)	0.594	< 0.001
Hematological indices				
hemoglobin, g/dL (male: 13.5–17.5, female: 12.0–16.0)	12.30 (10.50–13.70)	11.80 (10.20–13.30)	0.182	< 0.001
MCV, fL (80–100)	91.00 (87.00–95.00)	92.00 (88.00–98.00)	0.191	< 0.001
RBC, $\times 10^{12}$/L (male: 4.5–6.0, female: 4.5–5.5)	4.07 (3.54–4.56)	3.91 (3.35–4.40)	0.224	< 0.001
RDW, % (11.5–04.5)	14.20 (13.30–15.50)	14.70 (13.70–16.50)	0.313	< 0.001
Predictors				
lymphocyte, % (20–40)	19.64 (7.10–28.65)	21.50 (9.30–28.93)	0.103	< 0.001
NLR (1–3)	0.28 (0.09–0.45)	0.32 (0.12–0.46)	0.008	< 0.001
PLR (50–300)	124.78 (105.73–219.58)	129.68 (107.47– 238.07)	0.089	0.012
LAR	1.12 (0.82–1.45)	1.19 (0.90–1.63)	0.225	< 0.001
Outcomes				
LOS ICU, days	3.18 (1.90–6.71)	3.56 (1.92–7.37)	0.043	0.041

SMD – standardized mean difference; CCI – Charlson Comorbidity Index; WBCs – white blood cells; bpm – beats *per* minute; MBP – mean blood pressure; SpO_2 – peripheral capillary oxygen saturation; SOFA – Sequential Organ Failure Assessment; APSIII – Acute Physiology and Chronic Health Evaluation III; MCV – mean Corpuscular volume; RBC – red blood cells; RDW – red cell distribution width; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; LAR – lymphocyte-to-adrenal ratio; LOS ICU – Length of Stay in Intensive Care Unit.

Values in brackets in the first column indicate the reference range for each respective parameter. Group values are expressed as numbers (percentages) or median (interquartile range).

Vital parameters at admission also exhibited some differences. The median heart rate for survivors was 97.17 beats *per* minute (bpm) (IQR: 90.47–104.13) as opposed to 97.52 bpm (IQR: 89.68–104.14) for non-survivors. The median SOFA score at admission, a critical measure of organ failure, was equal in both groups, 4.00 (IQR: 3.00–5.00). Similarly, there was no significant difference in the APS III, with a median of 50.00 (IQR: 39.00–63.00) for the survivor group and 62.00 (IQR: 48.00–79.00) for the non-survivor group. In terms of blood count, hemoglobin levels were slightly elevated in the survivor group, with a median of 12.30 g/dL (IQR: 10.50–13.70), compared to the non-survivor group, which had a median of 11.80 g/dL (IQR: 10.20–13.30). The mean corpuscular volume was marginally lower in the surviving patients, with a median value of 91.00 fL (IQR: 87.00–95.00) vs. 92.00 fL (IQR: 88.00–98.00) in those who died. The analysis of predictors such as lymphocyte percentage showed a median of 19.64% (IQR: 17.10–26.85) for survivors compared to 21.50% (IQR: 9.30–28.93) for non-survivors. Inflammatory markers like the neutrophil-to-lymphocyte ratio (NLR) and PLR were also considered, showing that survivors had a median NLR of 0.28 (IQR: 0.09–0.45) and PLR of 124.78 (IQR: 105.73–219.58).

Ultimately, the duration of ICU stay was reduced for survivors, averaging 3.18 days (IQR: 1.90–6.71) compared to 3.56 days (IQR: 1.92–7.37) for non-survivors. This comprehensive data analysis underscores the complex nature of sepsis outcomes and highlights the importance of specific clinical and laboratory factors in predicting patient survival.

In evaluating the association between the PLR and the duration of ICU stay, our analyses reveal a trend that warrants attention. Figure 1 suggests a distribution where higher PLR values correlate with a prolonged length of stay in the ICU, as indicated by the concentration of data points and the trend line. This scatterplot visually demonstrates the potential utility of PLR as a predictor for ICU length of stay, with higher PLR values indicating a longer anticipated duration of stay in the ICU.

The logistic regression models further substantiate the influence of PLR on patient survival (Table 2). Model 1**,** which includes the PLR as the sole predictor, yields an odds ratio (OR) of 0.890 [95% confidence interval (CI): 0.810–0.970, $p < 0.001$], indicating that elevated PLR levels are modestly correlated with a decrease in the odds of survival. Model 2, controlling for demographic characteristics such as age, gender, and race, demonstrates a marginally elevated OR of 0.920 (95% CI: 0.850–0.995, $p < 0.001$). Model 3 incorporates comorbidities and biochemical test results recorded 24 hrs after ICU admission, producing an OR of 0.880 (95% CI: 0.800–0.960, $p = 0.0273$). Comprehensive Model 4**,** which adds vital signs parameters to the previous model, demonstrates an OR of 0.860 (95% CI: 0.780–0.940, $p = 0.0301$). This indicates that when vital signs are considered alongside PLR, a higher PLR is significantly associated with decreased survival odds. Model 4 shows the strongest association between PLR and mortality (lowest OR value) and therefore appears to be the most clinically useful model for predicting sepsis outcomes. These results highlight the potential of PLR as a prognostic indicator in ICU patients with sepsis, warranting further investigation into its role as a biomarker for clinical outcomes.

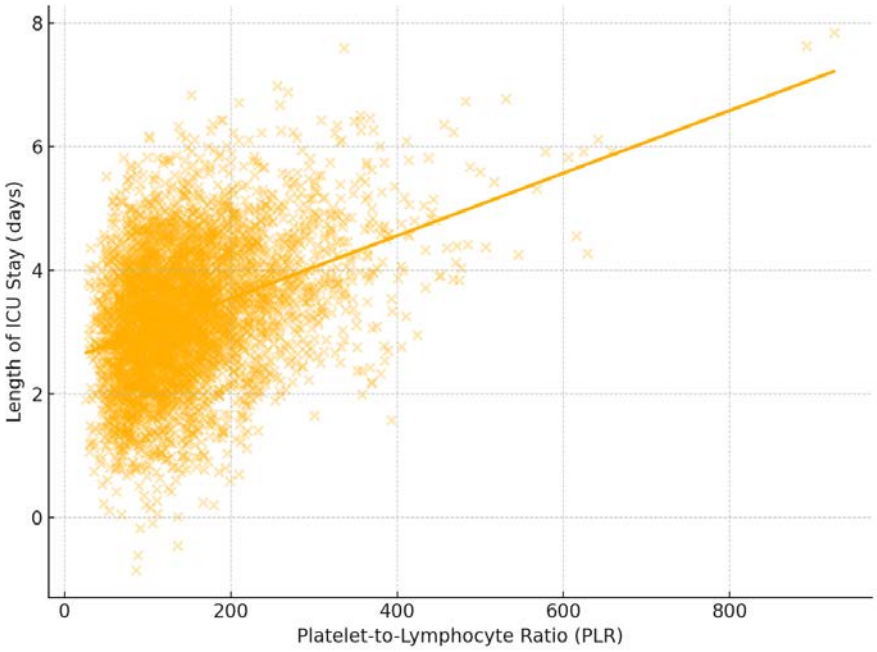

Fig. 1 – The relationship between PLR and length of ICU stay.
PLR – platelet-to-lymphocyte ratio; ICU – intensive care unit.
***Note*: this scatterplot depicts the correlation between PLR values at ICU admission and the duration of ICU stay in 4,624 sepsis patients from the Medical Information Mart for Intensive Care (MIMIC)-IV database. A trend line has been added to highlight the positive correlation observed – higher PLR levels are associated with a tendency toward longer ICU stays. Although the association did not reach statistical significance in the regression model, this pattern suggests PLR may offer prognostic utility in predicting ICU resource use.**

Table 2

The results of logistic regression between the PLR and the survival outcome

Model	PLR survival outcome [OR (95% CI)]	*p*-value
1	0.890 (0.810–0.970)	< 0.001
2	0.920 (0.850–0.995)	< 0.001
3	0.880 (0.800–0.960)	0.0273
4	0.860 (0.780–0.940)	0.0301

PLR – platelet-to-lymphocyte ratio; OR – odds ratio; CI – confidence interval.
***Note*: results of multiple logistic regression models analyzing the association between the PLR and survival outcome. Model 1 is the unadjusted model with PLR as the sole predictor. Model 2 adjusts for demographic factors including age, gender, and race. Model 3 further adjusts for comorbidities and biochemical test results recorded after 24 hrs of intensive care unit admission. Model 4 additionally incorporates vital signs parameters, including heart rate, mean blood pressure, respiration rate, peripheral capillary oxygen saturation, and temperature. ORs along with 95% CIs and *p*-values are reported for each model. A *p*-value < 0.05 is considered statistically significant, representing a significant association between PLR and survival outcome after adjusting for the included covariates in that particular model.**

Discussion

This study revealed that PLR is a key biomarker for the prognosis of patients with sepsis. This study examined the demographic and clinical features of septic patients upon admission, along with the impact of PLR on individual survival and duration of stay in the ICU. Several previous studies have examined the diagnostic and prognostic value of PLR in sepsis [4–8]. A recent systematic review and meta-analysis by Wang et al. [6] analyzed 16 studies with 2,403 septic patients and found that PLR levels were significantly higher in non-survivors than in survivors. Furthermore, Zheng et al. [7] reported a U-shaped relationship between baseline PLR and in-hospital mortality, indicating that both low

and high PLR values are associated with poorer outcomes. Our findings align with these previous studies and provide additional evidence from a larger dataset using the newer MIMIC-IV database.

The observed differences in demographic and clinical parameters between surviving and non-surviving patients underline the multifactorial nature of sepsis outcomes. Notably, survivors tended to be slightly younger and had lower CCI scores upon admission. While these findings align with previous literature, the significance lies in the confirmation of these trends within our cohort [7, 13]. Socioeconomic and clinical risk factors, such as race, education, hospital type, and delirium duration, are linked to worse long-term cognitive impairment after an ICU stay, particularly CCI [13]. CCI has been reported to synergize with age to affect patient survival [7].

Our analyses revealed intriguing insights into the predictive value of PLR in sepsis prognosis. Higher PLR values were associated with decreased odds of survival, even after adjusting for demographic variables, comorbidities, and vital signs. Previous studies have also demonstrated that higher PLR levels upon admission in patients with various conditions correlate with increased morbidity and mortality [3, 14]. For instance, Zheng et al. [7] found a significant difference in PLR change over time between survivors and non-survivors in sepsis patients. Similarly, studies examining PLR in patients diagnosed with coronavirus disease 2019 have shown that higher levels correlate with disease severity and poorer outcomes [6].

Nevertheless, due to the poor quality of this evidence, more research on the PLR threshold is required [15]. These findings corroborate previous studies suggesting PLR as a potential biomarker for adverse outcomes in sepsis patients [6]. Further research is necessary to clarify the processes behind this connection and to assess the applicability of PLR in treatment. The association between PLR and duration of ICU stay also merits attention. While our analyses indicated a trend towards a prolonged ICU stay with higher PLR values, the clinical implications of this observation warrant further investigation. The lack of statistical significance does not necessarily indicate no effect, but rather reflects the limitations of our current dataset and analytical approach. Future studies with larger sample sizes and possibly different analytical methods might reveal a statistically significant relationship.

Understanding the relationship between PLR and ICU outcomes could inform risk stratification and resource allocation strategies in sepsis management [16]. This research enhances the existing knowledge on prognostic indicators and therapeutic approaches in sepsis. By examining multiple models with varying degrees of adjustment for confounding factors, we have demonstrated that Model 4, which includes demographic variables, comorbidities, biochemical test results, and vital signs, provides the best assessment of PLR's relationship with mortality. This comprehensive model could be valuable for clinical decision-making, offering a more precise prediction of patient outcomes than simpler models or PLR alone.

Variables influencing patient outcomes were carefully analyzed by combining demographic, clinical, and laboratory data. Identifying PLR as a potential prognostic predictor underscores the significance of examining inflammatory markers in determining the treatment of sepsis and the probability of recurrence. Nevertheless, our study is not without limitations. Our findings may not be as broadly applicable as they may be due to the retrospective nature of our research and our reliance on a single-center dataset. Additionally, while we adjusted for various confounding factors in our analysis, including demographic variables, comorbidities, biochemical markers, and vital signs, the presence of unmeasured confounders cannot be entirely excluded. Future studies incorporating larger, multi-center datasets and prospective designs are required to corroborate our results and elucidate the PLR clinical implications in sepsis management.

Conclusion

This study provides valuable insights into the relationship between PLR and sepsis outcomes, contributing to the growing body of literature on prognostic markers and treatment strategies in sepsis. Our findings suggest that PLR could be a valuable tool in risk stratification and prognostic evaluation for sepsis patients. Furthermore, our analysis of four different models indicates that comprehensive consideration of demographic factors, comorbidities, biochemical parameters, and vital signs alongside PLR (Model 4) provides the most robust approach for predicting mortality in sepsis patients.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Angus DC, van der Poll T.* Severe sepsis and septic shock. N Engl J Med 2013; 369(9): 840–51. Erratum in: N Engl J Med 2013; 369(21): 2069.
2. *Gaieski DF, Edwards JM, Kallan MJ, Carr BG.* Benchmarking the incidence and mortality of severe sepsis in the United States. Crit Care Med 2013; 41(5): 1167–74.
3. *Póvoa P, Coelho L, Dal-Pizzol F, Ferrer R, Huttner A, Conway Morris A,* et al. How to use biomarkers of infection or sepsis at the bedside: guide to clinicians. Intensive Care Med 2023; 49(2): 142–53.
4. *Kriplani A, Pandit S, Chawla A, de la Rosette JJMCH, Laguna P, Jayadeva Reddy S,* et al. Neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and lymphocyte-monocyte ratio (LMR) in predicting systemic inflammatory response syndrome (SIRS) and sepsis after percutaneous nephrolithotomy (PNL). Urolithiasis 2022; 50(3): 341–8.

5. *Biyikli E, Kayipmaz AE, Kavalci C.* Effect of platelet-lymphocyte ratio and lactate levels obtained on mortality with sepsis and septic shock. Am J Emerg Med 2018; 36(4): 647–50.
6. *Wang G, Mivefroshan A, Yaghoobpoor S, Khanzadeh S, Siri G, Rahmani F,* et al. Prognostic Value of Platelet to Lymphocyte Ratio in Sepsis: A Systematic Review and Meta-analysis. Biomed Res Int 2022; 2022: 9056363.
7. *Zheng R, Shi YY, Pan JY, Qian SZ.* Decrease in the Platelet-to-Lymphocyte Ratio in Days after Admission for Sepsis Correlates with In-Hospital Mortality. Shock 2023; 59(4): 553–9.
8. *Shen Y, Huang X, Zhang W.* Platelet-to-lymphocyte ratio as a prognostic predictor of mortality for sepsis: interaction effect with disease severity-a retrospective study. BMJ Open 2019; 9(1): e022896.
9. *Pollard TJ, Johnson AEW, Raffa JD, Celi LA, Mark RG, Badawi O.* The eICU Collaborative Research Database, a freely available multi-center database for critical care research. Sci Data 2018; 5: 180178.
10. *Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M,* et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016; 315(8): 801–10.
11. *Van Vught LA, Wiewel MA, Hoogendijk AJ, Frencken JF, Scicluna BP, Klouwenberg PMC,* et al. The host response in patients with sepsis developing intensive care unit-acquired secondary infections. Am J Respir Crit Care Med 2017; 196(4): 458–70.
12. *Johnson AEW, Aboab J, Raffa JD, Pollard TJ, Deliberato RO, Celi LA,* et al. A Comparative Analysis of Sepsis Identification Methods in an Electronic Database. Crit Care Med 2018; 46(4): 494–9.
13. *Haddad DN, Mart MF, Wang L, Lindsell CJ, Raman R, Nordness MF,* et al. Socioeconomic Factors and Intensive Care Unit-Related Cognitive Impairment. Ann Surg 2020; 272(4): 596–602.
14. *Sarkar S, Kannan S, Khanna P, Singh AK.* Role of platelet-to-lymphocyte count ratio (PLR), as a prognostic indicator in COVID-19: A systematic review and meta-analysis. J Med Virol 2022; 94(1): 211–21.
15. *Simadibrata DM, Pandhita BAW, Ananta ME, Tango T.* Platelet-to-lymphocyte ratio, a novel biomarker to predict the severity of COVID-19 patients: A systematic review and meta-analysis. J Intensive Care Soc 2022; 23(1): 20–6.
16. *Muşat F, Păduraru DN, Bolocan A, Palcău CA, Copăceanu AM, Ion D,* et al. Machine Learning Models in Sepsis Outcome Prediction for ICU Patients: Integrating Routine Laboratory Tests-A Systematic Review. Biomedicines 2024; 12(12): 2892.

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The impact of focused attention on bilateral sensory threshold adaptation during unilateral short-term tactile stimulation

Uticaj usmerene pažnje na bilateralnu adaptaciju senzornog praga nadražaja tokom jednostrane kratkotrajne taktilne stimulacije

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Abstract

Background/Aim. Neuroplasticity of the somatosensory system can be manifested after short-term or long-term peripheral tactile stimulation. Focused attention has been well established as a modulator of neural processing in the visual and auditory systems. However, its role in the primary somatosensory cortex is insufficiently elucidated. The aim of this study was to examine the effect of focused attention on short-term somatosensory neuroplasticity following repeated tactile stimulation of different intensity over identical locations on the hands and shoulders. The aim of the study was also to determine whether repeated tactile stimulation of different intensity in the shoulder area of the non-dominant hand leads to a reduction in the stimulus threshold and to assess whether similar changes occur in the contralateral, unstimulated shoulder somatotopically identical location. **Methods.** This study included 30 healthy volunteers of both sexes. The contingent negative variation (CNV) wave and the Go/NoGo paradigm for measuring reaction time were used to objectively register the stimulus threshold for light touch, before and after sensory stimulation. The CNV wave was registered within the paradigm with two known stimuli, the first of which was tactile and the second visual in the form of a green or red circle that appeared randomly on the screen. Peripheral sensory stimulation was conducted only over the non-dominant hand and shoulder using multiple series with 12 tactile stimuli of varying intensities. **Results.** The results showed statistically significant decrease in the stimulus threshold for light touch on both shoulders after tactile stimulation performed only on the non-dominant shoulder. In addition, whenever CNV waves were detected within the Go/NoGo paradigm, reaction times of the subjects were significantly shorter, which served as an objective validation of the initial detection of tactile thresholds before and after peripheral sensory stimulation. **Conclusion.** Short-term, unilateral tactile stimulation leads to bilateral, functional adaptation of the proximal regions of the upper extremities, which suggests interhemispheric homologous transfer within the somatosensory system, supporting the principle of somatotopic organization in somatosensory neuroplasticity.

Key words:
cerebral cortex; electroencephalography; neuronal plasticity; sensory thresholds.

Apstrakt

Uvod/Cilj. Neuroplastičnost somatosenzornog sistema može se manifestovati nakon kratkotrajne ili dugotrajne periferne taktilne stimulacije. Fokusirana pažnja je dobro poznat modulator neuralne obrade u vizuelnim i auditivnim sistemima. Međutim, njena uloga u primarnom somatosenzornom korteksu je nedovoljno razjašnjena. Cilj rada bio je da se ispita efekat fokusirane pažnje na kratkoročnu somatosenzornu neuroplastičnost nakon ponovljenih serija taktilnih stimulusa različitog intenziteta na identičnim mestima na šakama i ramenima. Cilj rada je takođe bio da se utvrdi da li ponovljeni taktilni stimulusi različitog intenziteta u predelu ramena nedominantne ruke dovode do smanjenja praga nadražaja i da se proceni da li slične promene nastaju i u kontralateralnom, nestimulisanom ramenu, na somatotopski identičnoj lokaciji. **Metode.** U ovu studiju je bilo uključeno 30 zdravih dobrovoljaca oba pola. Talas kontingentne negativne varijacije (*contingent negative variation* – CNV) i *Go/NoGo* paradigma za merenje reakcionog vremena bili su korišćeni kako bi se objektivno registrovao prag nadražaja za lak dodir, pre i nakon senzorne stimulacije. CNV talas bio je registrovan u okviru paradigme sa dva poznata stimulusa, od kojih je prvi bio taktilni a drugi vizuelni u vidu zelenog ili crvenog kruga koji su se nasumično pojavljivali na ekranu. Periferna taktilna stimulacija bila je sprovedena samo na nedominantnoj šaci i ramenu, i to

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kroz više serija sa 12 taktilnih stimulusa različitih intenziteta. **Rezultati.** Rezultati su pokazali statistički značajno sniženje praga nadražaja za lak dodir na oba ramena nakon taktilne stimulacije sprovedene samo na nedominantnom ramenu. Pored toga, kada god su bili detektovani CNV talasi u okviru *Go/NoGo* paradigme, reakciona vremena ispitanika bila su značajno kraća, što je služilo kao objektivna potvrda detekcije praga nadražaja pre i nakon periferne senzorne stimulacije. **Zaključak.** Kratkotrajna, unilateralna taktilna stimulacija dovodi do bilateralne, funkcionalne adaptacije proksimalnih regija gornjih ekstremiteta, što sugeriše interhemisferični homologni transfer u okviru somatosenzornog sistema, podržavajući princip somatotopskog organizovanja u somatosenzornoj neuroplastičnosti.

Ključne reči:
mozak, veliki, kora; elektroencefalografija; neuroplastičnost; prag nadražaja.

Introduction

Studies have shown that the organization of the somatosensory system is prone to prompt adaptation as a response to changes in peripheral input [1–3]. In most of them, a deafferentation model was used in which peripheral sensory afferents were either temporarily suppressed or permanently eliminated [4]. However, research employing electrical stimulation of peripheral nerves has also demonstrated the induction of cortical plasticity, even in the absence of afferent deprivation [5, 6]. Additionally, studies focusing on repetitive tactile stimulation of peripheral mechanoreceptors have reported enhancements in somatosensory function, confirming that various stimulation paradigms can induce training-related perceptual learning [5, 7].

One of the main questions in the research of somatosensory systems involves the role of attention. A previous publication showed that anticipating a stimulus can improve overall perception by amplifying relevant stimuli and/or suppressing irrelevant ones [8]. In the visual and auditory systems, attention has an important role in filtering out unwanted and facilitating relevant afferent information; however, the picture is less clear for the somatosensory cortex, for which there is still a scientific debate whether this occurs at the level of primary somatosensory cortex (SI), secondary somatosensory cortex (SII) or both [9, 10].

Studies have used different parameters and spatial-temporal characteristics in repetitive tactile stimulation protocols [5].

The aim of this study was to explore the effects of focused attention on short-term peripheral tactile stimulation of the somatosensory system using tactile cued attention training. We employed a psychophysiological research paradigm combined with electroencephalography (EEG) recordings, with specific aims: to determine whether short-term tactile stimulation can reduce touch thresholds at the shoulder, and to examine whether a similar change occurs on the contralateral, non-stimulated shoulder, potentially indicating interhemispheric sensory modulation.

Methods

Participants

Thirty healthy volunteers (9 males and 21 females) between 18 and 55 years of age [mean ± standard deviation (SD): 35.4 ± 8.9 years] participated in our study. Participants were recruited from the general population and provided a detailed explanation of the study protocol before enrollment. We included subjects of all ethnic groups who could comprehend and had intact and normal manual dexterity to perform specific tasks during this research. According to the modified Edinburgh scale, 4 participants were left-handed and 26 had right-hand dominance [11]. All subjects self-declared that they had no prior history of any neurological, psychiatric, or cognitive impairment that might interfere with somatosensory perception and study interpretation. This study was approved by the Institutional Review Board of NYU School of Medicine, New York, USA (No. i14-01734; from January 13, 2015). Before participating, all volunteers provided written informed consent following the Declaration of Helsinki. All participants completed the study with no adverse events.

Location and experimental conditions

The measurements were conducted at the Clinical Neurophysiology Laboratory at the Hospital for Joint Diseases, NYU Langone Health, New York, USA. The temperature of the testing environment was maintained at a comfortable 25 ºC. To ensure consistency across participants, all experimental recordings were performed at a time of day when each participant reported feeling most alert and focused. All experimental testing was conducted in a single session lasting approximately 4 hrs. Participants were asked to abstain from consuming any psychoactive substances, such as caffeinated drinks and/or alcohol, at least 24 hrs before the measurements took place [12, 13].

Equipment

A tactile stimulator, TS 120 (SBMEDIC Electronics, Solna, Sweden), served as a generator of light touch. The actual tactile stimuli were delivered by a flat, round, 2 mm diameter plastic tip with blunt edges within the displacement-controlled system of the stimulator. The plastic tip had an adjustable displacement range of 1 µm to 1 mm with a resolution of 1 µm. Its duration of stimulation was 80 milliseconds (ms), and the speed of skin indentation was between 80 and 100 µm/ms. An EEG recorder (Nicolet Biomedical, Madison, WI, USA) was used for the registration of contingent negative variation (CNV) waves, and a desktop computer with the open-source application PsychoPy version 2020.1.2 (Open Science Tools Ltd., Nottingham, England) provided the recording of reaction time (RT) after the Go/NoGo decision task paradigm with dissimilar visual stimuli. The tactile stimulator and desktop computer were connected to an EEG recorder, which was a signal generator in this closed circuit.

Experimental design

Recordings were conducted on four target locations: the thenar eminence and the lateral shoulder region bilaterally. Before all measurements, a participant took the most comfortable sitting position in an adjustable reclining chair. The arm upon which the trials were conducted was placed in a vacuum fixation pillow (AB Germa, Kristianstad, Sweden), which secured a stable position during the experiment. A mechanical multi-jointed device (Foba AG, Wettswil, Switzerland), to which the head of the tactile stimulator was attached, permitted fixation at any desired position and angle in relation to the skin surface. To ensure permanent contact during interval displacements of the stimulating probe tip, the probe was placed perpendicularly to the skin surface with constant indentation of approximately 1–3 mm. Over the range of amplitudes needed to activate skin mechanoreceptors of the shoulder, the head of this stimulator produced a sound that could have affected both CNV and RT recordings during light touch stimuli trials. To avoid this confounding element, all participants wore noise-canceling headphones (Bose, Framingham, MA, USA) through which a computer-generated pink noise with a sufficient volume to mask all surrounding room noise was generated. Before measuring the shoulders, we conducted a two-point discrimination test using an aesthesiometer (Healthcare Fitness Products, Cottage Grove, WI, USA) to determine the width of the mechanoreceptor fields [14]. Volunteers were asked not to move or blink their eyes except between trials.

Contingent negative variation and reaction time recordings

To objectively assess light touch perception, we used the CNV paradigm as a quantitative psychophysiological procedure [15, 16]. The CNV traces were recorded with gold cup surface electrodes (Natus Medical Incorporated, Middleton, WI, USA) placed according to the international 10–20 system. Before placing the electrodes, we prepped the skin with alcohol and abrasive gel, and to decrease impedance, we used conductive paste (Weaver and Company, Houston, TX, USA). We used midline central (Cz), frontal left (F3), frontal right (F4), and left mastoid as recording electrodes, while the midline frontal (Fz) electrode served as a reference. The ground electrode was placed on the forehead. Electrode impedances were kept below 5 kiloohm (kΩ), and the evoked potentials were recorded with a filter bandwidth of 0.2 Hz to 30 Hz. The CNV was recorded by applying a tactile stimulus followed by a visual stimulus with a one second interval between them, during which CNV was generated. For each stimulating tip displacement value, a pair of tactile-visual stimuli were administered 12 times, and all CNV traces were averaged and recorded during one trial [16]. The averaged CNV amplitudes in microvolt (μV) were measured for each trial, and inter-trial intervals varied randomly from 3 to 8 s. The trial had no start cue, and participants waited for the new tactile stimulus after the last visual stimulus, therefore maintaining their focused attention during the experiment. The participants were given a 3- to 5-min break after each location was tested to avoid mental fatigue, maintain concentration, and prepare the equipment for the following stimulation site. For the analysis of CNV waves, we assessed the presence of early CNV (eCNV) and/or late CNV (lCNV) wave components. An early component was determined based on the individual maximum amplitude between 350 and 650 ms after tactile stimulus, and a late component with a maximum amplitude in a 200 ms interval preceding visual stimulus [17]. All CNV amplitudes were calculated offline after the measurements were done. During the recordings of all trials, we subjectively determined only the presence or absence of CNV responses. CNV recordings containing eye movement, blinking, or facial muscle artifacts were excluded from further analysis. RTs were recorded using the Go/NoGo paradigm, randomly providing one of the two dissimilar visual stimuli (green or red). Either a green or red circle was automatically shown on a computer screen, placed approximately 1.5 m in front of a subject, precisely one second after each tactile stimulus was exerted. Subjects were instructed to press the space key on a computer keyboard with the contralateral, non-stimulated hand as quickly as possible, only after the green light was shown on the screen, and to do nothing when the red light appeared. The result of this action was a measure of their RT in milliseconds, which was the period between the onset of visual stimulus and pressing the keyboard. The basic concept of the CNV paradigm and the study design were thoroughly explained to the subjects before the trials began. The sequence of the CNV paradigm and Go/NoGo decision task model is summarized in Figure 1.

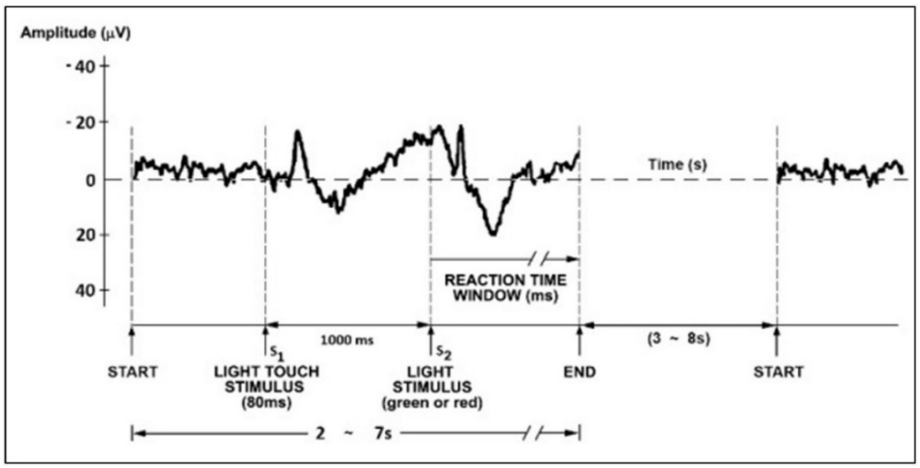

Fig. 1 – Schematic representation of the CNV paradigm and the Go/NoGo decision task model. CNV– contingent negative variation; S1– tactile stimulus; S2 – visual stimulus.

Determination of baseline tactile thresholds

To determine light touch thresholds, we tested the thenar eminence and shoulder of the non-dominant arm, followed by the thenar eminence and shoulder of the dominant arm, with the assessment of CNV and RT. For the initial threshold determination procedure, we used the method of descending levels, which utilizes stimuli of predetermined levels of intensity and duration [18]. The first displacement of the stimulating tip was set to a value well above the expected perception threshold of the tested skin region (50 μm and 150 μm for the thenar and shoulder, respectively), with the step size of changes in the intensity of tactile stimulation of 2 μm for the thenar and 20 μm for the shoulder. A set of 12 stimulations *per* trial was delivered, with one to three second of pseudorandom delay between stimulations, and CNV with RT values were recorded. After every stimulus, a participant was asked to report whether a tactile stimulus was perceived, and this process was repeated, with a subsequent decrease of stimulating tip amplitudes for every trial, until a participant reported feeling 6 to 8 touches out of 12 repetitions. The touch threshold was considered the displacement of the stimulating tip (in μm), which still produced a clear CNV recognizable in the record, even if it was of lower amplitude than the CNV related to stronger stimuli [16]. The same approach was repeated for the measurements over other locations as well.

Peripheral sensory stimulation and determination of new tactile thresholds

Peripheral sensory stimulation was conducted over the lateral shoulder surface of the non-dominant arm only. To avoid confounding results within the Go/NoGo RT paradigm, participants had to use their dominant hand to press the keyboard. We used the exact positioning of participants and equipment and the same concept of the CNV paradigm and Go/NoGo decision task compared to determination of baseline tactile threshold. Regarding the stimulus intensity, we started from the confirmed threshold levels and then randomly stepwise changed the displacement of the stimulating tip up and down, with an overall decrease of stimulating amplitudes toward sub-threshold levels. The step size change was 20 μm, identical for all 30 participants. On average, before reaching new tactile thresholds for all participants, we had to repeat 6 to 8 trials of 12 tactile stimuli. When the CNV was obtained, the lowest stimulus intensity was taken as a new objective threshold for light touch on the stimulated shoulder.

Tactile threshold assessment of non-stimulated shoulders

After a series of peripheral sensory stimulations, we assessed whether any changes occurred for the contralateral (non-stimulated) shoulder. The setup of the equipment and participants was the same, except here, we performed only two trials with 12 stimulus repetitions, as we wanted to avoid the effects of direct peripheral sensory stimulation on this shoulder. For these two trials, we used levels of tactile stimulus intensity comparable to when we recorded a new touch threshold over the non-dominant shoulder. If the CNV was acquired during the first trial, we took that as a new objective touch threshold over the non-stimulated shoulder. After that, we conducted a second trial with the tip displacement reduced by 20 μm to confirm the absence of the CNV at sub-threshold levels of tactile intensity. On the other hand, if the CNV was not recorded during the first trial, we increased the tip displacement by 20 μm, then recorded clear CNV and took that level of tactile stimulation as the new threshold.

Statistical analysis

Volunteers were assigned with record numbers only, and the following data parameters were recorded: gender, age, hand dominance, amplitude of stimulating tip displacement (in μm), CNV recordings (latency values in ms, amplitude values in μV), RT values within the Go/NoGo decision task paradigm (in ms) and number of subjectively perceived tactile stimuli. All statistical analyses were conducted using SPSS Statistics version 21. Results are given as mean values ± SD. To compare two means, a Student's *t*-test was used for independent or paired samples, with the statistical significance level set at $p < 0.05$. The one-way analysis of variance (ANOVA) and two-way repeated measures ANOVA were used to determine the differences with significance levels established at $p < 0.05$. Before applying ANOVA, Shapiro-Wilk's test was used to assess the assumptions of normality, and Mauchly's test of sphericity was used to test the homogeneity of variances. When necessary, a logarithmic transformation was applied ($p < 0.05$), and the procedure was repeated. The Student-Newman-Keuls method was used as a *post-hoc* test whenever the ANOVA revealed a significant difference between three or more sample means ($p < 0.05$). Bonferroni correction was used when several statistical tests were performed simultaneously. Spearman's rank-order correlation was used to determine the relationship between two parameters.

Results

Determination of baseline tactile thresholds

Mean values and SDs for baseline tactile threshold measurements (stimulating tip displacements in μm) are presented in Table 1.

Relationship of contingent negative variation and reaction time recordings before peripheral sensory stimulation

Before peripheral sensory stimulation, CNV traces were present for all supra-threshold and threshold measurements. This relationship was confirmed with the *t*-test, which showed significant differences in the number of subjective verifications of light touch stimuli when the CNV traces

were generated and when they were absent in all four locations we assessed [non-dominant thenar: t (88) = 30.74, p = 0.0001; non-dominant shoulder: t (88) = 34.57, p = 0.0001; dominant thenar: t (88) = 34.93, p = 0.0001; dominant shoulder: t (88) = 25.97, p = 0.0001]. The one-way ANOVA did not reveal statistically significant differences in CNV amplitudes between all four locations on which we conducted measurements for both supra-threshold ($F_{3,116}$ = 0.391, p = 0.761) and threshold ($F_{3,116}$ = 0.018, p = 0.997) levels of tactile stimuli.

Further analysis (Figure 2) demonstrated statistically significant shorter RT recordings when CNVs were present (non-dominant thenar: $F_{1,29}$ = 78.853, p = 0.001; non-dominant shoulder: $F_{1,29}$ = 79.032, p = 0.001; dominant thenar: $F_{1,29}$ = 80.575, p = 0.001; dominant shoulder: $F_{1,29}$ = 56.387, p = 0.001). Additionally, it was confirmed by one-way ANOVA that the applied intensity of touch stimuli significantly influenced the duration of RT recordings (non-dominant thenar: $F_{2,87}$ = 30.83, p = 0.001; non-dominant shoulder: $F_{2,87}$ = 21.95, p = 0.001; dominant thenar: $F_{2,87}$ = 16.02, p = 0.001; dominant shoulder: $F_{2,87}$ = 19.51, p = 0.001). After *post-hoc* analysis, we found significantly shorter RT recordings [non-dominant thenar: t (88) = 7.39, p = 0.001; non-dominant shoulder: t (88) = 6.65, p = 0.001; dominant thenar: t (88) = 5.51, p = 0.001; dominant shoulder: t (88) = 6.27, p = 0.001] during threshold and supra-threshold levels of tactile stimulation compared to trials when participants reported no sensation at all (Figure 3).

Table 1

Values of stimulating tip displacement in micrometers (μm) for measuring baseline tactile thresholds

Location of measurement	Baseline tactile thresholds
Non-dominant thenar	8.7 ± 1.6 (5–10)
Non-dominant shoulder	75.3 ± 17.5 (40–100)
Dominant thenar	8.3 ± 1.7 (5–12)
Dominant shoulder	78 ± 20.1 (40–110)

Values are presented as mean ± standard deviation and range.
ANOVA: $p < 0.05$; t-test: $p < 0.05$

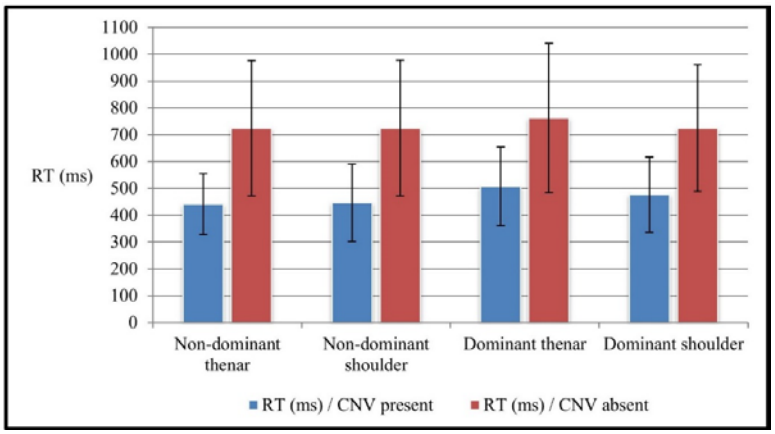

Fig. 2 – Relationship between CNV amplitude and RT before peripheral sensory stimulation.
CNV – contingent negative variation; RT – reaction time.
Values are presented as mean ± standard deviation; t-test: $p < 0.05$.

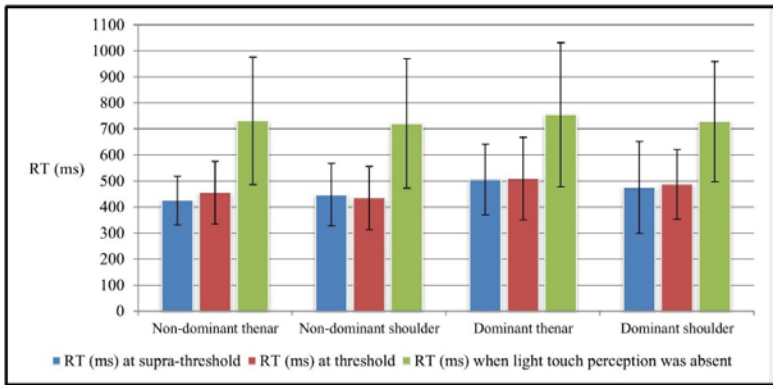

Fig. 3 – RT recordings at different thresholds across all measurement locations.
RT – reaction time.
Values are presented as mean ± standard deviation; t-test: $p < 0.05$;
***post-hoc* comparison for threshold and suprathreshold levels versus infrathreshold stimuli, $p = 0.001$.**

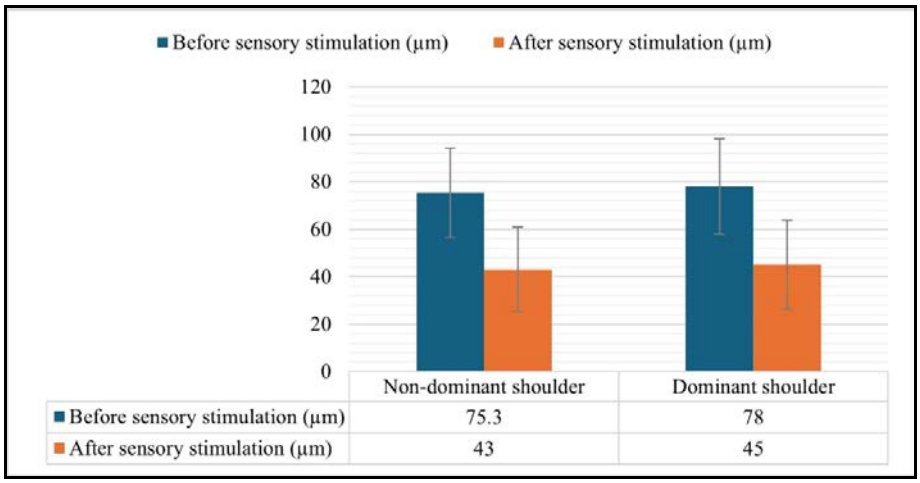

Fig. 4 – Reduction in tactile thresholds following peripheral sensory stimulation. Values are presented as mean ± standard deviation; *t*-test: $p < 0.01$.

Determination of tactile thresholds after peripheral sensory stimulation

Assessment of tactile thresholds after peripheral sensory stimulation conducted over the non-dominant shoulder showed changes on both shoulders in all 30 participants (Figure 4).

When we compared the data before and after sensory stimulation, we got a statistically significant decrease in tactile thresholds in both non-dominant ($F_{1,29}$ = 8.785, p = 0.006) and dominant ($F_{1,29}$ = 227.591, p = 0.001) shoulders. In addition to that, there was no statistically significant difference in the new tactile thresholds between both shoulders ($F_{1,29}$ = 1.812, p = 0.189). After peripheral sensory stimulation, we evaluated the presence of new tactile thresholds on both shoulders with recordings of CNV traces and RT values. CNV waves were present for all 30 participants, with varying amplitudes. The analysis of the relationship between generated CNV traces and RT values showed results comparable to those obtained before sensory stimulation. Whenever participants generated CNV traces of any amplitude, their RT values were significantly shorter [non-dominant shoulder: t (29) = 7.34, p = 0.001; dominant shoulder: t (29) = 5.98, p = 0.001].

Discussion

This study investigated the probability of tactile threshold changes after short-term peripheral sensory stimulation of skin mechanoreceptors.

Tactile thresholds decrease on the stimulated shoulders

Studies have shown that the processing of sensory inputs is not hard-wired but adapts to sensory experience. The adult cortex demonstrates reduced reorganization potential than during its developmental period, but still preserves the capacity for significant neuroplasticity [19, 20]. In addition to studies describing peripheral deafferentation as a model for investigating neural reorganization, experience-dependent neuroplasticity, and perceptual learning have emerged as frequently used paradigms for studying somatosensory changes at multiple levels [21]. Likewise, our study demonstrated a rapid and statistically significant change in somatosensory system processing after short-term sensory enrichment conducted at the exact location. The threshold of light touch changed from an average of 75.3 μm to 43 μm, a significant decrease of 32.3 μm on average over the area of stimulation. Other studies examining peripheral tactile stimulation in amputees have reported comparable changes, as seen in the publication by Dhillon et al. [22], where sensory input was presented to long-term upper limb amputees for only a short period (< 75 min throughout the study), resulting in enhanced sensory perception. That finding supported the view that the organization of the human brain is use-dependent and constantly adapting to demands and experiences.

The role of attentional engagement and behavioral relevance in sensory processing has been explored in studies on passive tactile co-activation, among others, some of which have yielded unexpected results. Ziemus et al. [23] investigated changes in the SI representation of the four fingers following a 40-min passive tactile co-activation task. Their results indicated a convergence of median and ulnar nerve cortical representations, implying that the cortical boundaries between activated regions moved closer together. However, they found no significant improvements in tactile perception, which they explained by the short duration of stimuli or lack of behavioral relevance in their experiment (subjects did not have to pay attention to the stimulation). On the contrary, Godde et al. [24] applied a co-activation task for three hrs, which was restricted to the tip of the right index finger, allowing simultaneous stimulation of all overlapping receptive fields. Even though participants in this study did not have to pay attention to stimulation, they found that several hrs of tactile co-activation protocol could improve perceptual performance. In this case, we might speculate that evident perceptual changes had occurred due to much longer tactile co-activation, which was focused on a single location with over-

lapping receptive field areas. Interestingly, the overall duration of peripheral stimulation in our setting lasted no more than 45 min. It was constantly delivered over the same receptive field on the shoulder area without co-activation. Compared to the most frequently used stimulation protocols [25], this duration of stimuli over the same receptive field, combined with behavioral-relevant study tasks, was sufficient to generate quick, adaptive changes in the somatosensory system in our experiment. For our study, the behavioral relevance of tactile peripheral stimulation was included in the Go/NoGo RT paradigm with dissimilar visual stimuli. The role of attention in cortex remodeling was clearly described [26]. We asked participants to remain attentive during the experiment and to focus on the tactile stimuli, which varied from supra- to sub-threshold intensities throughout the sensory enrichment we provided. Tactile stimulation served as a sensory preconditioning [27], combined with a goal-directed assignment in the Go/NoGo paradigm. Sensory preconditioning in this complex task was objectively confirmed by recordings of CNV, which reflects a tonic modulation of the EEG signal in the preparatory period between a warning signal (light touch, in our study) followed by a predictable and known interval before an imperative stimulus (visual stimulus, in our research) that cues an RT response [16]. We recorded a well-defined CNV where participants reported 6 to 8 perceived light touch stimuli in 12 repetitions, correlating with previously described data [16].

However, we did not register a significant difference between the CNV amplitudes before and after peripheral sensory training for both non-dominant and dominant shoulders. This implies that the intensity-response curve was not present in our CNV recordings. That was the expected result, which did not exclude the fact that whenever our participants felt light touch stimulation, they generated a CNV wave. In addition, we registered RT values that were functionally interconnected with CNV recordings, which served as another confirmation of light touch perception. Participants had faster RTs in the Go/NoGo paradigm task whenever they felt the tactile stimulation, compared with trials when the subjective perception of light touch was absent. In addition, we registered that whenever participants displayed an increased CNV amplitude, shorter RT was observed in that trial, but that correlation did not reach statistical significance. These findings suggest that participants were better prepared for the upcoming visual stimulus and motor response when tactile perception was heightened, demonstrating that a warning stimulus enhances response preparation and reduces RT, further reinforcing the functional interplay between tactile perception, attentional engagement, and motor response readiness.

Tactile thresholds decrease on opposite (non-stimulated) shoulders

It is well established that sensory input from one limb primarily modulates the contralateral SI, which represents the stimulated limb. However, reports during the last two decades showed that tactile information from the periphery reaches SI in both hemispheres [28–30] and that alteration of sensory input may influence the excitability of the ipsilateral somatosensory cortex as well [29–31]. Interhemispheric transfer of tactile information is particularly important in higher primates and humans. The cortical regions of bilateral hand and arm representations integrate somesthetic input during the bimanual and cooperative exploration and discrimination of tactile features [32]. The idea that the SI receives input from the ipsilateral hand initially came from Tamura [33] several decades ago. That hypothesis led to many behavioral, electromyographic, transcranial magnetic stimulation (TMS), and functional magnetic resonance imaging studies, which addressed the similar question of anatomical connection and transfer of information between homotopic cortical and subcortical areas [22, 26, 30]. Our study examined whether unilateral short-term stimulation of a proximal upper limb area would also enhance sensitivity in the homotopic region of the contralateral upper extremity. We applied a quantitative psychophysiological approach, incorporating cortical anticipation measures as an indirect indicator, in which, after the short-term peripheral sensory stimulation over the lateral surface of the shoulder, we observed equal changes in the contralateral, symmetrically opposite location. Statistical analysis showed almost no difference in the new touch thresholds, 45 μm and 43 μm, for the dominant/stimulated and nondominant/non-stimulated shoulders, respectively. This finding indirectly supports interhemispheric interactions after relatively short peripheral stimulation. Previous studies by Frank et al. [34] and Frank [35] supported the existence of interhemispheric interactions between symmetrically opposite body surface areas, which are somatotopically organized, and confirmed that tactile learning in humans is topographically distributed [7, 34, 36]. These crossed interactions between limbs are likely mediated, at least partially, by the fibers of the corpus callosum [37, 38]. Highlighting the significance of transcallosal pathways in influencing the functional state of the ipsilateral SI, studies showed that applying TMS to the parietal cortex on the same side as the stimuli led to heightened tactile sensitivity. It was suggested that this effect was due to TMS interfering with typical interhemispheric inhibition, a mechanism that suppresses mirror movements in the passive limb during one-handed tasks [39]. The functional significance of interhemispheric inhibition deactivation is reflected in everyday activities when individuals depend on sensory input from the fingers of one hand to recognize various textures. During unimanual exploration, the texture typically affects all fingers of that hand similarly. However, in tasks involving bimanual manipulation, each hand may receive distinct sensory information [28]. Besides the proposed transcallosal connections, other points along the ascending and descending pathways, including subcortical structures and segmental networks, may be implicated in the functional coupling between the upper limbs [40].

Indeed, understanding the mechanisms and time reference of afferent tactile information processing through the cortical and subcortical networks of the somatosensory system is of great interest. The review by Chipchase et al. [5] on peripheral electric stimulation and the induction of cortical plasticity mentioned several possible mechanisms that are

thought to underlie rapid plastic changes in the somatosensory cortex. These include the unmasking of latent horizontal connections, activation of silent synapses, modulation of activity-dependent synaptic plasticity, and generalized changes in the excitability of postsynaptic neurons. Another review [25] of tactile stimulation interventions reported that the transfer of plastic changes relies on the overlap of receptive fields and their cortical representations. Only if such an overlap is given among body parts can stimulation-induced effects spread between them.

In our study, the focused attention of all subjects could have represented an important additional factor for the short-term modulation within somatosensory system processing. Diminutive modifications of brain circuits and slight changes in synaptic strength across many neurons can be challenging to identify and measure. The contingent negative variation reveals sensorimotor integrative and preparatory processes, representing a long-latency, slow, and negative potential shift with cognitive and motor components [41]. It is present during response anticipation and was termed "expectancy wave" when it was first described by Walter et al. [15]. The CNV wave serves as an index of cortical arousal during orienting and attention and is related to higher mental functions involved in processing incoming sensory input [17], with high CNV amplitude indicating a high attentional state of a subject [42]. In addition, for the assessment of volitional inhibition or activation in neurophysiological studies, a Go/NoGo RT task has been frequently used [43, 44]. A Go/NoGo decision task model is based on the time required for a subject to respond and make a specific motor action to one class of stimuli (also known as the Go response) or to withhold from responding (the NoGo response) to a different stimulus type (decision of pressing a key for one stimulus while not pressing it for another stimulus). A study by Kropp et al. [17] showed that the negativity of the early CNV wave increased with decreasing RT, indicating that higher attentional involvement correlated with faster RT.

We might speculate that the influence of attention (top-down control) on the somatosensory system may involve gating and enhancing properties, depending on the task difficulty or the stimulus nature. Moreover, studies on animals and human subjects confirmed the existence of neurons in the postcentral gyrus with bilateral receptive fields in distal (hands) as well as in proximal parts of the body (upper arm, trunk), which might play a significant role in the somatotopic transfer of perceptual learning [7, 35, 45]. Harrar et al. [7] demonstrated that the generalization of tactile perceptual learning in hands was topographic, and the transfer was complete; topographically related fingers showed the same magnitude of improvement as the trained finger. Most importantly, investigations on human subjects [3] and experimental animals [46] confirmed that ipsilateral input can modify the SI and SII response to a subsequent contralateral stimulus.

The differences in communication between cerebral hemispheres and the spinal cord for proximal and distal muscle groups are well-documented [47]. Still, the similar effects of proximal limb somatosensory homologous transfer and its effect on bilateral communication, along with behavioral significance, have gained less attention. Aune et al. [48] investigated the hypothesis that bilateral learning transfer should be larger for proximal than for distal homologous effector muscles. They included 28 participants in three groups: training proximal effectors, training distal effectors, and a no-training control group. They found that both training groups showed similar improvements; however, the proximal training group exhibited greater transfer of learning than the distal one. Therefore, they concluded their hypothesis of a proximal-distal gradient in bilateral learning transfer between homologous effectors, suggesting that proximity to the body core may influence the extent of this communication. Similarly, our findings might raise the possibility that afferent signals from the repetitive shoulder stimulation modulated the response of the SI/SII upper arm region ipsilateral to tactile stimuli.

Without a doubt, more detailed studies are needed to elucidate this interesting question and offer further perspectives on how interhemispheric communication differs between proximal and distal limb somatosensory areas and how these differences might influence the transfer of learning.

Conclusion

Our results indicate that short-term unilateral peripheral stimulation leads to equivalent reductions in tactile thresholds on both shoulders. These findings provide indirect evidence for interhemispheric transfer of simple tactile stimuli, aligning with the somatotopic organization of somatosensory processing.

Conflict of interest

The authors declare no conflict of interest.

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R E F E R E N C E S

1. *Gao Z, Pang Z, Chen Y, Lei G, Zhu S, Li G,* et al. Restoring After Central Nervous System Injuries: Neural Mechanisms and Translational Applications of Motor Recovery. Neurosci Bull 2022; 38(12): 1569–87.
2. *Takase H, Regenhardt RW.* Motor tract reorganization after acute central nervous system injury: a translational perspective. Neural Regen Res 2020; 16(6): 1144–9.
3. *Tommerdahl M, Favorov OV, Whitsel BL.* Dynamic representations of the somatosensory cortex. Neurosci Biobehav Rev 2010; 34(2): 160–70.
4. *Lissek S, Wilimzig C, Stude P, Pleger B, Kalisch T, Maier C,* et al. Immobilization impairs tactile perception and shrinks somatosensory cortical maps. Curr Biol 2009; 19(10): 837–42.
5. *Chipchase LS, Schabrun SM, Hodges PW.* Peripheral electrical stimulation to induce cortical plasticity: a systematic review of stimulus parameters. Clin Neurophysiol 2011; 122(3): 456–63.
6. *Kowalewski R, Kattenstroth JC, Kalisch T, Dinse HR.* Improved acuity and dexterity but unchanged touch and pain thresholds following repetitive sensory stimulation of the fingers. Neural Plast 2012; 2012: 974504.
7. *Harrar V, Spence C, Makin TR.* Topographic generalization of tactile perceptual learning. J Exp Psychol Hum Percept Perform 2014; 40(1): 15–23.
8. *Carrasco M.* Visual attention: the past 25 years. Vision Res 2011; 51(13): 1484–525.
9. *Friedrich J, Verrel J, Kleimaker M, Münchau A, Beste C, Bäumer T.* Neurophysiological correlates of perception–action binding in the somatosensory system. Sci Rep 2020; 10(1): 14794.
10. *Van Ede F, de Lange FP, Maris E.* Anticipation increases tactile stimulus processing in the ipsilateral primary somatosensory cortex. Cereb Cortex 2014; 24(10): 2562–71.
11. *Veale JF.* Edinburgh Handedness Inventory - Short Form: a revised version based on confirmatory factor analysis. Laterality 2014; 19(2): 164–77.
12. *Ricupero S, Ritter FE.* Caffeine and cognition: a cognitive architecture-based review. Theor Issues Ergon Sci 2024; 25(6): 655–79.
13. *Fiani B, Zhu L, Musch BL, Briceno S, Andel R, Sadeq N,* et al. The Neurophysiology of Caffeine as a Central Nervous System Stimulant and the Resultant Effects on Cognitive Function. Cureus 2021; 13(5): e15032.
14. *Thube S, Shah MR, Kothari PH, Shah V.* Assessment of Two Point Discrimination on Hand in Adult Population: An Observational Study. Int J Health Res 2020; 10(5): 60–3.
15. *Walter WG, Cooper R, Aldridge VJ, Mccallum WC, Winter AL.* Contingent negative variation: an electric sign of sensorimotor association and expectancy in the human brain. Nature 1964; 203: 380–4.
16. *Prevec TS, Berić A.* Measurement of light touch perception threshold by contingent negative variation. Exp Brain Res 1991; 84(3): 643–8.
17. *Kropp P, Kiewitt A, Göbel H, Vetter P, Gerber WD.* Reliability and stability of contingent negative variation. Appl Psychophysiol Biofeedback 2000; 25(1): 33–41.
18. *Chong PS, Cros DP.* Technology literature review: quantitative sensory testing. Muscle Nerve 2004; 29(5): 734–47.
19. *Guidali G, Roncoroni C, Bolognini N.* Paired associative stimulations: Novel tools for interacting with sensory and motor cortical plasticity. Behav Brain Res 2021; 414: 113484.
20. *Schlieper S, Dinse HR.* Perceptual improvement following repetitive sensory stimulation depends monotonically on stimulation intensity. Brain Stimul 2012; 5(4): 647–51.
21. *Tamè L, Farnè A, Pavani F.* Spatial coding of touch at the fingers: Insights from double simultaneous stimulation within and between hands. Neurosci Lett 2011; 487(1): 78–82.
22. *Dhillon GS, Krüger TB, Sandhu JS, Horch KW.* Effects of short-term training on sensory and motor function in severed nerves of long-term human amputees. J Neurophysiol 2005; 93(5): 2625–33.
23. *Ziemus B, Huonker R, Haueisen J, Liepert J, Spengler F, Weiller C.* Effects of passive tactile co-activation on median ulnar nerve representation in human SI. Neuroreport 2000; 11(6): 1285–8.
24. *Godde B, Ehrhardt J, Braun C.* Behavioral significance of input-dependent plasticity of human somatosensory cortex. Neuroreport 2003; 14(4): 543–6.
25. *Parianen Lesemann FH, Reuter EM, Godde B.* Tactile stimulation interventions: influence of stimulation parameters on sensorimotor behavior and neurophysiological correlates in healthy and clinical samples. Neurosci Biobehav Rev 2015; 51: 126–37.
26. *Suzuki LY, Meehan SK.* Attention focus modulates afferent input to motor cortex during skilled action. Hum Mov Sci 2020; 74: 102716.
27. *Weinberger NM.* Dynamic regulation of receptive fields and maps in the adult sensory cortex. Annu Rev Neurosci 1995; 18: 129–58.
28. *Passmore SR, Mortaza N, Glazebrook CM, Murphy B, Lee TD.* Somatosensory Integration and Masking of Complex Tactile Information: Peripheral and Cortical Contributions. Brain Sci 2020; 10(12): 954.
29. *Hadoush H, Inoue K, Nakanishi K, Kurumadani H, Sunagawa T, Ochi M.* Ipsilateral primary sensorimotor cortical response to mechanical tactile stimuli. Neuroreport 2010; 21(2): 108–13.
30. *Tamè L, Braun C, Holmes NP, Farnè A, Pavani F.* Bilateral representations of touch in the primary somatosensory cortex. Cogn Neuropsychol 2016; 33(1–2): 48–66.
31. *Valyear KF, Philip BA, Cirstea CM, Chen PW, Baune NA, Marchal N,* et al. Interhemispheric transfer of post-amputation cortical plasticity within the human somatosensory cortex. NeuroImage 2020; 206: 116291.
32. *Tamè L, Pavani F, Braun C, Salemme R, Farnè A, Reilly KT.* Somatotopy and temporal dynamics of sensorimotor interactions: evidence from double afferent inhibition. Eur J Neurosci 2015; 41(11): 1459–65.
33. *Tamura K.* Ipsilateral somatosensory evoked responses in man. Folia Psychiatr Neurol Jpn 1972; 26(1): 83–94.
34. *Frank SM, Otto A, Volberg G, Tse PU, Watanabe T, Greenlee MW.* Transfer of Tactile Learning from Trained to Untrained Body Parts Supported by Cortical Coactivation in Primary Somatosensory Cortex. J Neurosci 2022; 42(31): 6131–44.
35. *Frank SM.* Transfer of Tactile Learning to Untrained Body Parts: Emerging Cortical Mechanisms. Neuroscientist 2025; 31(1): 98–114.
36. *Williamson JN, Sikora WA, James SA, Parmar NJ, Lepak LV, Cheema CF,* et al. Cortical Reorganization of Early Somatosensory Processing in Hemiparetic Stroke. J Clin Med 2022; 11(21): 6449.
37. *Morrone M, Martinez G, Achene A, Scaglione M, Masala S, Manca A,* et al. Size and site matter: the influence of corpus callosum subregional lesions on the magnitude of cross-education of strength. Front Physiol 2025; 16: 1554742.
38. *Pauwels L, Gooijers J.* The Role of the Corpus Callosum (Micro)Structure in Bimanual Coordination: A Literature Review Update. J Mot Behav 2023; 55(5): 525–37.
39. *Kičić D, Lioumis P, Ilmoniemi RJ, Nikulin VV.* Bilateral changes in excitability of sensorimotor cortices during unilateral movement: Combined electroencephalographic and transcranial magnetic stimulation study. Neuroscience 2008; 152(4): 1119–29.

40. *Carson RG.* Neural pathways mediating bilateral interactions between the upper limbs. Brain Res Brain Res Rev 2005; 49(3): 641–62.
41. *Nagai Y, Critchley HD, Featherstone E, Fenwick PBC, Trimble MR, Dolan RJ.* Brain activity relating to the contingent negative variation: an fMRI investigation. Neuroimage 2004; 21(4): 1232–41.
42. *Kropp P, Linstedt U, Niederberger U, Gerber WD.* Contingent negative variation and attentional performance in humans. Neurol Res 2001; 23(6): 647–50.
43. *Raud L, Westerhausen R, Dooley N, Huster RJ.* Differences in unity: The go/no-go and stop signal tasks rely on different mechanisms. Neuroimage 2020; 210: 116582.
44. *Isfahani SA, McGurrin P, Vial F, Hallett M.* Patterns of brain activity in choice or instructed go and no-go tasks. Exp Brain Res 2025; 243(3): 73.
45. *Bundy DT, Leuthardt EC.* The Cortical Physiology of Ipsilateral Limb Movements. Trends Neurosci 2019; 42(11): 825–39.
46. *DeCosta-Fortune TM, Ramshur JT, Li CX, de Jongh Curry A, Pellicer-Morata V, Wang L,* et al. Repetitive microstimulation in rat primary somatosensory cortex (SI) strengthens the connection between homotopic sites in the opposite SI and leads to expression of previously ineffective input from the ipsilateral forelimb. Brain Res 2020; 1732: 146694.
47. *Jablonka JA, Binkowski R, Kazmierczak M, Sadowska M, Sredniawa W, Szlachcic A,* et al. The Role of Interhemispheric Interactions in Cortical Plasticity. Front Neurosci 2021; 15: 631328.
48. *Aune TK, Aune MA, Ingvaldsen RP, Vereijken B.* Transfer of Motor Learning Is More Pronounced in Proximal Compared to Distal Effectors in Upper Extremities. Front Psychol 2017; 8: 1530.

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Consumption of diclofenac and health outcomes in outpatients with or at high risk for cardiovascular diseases in Montenegro after implementation of the innovative risk minimization digital tool

Potrošnja diklofenaka i zdravstveni ishodi kod ambulantnih bolesnika obolelih ili sa visokim rizikom od kardiovaskularnih bolesti u Crnoj Gori nakon implementacije inovativnog digitalnog alata za minimizaciju rizika

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Abstract

Background/Aim. Risk minimization measures (RMMs) for medicines are one of the most important interventions in maintaining a positive benefit–risk ratio and ensuring their safe use. Systemic formulations of diclofenac are medicines with routine and additional RMMs in place for its well-established cardiovascular (CV) safety risk. In 2021, an innovative digital tool (IDT) was implemented in the Montenegrin Information System of Primary Health Care (PHCIS). The aim of this study was to analyze diclofenac consumption in CV risk patients who did not use diclofenac before the introduction of this new IDT for RMM (diclofenac-naïve patients), taking into account their demographic (gender and age) and clinical characteristics [CV diseases (CVD)/risk factors of CVD]. **Methods.** Patients with CVD/diseases posing a risk for CVD development (conditions that are contraindications/precautions for prescribing diclofenac) were selected after an automated screening of all diagnoses, classified in accordance with the International Classification of Diseases, 10th revision, from their electronic medical records. These patients also had medical diagnoses that served as indications for prescribing nonsteroidal anti-inflammatory drugs such as diclofenac. These patients were monitored for a period of one year after the introduction of the IDT (October 4, 2021 – October 4, 2022). Diclofenac consumption was analyzed using the standard methodology of the World Health Organization based on daily defined doses/1,000 inhabitants/day and the Anatomical Therapeutic Chemical classification of medicines, as well as the total number of prescriptions and prescribed packages of the drug, taking into account their gender, age, and high-risk CV diagnoses. **Results.** It was shown that diclofenac was prescribed more frequently to women and patients aged 45–64. Regarding medical diagnoses that are contraindications/precautions for diclofenac use, the drug was most often prescribed to patients with ischemic heart disease (38.15%) and hypertension (71.00%). Following the introduction of the drug into therapy, there was an increase in the number of patients with diagnoses (CVD) that are contraindications for diclofenac use. The largest increases were recorded in patients with diseases of arteries, small arteries and capillaries (41.77%), and congestive heart failure (28.57%). **Conclusion.** After the introduction of the IDT as a new RMM for adverse CV effects of diclofenac into the Montenegrin PHCIS, the drug was most frequently prescribed to female diclofenac-naïve patients, individuals aged 45–64 years, and those with high-risk CV diagnoses that required precautions for its use. The use of diclofenac led to an increase in the number of patients with CVD, indicating the need to introduce new measures to reduce the risk of its adverse CV effects.

Key words:
cardiovascular diseases; delivery of health care; diclofenac; drug prescriptions; drug utilization; montenegro; pharmacovigilance.

Apstrakt

Uvod/Cilj. Mere za minimizaciju rizika (*risk minimization measures* – RMMs) za lekove su jedna od najvažnijih intervencija u održavanju pozitivnog odnosa korist–rizik i omogućavanju njihove bezbedne upotrebe. Sistemske formulacije diklofenaka su lekovi sa rutinskim i dodatnim RMMs zbog njihovog dobro utvrđenog kardiovaskularnog

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(KV) bezbednosnog rizika. U crnogorski Informacioni sistem primarne zdravstvene zaštite je 2021. godine implementirana inovativna digitalna alatka (IDA). Cilj rada bio je da se analizira potrošnja diklofenaka kod bolesnika sa KV rizikom koji nisu koristili diklofenak pre uvođenja nove IDA za RMM (*diclofenac-naïve* bolesnici) uzimajući u obzir njihove demografske (pol i starost) i kliničke karakteristike [KV bolesti (KVB)/faktori rizika od KVB]. **Metode**. Oboleli od KVB/bolesti koje predstavljaju rizik od razvoja KVB (stanja koje su kontraindikacije/stanja koja zahtevaju mere predostrožnosti za propisivanje diklofenaka) odabrani su nakon automatizovanog skriniga svih dijagnoza, klasifikovanih u skladu sa Međunarodnom klasifikacijom bolesti, 10. revizija, iz njihove elektronske medicinske dokumentacije. Ovi bolesnici imali su i medicinske dijagnoze koje su bile indikacije za propisivanje nesteroidnih antizapaljenskih lekova kao što je diklofenak. Bolesnici su praćeni u periodu od godinu dana nakon uvođenja IDA (4. oktobar 2021 – 4. oktobar 2022). Potrošnja diklofenaka analizirana je po standardnoj metodologiji Svetske zdravstvene organizacije na osnovu broja definisanih dnevnih doza na 1 000 stanovnika dnevno i Anatomsko terapijsko-hemijske klasifikacije lekova, kao i ukupnog broja recepata i propisanih pakovanja leka, uzimajući u obzir pol, godine života i dijagnoze bolesnika sa visokim KV rizikom. **Rezultati.** Pokazano je da se diklofenak češće propisivao ženama i bolesnicima starosti 45–64 godina. Kada je reč o medicinskim dijagnozama koje su kontraindikacije/ zahtevaju mere predostrožnosti za primenu diklofenaka, lek je najčešće bio propisivan bolesnicima sa ishemijskom bolešću srca (38,15%), odnosno hipertenzijom (71,00%). Nakon uvođenja leka u terapiju povećao se broj bolesnika sa dijagnozama (KVB) koje su kontraindikacije za primenu diklofenaka. Najveći porast zabeležen je kod bolesnika sa bolestima arterija, malih arterija i kapilara (41,77%) i kongestivnom srčanom insuficijencijom (28,57%). **Zaključak.** Nakon uvođenja IDA kao nove RMM od neželjenih KV efekata diklofenaka u Informacioni sistem primarne zdravstvene zaštite Crne Gore, kod bolesnika sa bezbednosno rizičnim KV dijagnozama koji ga ranije nisu primali, lek se najčešće propisivao *diclofenac-naïve* bolesnicama, osobama starosti 45-64 godine i onima koji su imali dijagnoze koje su zahtevale mere opreza prilikom njegove upotrebe. Upotreba diklofenaka dovela je do povećanja broja bolesnika sa KVB što ukazuje na potrebu uvođenja novih mera za smanjenje rizika od njegovih štetnih KV efekata.

Ključne reči:
kardiovaskularne bolesti; primarno zdravstveno zbrinjavanje; diklofenak; lekovi, propisivanje; lekovi, korišćenje; crna gora; farmakovigilanca.

Introduction

The risk management system in pharmacovigilance (PV) includes PV activities and risk minimization intervention with the ultimate goal of identifying, characterizing, preventing, or minimizing the risk of medicines use, including the evaluation of their effectiveness and impact in clinical practice. The implementation of risk minimization measures (RMMs) poses a great challenge for all stakeholders in the PV system [1]. RMMs consist of two components: RMM message and RMM tool. An RMM message is the key information about the risk and the actions intended to be taken by the healthcare professional (HCP) or the patient for minimizing the risk. An RMM tool is a tool used to disseminate RMM messages and to support or monitor adherence to the intended risk-minimization actions. It can belong to either the category of routine or additional RMM tools [2]. The medicines regulation in Montenegro (MNE) [3], like that in the European Union (EU), lays down the assessment of RMMs' effectiveness in clinical practice, as mandatory for marketing authorization holders and regulatory authorities [4–6]. One example is the assessment of the impact of imposed restrictions on the use of certain medicines on their further prescriptions and consumption [7]. There are a few examples of assessing the influence of PV intervention on patient-relevant health outcomes, which play a key role in the PV system that makes a difference to patients [8].

Systemic formulations of diclofenac (hereinafter: diclofenac) are one of the examples of medicines with routine and additional RMMs in place for its identified, well-established cardiovascular (CV) safety risk. These RMMs have been legally binding in the EU since 2013 [9–11], and were approved by the Institute for Medicines and Medical Devices (CInMED) of MNE in 2015. The routine RMMs referred to the changes in the diclofenac reference information (summary of product characteristics and the patient information leaflet). These changes mostly affected the safety sections in these documents (contraindications and precautions/warnings). However, the sections related to posology and route of administration were also modified, with new recommendations on maximum daily dose and duration of its use. Namely, established congestive heart failure, ischemic heart disease, peripheral arterial disease, and cerebrovascular diseases were introduced as new contraindications for prescribing diclofenac, while hypertension, hyperlipidaemia, and diabetes mellitus, which are risk factors for CV diseases (CVD), were introduced as new precautions/warnings for its prescribing. Besides routine measures, additional RMMs were introduced with the aim to proactively and timely inform relevant HCPs about the new CV safety restrictions for diclofenac use, through an official Dear Healthcare Professional Communication. This communication was disseminated to HCPs by CInMED and all marketing authorization holders for diclofenac *via* various traditional channels: post, e-mail, in person, and publication on CInMED web pages [12, 13].

Unfortunately, the implemented routine and additional measures to minimize the risk of adverse CV effects of diclofenac did not give the expected result. In the research that covered the period from 2016 to 2020, it was shown that diclofenac was still widely prescribed and that even 15–24% of outpatients who were prescribed diclofenac belong to the risk groups of patients with CVD or diseases that represent a high risk of CVD occurrence [14].

PV guidelines, relevant for RMMs, for the first time emphasize the possibility of using different digital tools in order to improve the effectiveness and impact of RMMs in clinical practice, as an additional method to the existing, traditional ones [2]. Based on the CInMED initiative, for the purpose of better impact of RMMs for diclofenac in clinical practice, an innovative digital tool (IDT) was implemented in the Primary Health Care (PHC) Information System – PHCIS. It was introduced at the level of diclofenac prescribing on October 4, 2021. Before the implementation of IDT in PHCIS, HCPs from PHC institutions, as prescribers of diclofenac, were educated about the functionality and purpose of this new, innovative means of RMMs dissemination. Education was organized by CInMED and included educational material and a practical demonstration of IDT.

RMMs for reducing CV risk of diclofenac remained unchanged, but a new RMM tool was introduced as a means of disseminating RMM messages.

This new digital tool for reducing the risk of adverse CV effects of diclofenac is based on the introduction of relevant safety data of this drug contained in its summary of product characteristics, into the PHCIS. These data appeared as warning messages on physicians' computer screens at the moment of their intention to prescribe diclofenac on an electronic prescription to patients with contraindications or warnings/precautions for its use. Based on the text of those warning messages, physicians were alerted of their adverse CV effects/contraindications and precautions.

Accordingly, it was expected that this IDT would lead to a greater decrease in diclofenac consumption, especially among high-risk groups of patients, and thus better prevention of its adverse CV effects. However, the study we conducted comparing the consumption of diclofenac in those patients one year before (October 4, 2020 – October 4, 2021) and one year after the introduction of the IDT (October 4, 2021 – October 4, 2022) showed that, although in a smaller extent, the drug continued to be prescribed even in patients for whom its use was contraindicated. The study also showed that the drug had been prescribed to patients with CVD/at risk for CVD who had not received it before the introduction of this new RMM (diclofenac-naïve patients) [15].

The aim of this study was to analyze in detail the consumption and health outcomes of diclofenac use in a group of patients who were diclofenac-naïve, one year before IDT implementation, but to whom diclofenac was prescribed in the year following IDT implementation (new diclofenac users).

Methods

This study was a continuation of previous research, the results of which have already been published, where the method of patient selection was described in detail [15]. Briefly, new diclofenac users were selected after automated screening of all diagnoses in their electronic medical records, classified according to the International Classification of Diseases, 10th revision (ICD-10) [16], to detect those with CVD or diseases that increase the risk of developing CVD (a list of ICD diagnoses relevant for the study is given in the Supplement). These patients also had medical diagnoses that indicated the need for prescribing nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, but had not received the drug in the year prior to the introduction of the IDT (October 4, 2020 – October 4, 2021), which served as a new tool for RMM dissemination.

The patients were monitored for a period of one year after the introduction of the IDT (October 4, 2021 – October 4, 2022). Diclofenac consumption in patients was analyzed using the standard methodology of the World Health Organization based on defined daily doses/1,000 inhabitants/day and Anatomical Therapeutic Chemical classification of medicines [17], as well as through the total number of prescriptions and prescribed drug packages. Analysis was performed regarding the gender and age of the patients. We also analyzed diclofenac prescribing in new diclofenac users in relation to their CV diagnoses. Health outcomes in these patients were evaluated based on changes in their CV diagnoses during the year following the start of diclofenac use.

The Health Insurance Fund of MNE gave permission for using patient medical data from PHCIS (No. 04-2294, from June 4, 2020). The data of the patients were anonymous.

Statistical analysis

The statistical computer program IBM SPSS version 26.0 was used for statistical data processing. The data are presented in the form of absolute and relative values. Significant differences between groups were tested using the Mann-Whitney *U* test and the Kruskal-Wallis test. Results obtained were considered statistically significant at the level of $p < 0.05$.

Results

Diclofenac consumption in patients with CVD or risks for CVD, who were new diclofenac users in the time period of one year after implementation of the IDT, is given in Table 1. It was shown that the consumption of diclofenac was higher in women compared to men and was the highest in the youngest age group observed (45–64 years) (Table 1).

Specific comorbidities of the patients in this analysis were medical diagnoses (in the Supplement of this article), which are contraindications and precautions/warnings for prescribing diclofenac. Considering contraindications, diclofenac was most commonly prescribed to patients with ischemic heart diseases (i.e., angina pectoris, infarct myocardi), other heart diseases (i.e., cardiomyopathy), and cerebrovascular diseases (i.e., hemorraghia, infarctus cerebri, apoplexia cerebri). Considering the precautions/warnings for its prescribing, diclofenac was most commonly prescribed to patients with hypertension. In most cases, the drug was more often prescribed to female patients (Table 2) and those younger than 75 years (Table 3). Exceptionally, the drug was most frequently prescribed to the oldest patients (≥ 75) who had congestive heart disease and other CVD (Table 3).

Table 1

Consumption of diclofenac in new diclofenac users with CVD/risks for CVD who were prescribed diclofenac, by gender and age

Consumption	Group			*p**	Age, years			*p***
	total	male	female		45–64	65–74	≥ 75	
DDD/1,000 inhabitants/day	2.36	0.94	1.42	0.067	1.02	0.82	0.51	< 0.001
Number of prescriptions	20,361	8,080	12,281	0.119	9,008	7,033	4,320	< 0.001
Number of packages	24,401	9,727	14,674	0.056	10,603	8,472	5,326	< 0.001

CVD – cardiovascular diseases; DDD – daily defined dose.
All values are given as numbers.
*** – Mann-Whitney *U* test was used; ** – Kruskal-Wallis test was used.**

Table 2

Number of new diclofenac users with CVD/risk for CVD who were prescribed diclofenac, by gender

Conditions	Group	Number of patients
Contraindications		
congestive heart failure	total	28
	male	16
	female	12
ischemic heart diseases	total	460
	male	235
	female	225
other heart diseases	total	277
	male	130
	female	147
diseases of arteries, small arteries and capillaries	total	158
	male	84
	female	74
cerebrovascular diseases	total	211
	male	94
	female	117
diseases of the heart of pulmonary origin and diseases of the blood vessels of the lungs	total	39
	male	15
	female	24
Precautions		
hypertension	total	5,916
	male	2,258
	female	3,658
hyperlipidemia	total	701
	male	253
	female	448
diabetes mellitus	total	1,510
	male	743
	female	767

CVD – cardiovascular diseases.

Analysis of the number of new diclofenac users with CVD, which contraindicates its use, showed an increase during the year after the drug was introduced into their therapy. The greatest increase was recorded in the number of patients with diseases of arteries, small arteries and capillaries (41.77%), and congestive heart failure (28.57%), then in the number of patients with cerebrovascular diseases (12.80%) and ischemic heart diseases (10.65%), and the least in the number of patients with diseases of the heart of pulmonary origin and diseases of blood vessels of the lungs (5.13%) and other heart diseases (3.25%) (Table 4).

Table 3

Number of new diclofenac users with CVD/risk for CVD who were prescribed diclofenac, by age

Conditions	Age, years	Number of patients	Total patients
Contraindications			
congestive heart failure	45–64	5	28
	65–74	7	
	≥ 75	16	
ischemic heart diseases	45–64	196	460
	65–74	166	
	≥ 75	98	
other heart diseases	45–64	37	277
	65–74	86	
	≥ 75	154	
diseases of arteries, small arteries and capillaries	45–64	64	158
	65–74	65	
	≥ 75	29	
cerebrovascular diseases	45–64	81	211
	65–74	73	
	≥ 75	57	
diseases of the heart of pulmonary origin and diseases of the blood vessels of the lungs	45–64	18	39
	65–74	14	
	≥ 75	7	
Precautions			
hypertension	45–64	2,648	5,916
	65–74	2,043	
	≥ 75	1,225	
hyperlipidemia	45–64	442	701
	65–74	198	
	≥ 75	61	
diabetes mellitus	45–64	623	1,510
	65–74	597	
	≥ 75	290	

CVD – cardiovascular diseases.

Table 4

Number of new diclofenac users with cardiovascular diseases (for which diclofenac use is contraindicated) before and after introducing the drug into their therapy

Diagnoses	Number of patients		Change in number of patients, (%)
	before using diclofenac	after using diclofenac	
Congestive heart failure	28	36	+ 28.57
Ischemic heart diseases	460	509	+ 10.65
Other heart diseases	277	286	+ 3.25
Diseases of arteries, small arteries and capillaries	158	224	+ 41.77
Cerebrovascular diseases	211	238	+ 12.80
Diseases of the heart of pulmonary origin and diseases of the blood vessels of the lungs	39	41	+ 5.13

Discussion

The results of the impact study conducted earlier in MNE [15] showed that the introduction of the IDT in the PHCIS, as a new tool for RMM for diclofenac adverse CV effects, made an impact in the cohort of patients with CVD/risk for CVD who were prescribed diclofenac one year before the introduction of the IDT. The prescribing of diclofenac one year after the introduction of the IDT decreased by 38.79%, 37.62%, and 29.85% in patients with other heart diseases (mostly cardiomyopathy), cerebrovascular diseases, and ischemic heart diseases, respectively, which are contraindications for its prescribing. Diclofenac was less prescribed, by 22.86%, 23.61%, and 26.32% in patients with hypertension, hyperlipidemia, and diabetes mellitus, respectively, which are warnings/precautions for prescribing the

drug. It was also shown that, among new diclofenac users, diclofenac was prescribed significantly less often compared with patients who had received the drug before the introduction of the IDT.

In this study, diclofenac consumption in new diclofenac users during the year following the introduction of the IDT in the PHCIS was analyzed in more detail, taking into account gender and age of these patients as well as their specific comorbidities (medical diagnoses/diseases) that were supposed to limit its use. Additionally, the impact of initiating diclofenac therapy on these patients and CV morbidity was assessed as an indicator of its potential adverse CV effects.

Our analysis revealed that diclofenac was more commonly prescribed to women than men, and it was most frequently prescribed in the 45–64 age group, followed by the 65–74 age group, and least frequently in the ≥ 75 age group.

Regarding CVD, which are contraindications for diclofenac use, the drug was the most commonly prescribed in patients with ischemic heart disease (38.15%), other heart disease, mostly cardiomyopathy (21.44%), and cerebrovascular diseases (17.84%). For diseases listed as precautions or warnings, diclofenac was most often prescribed to patients with hypertension (71.00%).

For CVD (contraindications), diclofenac was slightly more frequently prescribed to men than women with diseases of arteries, small arteries and capillaries, ischemic heart disease, and congestive heart failure. In contrast, for other diseases, women received diclofenac more often than men. For conditions listed under precautions/warnings, women had higher prescription rates across all comorbidities (hypertension, hyperlipidemia, and diabetes mellitus). Regarding age groups and CVD (contraindications) for prescribing diclofenac, the drug was most commonly prescribed to patients aged 45–64 with ischemic heart diseases, cerebrovascular diseases, and diseases of the heart of pulmonary origin and diseases of the blood vessels of the lungs, those aged 65–74 who had diseases of arteries, small arteries and capillaries, and to patients aged ≥ 75 who had congestive heart failure and other heart diseases. Concerning all precautions/warnings, the highest prescription rate was in the youngest age group observed (45–64 years).

Other studies have also shown that drugs from the NSAID group are most often prescribed to women and younger people [18–20].

In a non-interventional retrospective cohort study investigating patterns of use for selected NSAIDs, including diclofenac, data were obtained from healthcare databases in the United States of America (MarketScan) and the United Kingdom (Clinical Practice Research Datalink – CPRD). The aim was, among other issues, to describe the demographic characteristics and specific comorbidities of patients who were on NSAID therapy [18, 19].

In both observed populations, the prevalence of use of all selected NSAIDs was higher in women, compared to men, in all age groups. For instance, in the MarketScan population of new diclofenac users, the proportion of women was 55.2% and men 44.8%. In the CPRD population, this ratio was 51.7% for women and 48.3% for men. Regarding the specific type of comorbidity in patients taking diclofenac, in the MarketScan population, the most common comorbidities were circulatory disorders, followed by ischemic heart disease and cerebrovascular diseases.[18]. The results were similar in the CPRD population, as well [19].

Morales et al. [20] analyzed diclofenac prescribing practice in the period after the EU made the prescribing limitations legally binding. Four countries were included: Denmark, the Netherlands, England, and Scotland. Results showed that women were more often prescribed diclofenac than men, and the decrease in prescribing the drug had the strongest impact on the elderly population.

It can be assumed that the greater use of NSAIDs in women is a consequence of the greater prevalence of rheumatic diseases and other painful conditions for which these drugs are indicated in that gender [21–23].

Based on the results of our study, it can be concluded that diclofenac was least prescribed to the oldest patients, which is understandable considering that they represent an extremely vulnerable group. Obviously, even physicians who, despite warnings about the use of diclofenac, prescribed the drug to such patients, keep these facts in mind because they mostly prescribed it to patients in the youngest age group observed. In addition, the results of our study showed that diclofenac was almost seven times more prescribed to patients with diagnoses that require caution in its use than to patients with CVD in whom diclofenac should not be used. This also leads to the conclusion that the introduction of the IDT influenced HCPs to be more careful in prescribing diclofenac to high-risk patient groups.

However, the fact that the drug was prescribed even to patients for whom its use was contraindicated, and that the number of CVD patients increased only one year after its introduction in the treatment of new diclofenac users, is worrying. This suggests that the RMMs undertaken in MNE in the case of diclofenac are still insufficient and should be strengthened. One of the solutions could be the improvement of the existing IDT, as a new tool for RMM dissemination, with data on the use of safer NSAIDs (e.g., naproxen and ibuprofen) in the indications in which diclofenac is most often prescribed. Besides, one cycle of education of HCPs from PHC that preceded the introduction of IDT in PHCIS did not prove efficient in their adherence to the RMM for diclofenac. More intensive work on educating HCPs in PHC about the safe use of diclofenac and other drugs is necessary. Results of a study in Norway showed that an educational program for HCPs in PHC on the rational prescribing of NSAIDs aimed to promote the use of naproxen as the first choice among NSAIDs. The message of the program was that diclofenac should be avoided due to adverse CV effects, which led to a significant decrease in diclofenac prescribing immediately after the education. [24].

The advantage of this study is that it included the entire MNE primary healthcare system, where diclofenac is predominantly used based on its “prescription-only” status. Additionally, this is the first MNE study with the aim of measuring the effectiveness of a digital regulatory intervention introduced at the level of drug prescribing on patient health outcomes.

The limitation of this study is certainly its short duration (one year), although there are data that the effects of undertaken RMMs, as above-mentioned, are most pronounced immediately after their introduction [20, 24]. Besides, this research started immediately after the new IDT was implemented, while changes in healthcare, including changes in HCPs behavior, need time to be understood and implemented in clinical practice. According to the relevant Good Pharmacovigilance Practice guideline, an initial evaluation of RMM should be conducted within 12–24 months after regulatory implementation, to allow the possibility of necessary changes in healthcare. A comprehensive effectiveness evaluation should follow within four years of implementation, which, where applicable, can also inform the assessment for marketing authorization renewal. Therefore, it would be of utmost interest to continue researching this problem. Moreover, diclofenac dispensing data were not used, and consequently, there is a potential underestimation of real diclofenac use, as prescription data were used to measure drug utilization. Additionally, our research was conducted during the coronavirus disease 2019 pandemic, which could also have influenced the results obtained.

Conclusion

After the introduction of the innovative digital tool as a new measure to reduce the risk of adverse cardiovascular effects of diclofenac into the PHCIS of Montenegro, the drug was still prescribed to patients who had not received it before. Some of these patients had existing cardiovascular diseases, making diclofenac contraindicated. Others could use diclofenac, but with increased caution, because they had diseases that increased the risk of developing cardiovascular diseases. In these patients, diclofenac was most often prescribed to women, people aged 45–64, and those with diagnoses that required caution when using it. The increase in the number of patients with cardiovascular diseases during one year from the beginning of diclofenac use indicates that new and/or improved digital interventions are needed for reducing its adverse cardiovascular effects.

REFERENCES

1. *European Medicines Agency* (*EMA*). Guideline on good pharmacovigilance practices (GVP). Module V – Risk management systems (Rev 2) [Internet]. Amsterdam, NL: European Medicines Agency; 2017 [accessed 2023 Dec 20; cited 2025 May 29]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-pharmacovigilance-practices-module-v-risk-management-systems-rev-2_en.pdf
2. *European Medicines Agency* (*EMA*). Guideline on good pharmacovigilance practices (GVP). Module XVI – Risk minimisation measures (Rev 3) [Internet]. Amsterdam, NL: European Medicines Agency; 2024 [accessed 2024 Sep 11; cited 2025 May 29]. Available from: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/guideline-good-pharmacovigilance-practices-gvp-module-xvi-risk-minimisation-measures-rev-3_en.pdf
3. *Institute for Medicines and Medical Devices*. Law on Medicines (Official Gazette of Montenegro 80/2020) [Internet]. Montenegro: Institute for Medicines and Medical Devices; 2020 [accessed 2023 Nov 20; cited 2025 May 29]. Available from: https://cinmed.me/wp-content/uploads/2023/01/Law-on-medicines-Official-Gazette-of-Montenegro-080-20-unofficial-translation.pdf
4. *European Commission*. Directive 2010/84/EU of the European Parliament and of the Council of 15 December 2010 amending as regards pharmacovigilance, Directive 2001/83/EC on the Community code relating to medicinal products for human use (Official Journal of the European Union. 348, 31.12.2010, 74-99) [Internet]. Brussels, BE: European Commission; 2010 [accessed 2023 Nov 10; cited 2025 May 29]. Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:348:0074:0099:EN:PDF
5. *European Commission*. Regulation 1235/2010 of the European Parliament and of the Council of 15 December 2010 amending, as regards pharmacovigilance of medicinal products for human use, Regulation 726/2004 and Regulation 1394/2007 (Official Journal of the European Union. 348, 31.12.2010, p. 1–16) [Internet]. Brussels, BE: European Commission; 2010 [accessed 2023 Nov 10; cited 2025 May 29]. Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:348:0001:0016:EN:PDF
6. *European Commission*. Commission Implementing Regulation 520/2012 of the European Parliament and of the Council of 19 June 2012, on the performance of pharmacovigilance activities provided for in Regulation 726/2004 of the European Parliament and of the Council and Directive 2001/83/EC of the European Parliament and of the Council (Official Journal of the European Union. 159, 20.06.2012, p. 5-25) [Internet]. Brussels, BE: European Commission; 2012 [accessed 2023 Nov 10; cited 2025 May 29]. Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:159:0005:0025:EN:PDF
7. *European Medicines Agency (EMA)*. PRAC Strategy on Measuring the Impact of Pharmacovigilance activities (Revision 2) [Internet]. Amsterdam, NL: European Medicines Agency; 2022 [accessed 2023 June 11; cited 2025 May 29]. Available from: https://www.ema.europa.eu/en/documents/other/prac-strategy-measuring-impact-pharmacovigilance-activities_en.pdf
8. *European Medicines Agency (EMA)*. The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology (Revision 11) [Internet]. Amsterdam, NL: European Medicines Agency; 2010 [accessed 2023 July 15; cited 2025 May 29]. Available from: https://encepp.europa.eu/document/download/f6e403a6-8033-4c22-a5ff-195ba3666299_en?filename=01.ENCePPMethodsGuideRev.11.pdf
9. *European Medicines Agency (EMA)*. Assessment report for Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and cardiovascular risk. Procedure no: EMEA/H/A-5(3)/1319 [Internet]. Amsterdam, NL: European Medicines Agency; 2012 [accessed 2021 Dec 3; cited 2025 May 29]. Available from: https://www.ema.europa.eu/en/documents/referral/assessment-report-article-53-procedure-non-steroidal-anti-inflammatory-drugs-nsaids-cardiovascular_en.pdf
10. *European Medicines Agency (EMA)*. New safety advice for diclofenac – CMDh endorses PRAC recommendation [Internet]. Amsterdam, NL: European Medicines Agency; 2013 [accessed 2021 Dec 25; cited 2025 May 29]. Available from: https://www.ema.europa.eu/en/news/new-safety-advice-diclofenac-cmdh-endorses-prac-recommendation
11. *European Medicines Agency (EMA)*. New safety advice for diclofenac – New measures aim to minimise cardiovascular

risks [Internet]. Amsterdam, NL: European Medicines Agency; 2013 [accessed 2021 Dec 25; cited 2025 May 29]. Available from: https://www.ema.europa.eu/en/documents/referral/diclofenac-article-31-referral-new-safety-advice-diclofenac_en.pdf

12. *Institute for Medicines and Medical Devices*. Summary of product characteristics. Diclofenac, extended-release tablets, 100 mg, Galenika A.D. Serbia [Internet]. Montenegro: Institute for Medicines and Medical Devices; 2023 [accessed 2023 Dec 20; cited 2025 May 29]. Available from: https://cinmed.me/wp-content/uploads/media-registri/1141743_diklofen%20100mg%20smpc_odobreni.docx (Montenegrin)
13. *Institute for Medicines and Medical Devices*. Healthcare professional communication on safety of medicines containing diclofenac [Internet]. Montenegro: Institute for Medicines and Medical Devices; 2015 [accessed 2023 Nov 11; cited 2025 May 29]. Available from: https://cinmed.me/wp-content/uploads/2022/12/DiklofenakDHPC.pdf (Montenegrin)
14. *Stanković M, Turković N, Dobrić S, Rančić N*. Prescription patterns of diclofenac in patients with cardiovascular diseases or at high risk for cardiovascular diseases at primary health care level in Montenegro: retrospective, national, drug utilization study. Vojnosanit Pregl 2023; 80(9): 778–88.
15. *Stanković M, Turković N, Dobrić S, Rančić N*. Evaluation of diclofenac utilization patterns before and after digital risk minimization intervention in outpatient settings in Montenegro. Drugs Ther Perspect 2024; 40: 90–100.
16. *World Health Organization (WHO)*. International Classification of Diseases (ICD) 10th Revision [Internet]. Geneva, CH: WHO; 2019 [accessed on 2024 May 19; cited 2025 May 29]. Available from: https://icd.who.int/browse10/2019/en
17. *WHO Collaborating Centre for Drug Statistics Methodology*. ATC/DDD Index 2016 [Internet]. Oslo, NO: Norwegian Institute of Public Health; 2016 [accessed on 2024 May 19; cited 2025 May 29]. Available from: http://www.whocc.no/atc_ddd_index/
18. *European Medicines Agency (EMA)*. Usage Patterns of Selected Systemic NSAIDs (including Diclofenac): A Retrospective Cohort Study [Internet]. Amsterdam, NL: European Medicines Agency; 2015 [accessed 2024 May 25; cited 2025 May 29]. Available from: https://catalogues.ema.europa.eu/node/1437/administrative-details
19. *European Medicines Agency (EMA)*. Usage Patterns of Selected Systemic NSAIDs (including Diclofenac): A Retrospective Cohort Study [Internet]. Amsterdam, NL: European Medicines Agency; 2015 [accessed 2024 May 25; cited 2025 May 29]. Available from: https://catalogues.ema.europa.eu/sites/default/files/document_files/vol458a2001--legacy-clinical-study-report-redacted.pdf
20. *Morales DR, Morant SV, MacDonald TM, Hallas J, Ernst MT, Pottegard A,* et al. Impact of EU regulatory label changes for diclofenac in people with cardiovascular disease in four countries: Interrupted time series regression analysis. Br J Clin Pharmacol 2021; 87(3): 1129–40.
21. *Dominick KL, Ahern FM, Gold CH, Heller DA*. Gender differences in NSAID use among older adults with osteoarthritis. Ann Pharmacother 2003; 37(11): 1566−71.
22. *Mauvais-Jarvis F, Bairey Merz N, Barnes PJ, Brinton RD, Carrero JJ, DeMeo DL,* et al. Sex and gender: modifiers of health, disease and medicine. Lancet 2020; 396(10250): 565−82. Erratum in: Lancet 2020; 396(10252): 668.
23. *Barnabe C, Bessette L, Flanagan C, Leclercq S, Steiman A, Kalache F,* et al. Sex differences in pain scores and localization in inflammatory arthritis: a systematic review and metaanalysis. J Rheumatol 2012; 39(6): 1221−30.
24. *Langaas HC, Hurley E, Dyrkorn R, Spigset O*. Effectiveness of an academic detailing intervention in primary care on the prescribing of non-steroidal anti-inflammatory drugs. Eur J Clin Pharmacol 2019; 75(4): 577–86.

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SUPPLEMENT – International Classification of Diseases 10th revision (ICD-10) diagnoses of relevance for the study

ICD-10: I20
Diagnosis: Angina pectoris
ICD-10: I21
Diagnosis: Infarctus myocardii acutus
ICD-10: I22
Dijagnosis: Infarctus myocardii recidivus acutus
ICD-10: I23
Diagnosis: Complicatio acuta post infarctum cordis acutum
ICD-10: I24
Diagnosis: Morbi cordis ishaemici acuti alli
ICD-10: I25
Diagnosis: Morbus cordis ischaemicus chronicus
ICD-10: I26
Diagnosis: Embolia pulmonis
ICD-10: I27
Diagnosis: Morbi cordis pulmonales alii
ICD-10: I28
Diagnosis: Morbi vasorum pulmonis alii
ICD-10: I42
Diagnosis: Cardiomyopathia
ICD-10: I43
Diagnosis: Cardiomyopathia in morbis aliis
ICD-10: I50
Diagnosis: Insufficientia cordis
ICD-10: I60
Diagnosis: Haemorrhagia subarachnoidalis
ICD-10: I61
Diagnosis: Haemorrhagia cerebri
ICD-10: I62
Diagnosis: Haemorrhagia intracranialis non traumatica, alia
ICD-10: I63
Diagnosis: Infarctus cerebri
ICD-10: I64
Diagnosis: Apoplexia cerebri et haemorrhagia sive infarctus non specificata
ICD-10: I65
Diagnosis: Occlusio arteriae praecerebralis et stenosis arteriae praecerebralis sine infarctus cerebri
ICD-10: I66
Diagnosis: Occlusio arteriae cerebri et stenosis arteriae cerebri sine infarctu
ICD-10: I67
Diagnosis: Morbi cerebrovasculares alli
ICD-10: I68
Diagnosis: Morbi cerebrovasculares in morbis aliis
ICD-10: I69
Diagnosis: Sequelae morbi cerebrovascularis
ICD-10: I70
Diagnosis: Atherosclerosis
ICD-10: I71
Diagnosis: Aneurysma aortae et dissectio aortae
ICD-10: I72
Diagnosis: Aneurysmata alia
ICD-10: I73
Diagnosis: Morbi vasorum periphericorum alii
ICD-10: I74
Diagnosis: Embolia ateriarum et thrombosis arteriarum
ICD-10: I77
Diagnosis: Morbi arteriales et arteriolares alli
ICD-10: I79
Diagnosis: Morbi arteriales, arteriolares et capillares in morbis aliis
ICD-10: I10
Diagnosis: Hypertensio arterialis essentialis (primaria)
ICD-10: I11
Diagnosis: Morbus cordis hypertensivus

ICD-10: I12
Diagnosis: Morbus renalis hypertensivus
ICD-10: I13
Diagnosis: Morbus cordis et morbus renis hypertensivus
ICD-10: I15
Diagnosis: Hypertensio arterialis, secundaria
ICD-10: E10
Diagnosis: Diabetes mellitus ab insulino dependens
ICD-10: E11
Diagnosis: Diabetes mellitus ad insulino independens
ICD-10: E12
Diagnosis: Diabetes mellitus malnutritionalis
ICD-10: E13
Diagnosis: Diabetes mellitus alius, specificatus
ICD-10: E14
Diagnosis: Diabetes mellitus, non specificatus
ICD-10: E78
Diagnosis: Disordines metabolismi lipoproteiniet lipidaemiae alii

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Comparison of endovascular microwave ablation and traditional vein stripping for lower extremities varicose veins: a retrospective study

Poređenje endovaskularne mikrotalasne ablacije i tradicionalnog uklanjanja vena kod varikoznih vena donjih ekstremiteta: retrospektivna studija

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Abstract

Background/Aim. Varicose veins typically occur in the superficial veins of the lower extremities and are a significant manifestation of chronic venous disease. Patients' symptoms may vary depending on the pathogenesis, location, and severity of chronic venous disease. The aim of this study was to examine the efficacy of endovascular microwave ablation (EMA) and conventional high ligation with saphenous vein stripping in managing lower extremity varicose veins. **Methods.** This retrospective study included 100 patients diagnosed with unilateral varicose veins of the lower extremity. Of these, 50 underwent ultrasound-guided EMA (EMA group), and 50 received traditional vein stripping (traditional group). We assessed and compared operative duration, blood loss during surgery, number of incisions, hospital stay length, and postoperative complications across both groups. Additionally, the Venous Clinical Severity Score (VCSS) and Aberdeen Varicose Vein Questionnaire (AVVQ) were evaluated at 6 and 12 months postoperatively to determine treatment effectiveness. **Results.** All procedures were completed in the 100 cases. The EMA group had considerably reduced operating durations, less blood loss, fewer incisions, and abbreviated hospital stays relative to the traditional group ($p < 0.05$). Differences in subcutaneous bruising, hematoma formation, and skin burns across the groups were statistically significant ($p < 0.05$), while differences in local sensory changes and incision infection were not ($p > 0.05$). The preoperative and postoperative groups showed no substantial difference in VCSS and AVVQ scores ($p > 0.05$). However, both VCSS and AVVQ scores improved at 6 and 12 months post-surgery ($p < 0.05$). **Conclusion.** Clinical evaluation indicates that EMA's effectiveness is comparable to traditional vein stripping in treating lower extremity varicose veins. EMA also presents safety advantages, suggesting its broader adoption in clinical settings.

Key words:
ablation techniques; ligation; lower extremity; saphenous vein; treatment outcome; varicose veins.

Apstrakt

Uvod/Cilj. Varikozne vene se obično javljaju u površinskim venama donjih ekstremiteta i predstavljaju značajnu manifestaciju hronične venske bolesti. Simptomi kod bolesnika mogu se razlikovati u zavisnosti od patogeneze, lokacije i stepena hronične venske bolesti. Cilj rada bio je da se ispita efikasnost endovaskularne mikrotalasne ablacije (EMA) i konvencionalne visoke ligacije sa uklanjanjem vene safene u lečenju varikoznih vena donjih ekstremiteta. **Metode.** Ova retrospektivna studija obuhvatila je 100 bolesnika sa dijagnozom unilateralnih varikoznih vena donjih ekstremiteta. Od toga, 50 je podvrgnuto ultrazvučno-vođenoj EMA (EMA grupa), a 50 je podvrgnuto tradicionalnom uklanjanju vena (tradicionalna grupa). Procenjivano je i upoređivano trajanje operacije, gubitak krvi tokom operacije, broj rezova, dužina boravka u bolnici i postoperativne komplikacije u obe grupe. Pored toga, *Venous Clinical Severity Score* (VCSS) i *Aberdeen Varicose Vein Questionnaire* (AVVQ) procenjivani su 6 i 12 meseci nakon operacije kako bi se utvrdila efikasnost lečenja. **Rezultati.** Sve procedure su uspešno završene kod svih 100 slučajeva. EMA grupa imala je značajno kraće trajanje operacije, manji gubitak krvi, manje rezova i kraći boravak u bolnici u odnosu na tradicionalnu grupu ($p < 0,05$). Razlike u potkožnim modricama, formiranju hematoma i opekotinama kože među grupama bile su statistički značajne ($p < 0,05$), dok razlike u lokalnim senzitivnim promenama i infekciji reza nisu bile statistički značajne ($p > 0,05$). Preoperativne i postoperativne grupe nisu

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pokazale značajnu razliku u skorovima VCSS i AVVQ ($p > 0,05$). Međutim, i VCSS i AVVQ rezultati su se poboljšali 6 i 12 meseci nakon operacije ($p < 0,05$). **Zaključak.** Klinička procena ukazuje da je efikasnost EMA uporediva sa tradicionalnim uklanjanjem vena u lečenju varikoznih vena donjih ekstremiteta. EMA takođe pokazuje prednosti u pogledu bezbednosti, što sugeriše širu primenu ove procedure u kliničkoj praksi.

Ključne reči:
ablacija, tehnike; ligacija; donji ekstremiteti; v. saphena; lečenje, ishod; vene, varikozne.

Introduction

Lower extremity varicose veins (LEVV) are a prevalent chronic venous disorder in vascular surgery, particularly among middle-aged and elderly individuals. This condition is associated with multiple contributing factors, including genetic predisposition, prolonged standing or sitting, pregnancy, and obesity [1, 2]. In its early stages, patients may experience discomfort in the lower extremities, particularly after prolonged periods of standing or sitting. Additionally, visible clusters of twisted veins often appear due to compromised venous return [3, 4].

Without timely intervention, varicose veins can progress, leading to more severe symptoms. Advanced stages may involve complications such as localized itching in the lower leg area, likely due to underlying inflammatory responses [4]. Hyperpigmentation, or darkened skin, is another frequently observed symptom. Moreover, chronic blood stasis and inflammation can cause further complications, including skin induration, ulcers, and eczema [5, 6]. More severe issues, such as phlebitis and venous thrombosis, can lead to intense pain, swelling, and in rare cases, life-threatening pulmonary embolism [6, 7].

Historically, high ligation and stripping of the saphenous vein have been the primary interventions for LEVV. This surgical approach involves the removal of the affected vein while preserving healthy venous circulation [7, 8]. Although effective, this technique is more invasive, leading to extended recovery times and potential postoperative complications. Recently, advances in medical technology have introduced minimally invasive options, such as endovenous microwave ablation (EMA), which have garnered increasing interest [8, 9]. In EMA, a microwave probe is inserted into the vein, generating heat to occlude the damaged vein and restore normal blood flow. Compared to traditional surgery, EMA offers advantages in reduced trauma and expedited recovery [9].

LEVV warrant timely treatment to avoid progression to more severe complications. Contemporary medical advancements offer several treatment options, allowing patients to choose an approach tailored to their individual circumstances [9, 10].

The aim of this study was to compare the clinical outcomes of EMA with conventional high ligation and stripping of the great saphenous vein in the treatment of LEVV.

Methods

Study population

This study comprised 100 patients with LEVV admitted to the Department of Vascular Hernia Surgery, the First People's Hospital of Linping District, Hangzhou, Zhejiang, China, between August 2022 and March 2024. The cohort consisted of 56 men and 44 women, aged 23 to 72 years, with a mean age of 55.4 ± 10.1 years. Patients were randomly assigned to two groups using a computer-generated randomization sequence, with each group comprising 50 individuals: a group that received ultrasound-guided EMA (the EMA group) and a group that underwent traditional high ligation and stripping of the saphenous vein (the traditional group). The study was approved by the Ethics Committee of the First People's Hospital of Linping District (No. LDSTYU, from February 20, 2022).

Inclusion criteria included the following: clinical assessment of LEVV classified as C2–C6; unilateral onset of varicose veins, confirmed by Doppler ultrasound showing a varicose great saphenous vein; patients who provided informed consent before the surgical procedure on their own or *via* their relatives.

Exclusion criteria were as follows: significant cardiac, pulmonary, or renal failure preventing surgery; presence of deep venous valve insufficiency or deep vein thrombosis (deep venous insufficiency was diagnosed using duplex ultrasound examination, showing reflux duration > 1.0 second in deep veins with provocative maneuvers); prior surgical history of LEVV; pregnancy or lactation; long-term bed rest or high risk of thrombosis; coagulation disorders.

Procedural approach to preoperative preparation

Patients were instructed to walk for 20 min before surgery, with varicose veins marked based on their protrusion.

Endovenous microwave ablation under ultrasound guidance

A SonoScape S8 EXP ultrasound machine (Medsinglong Co., Ltd, Guangdong, China) with a 5–10 MHz probe was used. Patients were placed in a supine position and given epidural anesthesia. Epidural anesthesia was chosen to provide adequate regional anesthesia while allowing patient cooperation and minimizing systemic effects, which is particularly beneficial for elderly patients with comorbidities. The great saphenous vein was punctured 5 cm below the medial knee joint, and a PSI-6F-11-035-18G vascular sheath (Merit Medical Systems, Utah, United States) was introduced, followed by insertion of the ECO-100F-2016 microwave ablation catheter (Nanjing Yigao Medical Technology Co., Ltd., Zhejiang, China). Upon reaching the vein's entrance, the catheter was retracted 2.5 cm, and a tumescent solution (500 mL of normal saline, 10 mL of so-

dium bicarbonate injection, 0.5 mL of epinephrine, and 5 mL of lidocaine) was administered along the vein. The EMA was then performed with a microwave power setting between 30 and 60 W and catheter movement maintained at 1–2 millimeters *per* second, adjusted based on local vascular and skin conditions. Superficial varicose veins in the lower leg were treated using the ECO-100F-1213 microwave ablation needle (Nanjing Yigao Medical Technology Co., Ltd., Zhejiang, China) at 35 W according to premarked areas.

Traditional high ligation and stripping

Patients were positioned supine under epidural anesthesia. Incisions (1–2 cm) were made at the medial ankle and groin. After ligation and severance of the great saphenous vein, it was stripped using the 02R2000 catheter (Gamida Tech, Eaubonne, France). The excised location was compressed for 10 min to manage bleeding, followed by therapy along the vein. Superficial varicose veins in the lower leg were excised and locally stripped *per* preoperative markings.

Postoperative care

Patients received low-molecular-weight heparin calcium injections at a dosage of 4,100 anti-Xa (AXa) international units *per* day from the first postoperative day until discharge. This specific dosage was chosen based on current guidelines for venous thromboembolism prophylaxis in moderate-risk surgical patients [11]. Rivaroxaban (10 milligrams *per* day) was prescribed for anticoagulation for one month to prevent deep vein thrombosis and maintain venous patency during the critical healing period. Patients were advised to wear compression stockings (23–32 millimeters of mercury) for six months to reduce venous hypertension, prevent recurrence, and optimize long-term outcomes. The duration of compression therapy was based on the European Society for Vascular Surgery guidelines, which recommend extended compression therapy following varicose vein interventions to minimize recurrence rates and improve functional outcomes [12].

Outcomes and follow-up

Key outcome measures included operation time, number of incisions, blood loss, hospital stay, and postoperative complications (e.g., subcutaneous bruising, hematoma, paresthesia, incision infection, and skin burns). Ultrasound was used to assess the great saphenous vein on the first postoperative day. Venous Clinical Severity Score (VCSS) and Aberdeen Varicose Vein Questionnaire (AVVQ) scores were recorded at 6 and 12 months postoperatively to evaluate clinical effectiveness. All procedures were performed by experienced vascular surgeons with more than 10 years of experience in varicose vein surgery. Prophylactic antibiotics (cefazolin 1 g) were administered 30 min before surgery in both groups.

Statistical analysis

Data were compiled in WPS Office 2019 software (Kingsoft) and analyzed with SPSS 26.0. Data were analyzed across groups using the χ^2 test. Continuous data are expressed as mean ± standard deviation. The Shapiro-Wilk test for normality was followed by independent sample *t*-tests for normally distributed data, and the Mann-Whitney *U* test was used for non-normal data. The Wilcoxon signed-rank test was used for non-normally distributed data in intra-group comparisons, whereas paired *t*-tests were employed for normally distributed data. The threshold for statistical significance was established at $p < 0.05$.

Results

Comparison of general data between the two groups

There was no statistical significance in the general data between the two groups ($p > 0.05$), as shown in Table 1. In this study, the underlying disease history of the patients was compared between groups using a Chi-square test for the overall distribution of disease categories (including hypertension, diabetes mellitus, coronary artery disease, and no underlying disease), which showed no significant difference ($\chi^2 = 0.921$, $p = 0.105$). Additionally, smoking status was analyzed separately and also showed no significant difference between groups ($\chi^2 = 0.136$, $p = 0.885$). Clinical-Etiology-Anatomy-Pathophysiology (CEAP) grade classification was assessed ($p = 0.078$). The injured extremity showed a *p*-value of 0.304.

Observation index and follow-up

All patients underwent successful treatment. Postoperative ultrasonography confirmed that the great saphenous vein was effectively occluded in the EMA group, whereas it was not detectable in the traditional group. Key indicators, including operation time, intraoperative blood loss, number of incisions, and length of hospital stay, were significantly lower in the EMA group compared to the traditional group ($p < 0.05$) (Table 2). The treated veins included the great saphenous vein in all patients, with mean vein diameters of 6.8 ± 1.2 mm in the EMA group and 7.1 ± 1.4 mm in the traditional group ($p > 0.05$). The great saphenous vein diameter was measured at the saphenofemoral junction using duplex ultrasound in a standing position with the patient bearing weight on the contralateral extremity. No concomitant procedures for the small saphenous vein or accessory saphenous veins were performed in this study.

Following the operation, subcutaneous bruising was observed in 9 cases in the EMA group and 20 cases in the traditional group. Local paresthesia was noted in 6 cases in the EMA group and 12 cases in the traditional group. All cases of bruising and paresthesia gradually resolved without further treatment. The incidence of subcutaneous bruising was significantly higher in the traditional group compared to the EMA group ($p < 0.05$).

In the EMA group, five patients experienced skin burns, all located at the proximal thigh region where the vein was more superficial, which healed with the application of topical burn cream. In the traditional group, 7 cases developed subcutaneous hematomas, and 6 cases experienced incision infections, including one severe infection. These complications improved with symptomatic treatment, anti-inflammatory therapy, and local incision drainage. Significant differences were observed in the incidence rates of subcutaneous hematoma and skin burns between the two groups ($p < 0.05$) (Table 3).

Comparison of VCSS and AVVQ scores between the two groups

There were no significant differences in VCSS and AVVQ scores between the two groups before surgery ($p > 0.05$). However, statistically significant differences in both VCSS and AVVQ scores were observed before and after surgery within each group ($p < 0.05$). The VCSS and AVVQ scores at both 6 and 12 months post-surgery were significantly lower than the preoperative scores ($p < 0.05$). Additionally, there were no statistically significant differ-

Table 1

Comparison of general data between the two groups

Parameters	EMA group	Traditional group	t/χ^2 value	*p*-value
Age, years	57.3 ± 11.2	58.8 ± 10.9	0.873	0.214
Gender			0.265	0.781
male	26	28		
female	24	22		
Weight, kg	72.34 ± 12.65	73.21 ± 11.78	0.642	0.487
Course of disease, years	9.00 (4.00–18.00)	7.00 (2.00–12.00)	1.873	0.096
Underlying disease history			0.921	0.105
hypertension	8	9		
diabetes mellitus	5	6		
coronary artery disease	4	3		
Smoking	11	13	0.136	0.885
CEAP classification			3.168	0.078
C2	5	6		
C3	20	18		
C4	15	13		
C5	6	8		
C6	4	5		
Injured extremity			0.782	0.304
left	24	28		
right	26	22		

EMA – endovenous microwave ablation; CEAP – Clinical-Etiology-Anatomy-Pathophysiology.
All values are presented as numbers, mean ± standard deviation, or median (interquartile range).

Table 2

Comparison of intraoperative and postoperative observation indexes between the two groups

Parameters	EMA group	Traditional group	Z/χ^2	*p*-value
Time of operation, min	58.00 (51.00–66.00)	67.00 (55.00–83.00)	3.674	0.012
Peroperative bleeding, mL	23.00 (18.00–37.00)	73.00 (58.00–92.00)	8.432	0.006
Number of intraoperative incisions	1 (1.00–1.00)	6 (4.00–8.00)	9.241	0.004
Length of stay, days	4.00 (3.00–7.00)	8.00 (6.00–10.00)	6.345	0.009

EMA – endovenous microwave ablation.
All values are presented as medians (interquartile ranges).

Table 3

Comparison of postoperative complications between the two groups

Postoperative complications	EMA group	Traditional group	χ^2 value	*p*-value
Subcutaneous bruising	9	20	4.374	0.018
Subcutaneous hematoma	0	7	/	0.015
Paresthesia	6	12	2.481	0.095
Infection of the incisional wound	0	6	/	0.103
Skin burn	5	0	/	0.009

EMA – endovenous microwave ablation.
All values are presented as numbers.

Table 4

Comparison of VCSS and AVVQ scores between the two groups

Parameters	EMA group	Traditional group	*t*/Z	*p*-value
VCSS score				
before operation	7.00 (4.00–9.00)	8.00 (5.00–10.00)	1.263	0.104
6 months after surgery	3.00 (2.00–4.00)*	2.50 (2.00–3.00)*	1.487	0.097
12 months after surgery	2.00 (2.00–3.00)*	2.00 (1.00–3.00)*	0.873	0.146
AVVQ score				
before operation	12.38 ± 3.19	11.87 ± 2.87	0.784	0.263
6 months after surgery	4.67 ± 2.15*	6.42 ± 2.89*	1.625	0.083
12 months after surgery	3.04 ± 1.85*	4.16 ± 2.16*	1.324	0.099

VCSS – Venous Clinical Severity Score; AVVQ – Aberdeen Varicose Vein Questionnaire; EMA – endovenous microwave ablation.
All values are presented as median (interquartile range) or mean ± standard deviation.
***Note*: *$p < 0.05$ – compared with the same group before operation.**

ences between the scores recorded at 6 months and those recorded at 12 months post-surgery ($p > 0.05$) (Table 4).

Discussion

LEVV are a prevalent vascular condition marked by venous dilation, tortuosity, and functional impairment. This condition impacts not only the appearance of the extremities but also contributes to discomfort, fatigue, and progressive skin changes, which can lead to severe complications in advanced cases [13, 14]. For symptomatic varicose veins (CEAP C2–C6), treatment options include conservative management with compression therapy for patients with C2 disease, while endovenous thermal ablation or surgical intervention is recommended for those with more severe disease (C3–C6) or for patients with C2 disease who have failed conservative treatment [14–16]. Traditionally, the surgical approach involves high ligation and stripping of the saphenous vein, a method designed to remove the affected vein segments, thereby alleviating symptoms and minimizing the risk of further complications. However, this traditional method can be associated with significant tissue trauma, extended postoperative recovery, and risks such as pain, bruising, and other complications [16].

In recent years, advancements in medical technology have led to the emergence of minimally invasive techniques, such as EMA, as effective alternatives for treating LEVV. The EMA offers several advantages over conventional surgery, including reduced trauma, faster recovery, and fewer complications, resulting in improved clinical outcomes and a more aesthetically pleasing cosmetic appearance [17, 18]. By employing a microwave probe within the affected vein, EMA generates heat that effectively closes the dysfunctional vein, restoring normal blood flow with minimal impact on surrounding tissues. This method allows for a more targeted approach, reducing the likelihood of postoperative bruising and minimizing recovery time compared to traditional stripping and ligation procedures.

Our study demonstrates that EMA not only provides comparable efficacy to traditional high ligation and stripping but also offers advantages in terms of safety, with lower rates of complications, including subcutaneous bruising, subcutaneous hematoma, and skin burns. The relatively high complication rates observed in both groups may be attributed to the learning curve associated with these procedures, despite the surgeons' extensive experience. Skin burns in the EMA group (10%) occurred primarily in areas where the saphenous vein was more superficial, suggesting the importance of adequate tumescent anesthesia and careful power adjustment in these regions. These findings support EMA as a valuable treatment modality that may enhance patient outcomes and quality of life. Further research with larger patient cohorts and long-term follow-up is warranted to fully establish EMA as a standard treatment option for LEVV, potentially expanding its use in clinical practice.

The extended hospital stays observed in both groups (median 4 days for EMA and 8 days for traditional surgery) reflect our institutional protocol for postoperative monitoring and anticoagulation management. Although international standards typically advocate for same-day discharge after EMA procedures, our conservative approach was adopted due to the following: a) the need for careful monitoring of anticoagulation therapy initiation, b) patient education regarding compression therapy and ambulation, and c) local healthcare system requirements for insurance coverage. We acknowledge that shorter hospital stays are feasible and may be implemented as experience with these procedures increases.

High saphenous vein ligation combined with EMA is a practical approach for treating LEVV, offering efficacy similar to traditional stripping but with reduced tissue damage [19, 20]. Previous studies have highlighted that EMA provides better outcomes for saphenous vein occlusion, with a lower rate of postoperative recurrence compared to foam sclerotherapy [20, 21]. Additionally, research comparing EMA with endovenous laser ablation suggests that EMA is a safer and more effective alternative, associated with fewer complications and reduced recurrence rates in the management of LEVV [22–24].

In our study, EMA demonstrated advantages over traditional methods in terms of shorter operation times, reduced intraoperative blood loss, fewer incisions, and shorter hospital stays. Although no significant differences were observed in preoperative and postoperative VCSS and AVVQ scores between the EMA and traditional groups, both groups showed substantial improvements in these scores at 6 and 12 months post-surgery, consistent with findings reported by other researchers [25–29]. This suggests that both ultrasound-

guided EMA and traditional stripping effectively reduce the severity of LEVV, with EMA offering the added benefits of less tissue trauma and quicker recovery, contributing to an improved quality of life for patients [28, 29].

The primary postoperative complications observed in the EMA group included subcutaneous bruising and skin burns, along with occasional cases of local paresthesia. In the traditional group, complications such as subcutaneous bruising, local paresthesia, subcutaneous hematoma, and incision infections were noted. Our findings revealed statistically significant differences in the rates of subcutaneous bruising, hematoma, and skin burns between the two groups, while the incidence of incision infections did not differ significantly. The study's limited sample size may contribute to the lack of significance in infection rates.

EMA is a safe and effective alternative to traditional saphenous vein stripping for treating LEVV, providing comparable efficacy with fewer adverse effects. However, the retrospective nature of this study, combined with a small sample size from a single center, suggests the need for further validation through multicenter, prospective studies with larger patient cohorts.

Conclusion

Clinical studies demonstrate that EMA provides efficacy comparable to traditional saphenous vein stripping in the treatment of LEVV. However, EMA offers enhanced safety and fewer complications, making it a favorable option. Therefore, promoting EMA in clinical practice is recommended to improve patient outcomes and recovery in the management of varicose veins.

Conflicts of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Lu W, Jiang J, Wu H, Chen G, Zhang Q, Yang G.* Endovenous Microwave Ablation Versus Laser Ablation for Small Saphenous Vein Varicosis. Adv Ther 2024; 41(6): 2342–51.
2. *Tan J, Li J, Bai X, Wang C, Xu W.* One Year Follow-Up of Endovascular Microwave Ablation and Concomitant Foam Sclerotherapy in the Treatment of Primary Small Saphenous Vein Insufficiency. Ann Vasc Surg 2023; 96: 374–81.
3. *Anuforo A, Evbayekha E, Agwuegbo C, Okafor TL, Antia A, Adabale O,* et al. Superficial Venous Disease-An Updated Review. Ann Vasc Surg 2024; 105: 106–24.
4. *Yang X, Li J, Bai X, Zhou L, Xu W.* Endovenous Microwave Ablation Versus Radiofrequency Ablation for the Treatment of Lower Limb Varicose Veins. Ann Vasc Surg 2024; 98: 301–8.
5. *Li Y, Wu W, Li Y, Li J, Sun M.* Efficacy and safety of endovenous microwave ablation versus laser ablation for great saphenous vein varicosis: study protocol for a multicentre, randomised controlled non-inferiority trial. BMJ Open 2022; 12(5): e059213.
6. *Yang L, Wang XP, Su WJ, Zhang Y, Wang Y.* Randomized clinical trial of endovenous microwave ablation combined with high ligation versus conventional surgery for varicose veins. Eur J Vasc Endovasc Surg 2013; 46(4): 473–9.
7. *Mao J, Zhang C, Wang Z, Gan S, Li K.* A retrospective study comparing endovenous laser ablation and microwave ablation for great saphenous varicose veins. Eur Rev Med Pharmacol Sci 2012; 16(7): 873–7.
8. *Fan P, Cong L, Dong J, Han Y, Yang L.* Comparison of 5-year outcomes and quality of life between endovenous laser (980 nm) and microwave ablation combined with high ligation for varicose veins. Front Surg 2022; 9: 1022439.
9. *Dong F, Wu Y, Li W, Li X, Zhou J, Wang B,* et al. Advancements in microwave ablation for tumor treatment and future directions. iScience 2025; 28(4): 112175.
10. *Zhao N, Guo H, Zhang Y, Hu X, He JN, Wang D,* et al. Comparison of endovenous microwave ablation versus radiofrequency ablation for lower limb varicose veins. J Vasc Surg Venous Lymphat Disord 2024; 12(1): 101662.
11. *Kakkos SK, Gohel M, Baekgaard N, Bauersachs R, Bellmunt-Montoya S, Black SA,* Ten et al. Editor's Choice - European Society for Vascular Surgery (ESVS) 2021 Clinical Practice Guidelines on the Management of Venous Thrombosis. Eur J Vasc Endovasc Surg 2021; 61(1): 9–82.
12. *Wittens C, Davies AH, Bækgaard N, Broholm R, Cavezzi A, Chastanet S,* et al. Editor's Choice - Management of Chronic Venous Disease: Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). Eur J Vasc Endovasc Surg 2015; 49(6): 678–737. Erratum in: Eur J Vasc Endovasc 2020; 59(3): 495.
13. *Tang TY, Chan KKW, Yap CJQ, Chan SL, Soon SXY, Khoo V,* et al. Pilot single-arm study to investigate the efficacy and safety of endovenous Microwave ablations for treatment of varicose veins in Singapore - one year results of the *MAESTRO* registry. Phlebology 2022; 37(10): 709–20.
14. *Huang W, Zeng W, Lin XQ, Zhang LF, Wei HJ, He CS.* Comparison of one-year outcomes and quality of life between endovenous microwave ablation and high ligation and stripping of the great saphenous vein. Phlebology 2024; 39(2): 108–13.
15. *Subwongcharoen S, Praditphol N, Chitwiset S.* Endovenous microwave ablation of varicose veins: in vitro, live swine model, and clinical study. Surg Laparosc Endosc Percutan Tech 2009; 19(2): 170–4.
16. *Yang L, Wang X, Wei Z, Zhu C, Liu J, Han Y.* The clinical outcomes of endovenous microwave and laser ablation for varicose veins: A prospective study. Surgery 2020; 168(5): 909–14.
17. *Tan J, Chen Y, Huang J, Xu W.* A systematic review of endovenous ablation for the treatment of small saphenous varicose veins. Vasa 2023; 52(6): 355–65.
18. *Bachetta A, Cheung S, Moore ER, Nguyen D, Kiely MJ, Whiteley MS.* Defining the Parameters for Endovenous Microwave Ablation to Achieve Equivalence With Endovenous Laser Ablation, Using the Porcine Liver Model. Vasc Endovascular Surg 2024; 58(5): 491–7.
19. *Karnabatidis D, Papageorgiou C, Kitrou P, Spiliopoulos S.* One-year duplex ultrasound-assessed closure outcomes of percutaneous endovenous microwave ablation for the treatment of varicose veins of the lower limbs. Vascular 2023; 31(5): 1011–6.
20. *Li X, Feng Y, Liu Y, Zhang F.* Varicose veins of the lower extremity secondary to tricuspid regurgitation. Ann Vasc Surg 2019; 60: 477.e1–77.e6.
21. *Broderick C, Watson L, Armon MP.* Thrombolytic strategies versus standard anticoagulation for acute deep vein thrombosis of the lower limb. Cochrane Database Syst Rev 2021; 1(1): CD002783.
22. *Wang W, Li Q, Wen L.* An Unusual Cause of Left Lower Extremity Varicose Veins. Gastroenterology 2022; 162(4): e9–11.

23. *Gloviczki P, Lawrence PF, Wasan SM, Meissner MH, Almeida J, Brown KR,* et al. The 2022 Society for Vascular Surgery, American Venous Forum, and American Vein and Lymphatic Society clinical practice guidelines for the management of varicose veins of the lower extremities. Part I. Duplex Scanning and Treatment of Superficial Truncal Reflux: Endorsed by the Society for Vascular Medicine and the International Union of Phlebology. J Vasc Surg Venous Lymphat Disord 2023; 11(2): 231–61.e6. Erratum in: J Vasc Surg Venous Lymphat Disord 2024; 12(2): 101719.
24. *Gloviczki P, Lawrence PF, Wasan SM, Meissner MH, Almeida J, Brown KR,* et al. The 2023 Society for Vascular Surgery, American Venous Forum, and American Vein and Lymphatic Society clinical practice guidelines for the management of varicose veins of the lower extremities. Part II: Endorsed by the Society of Interventional Radiology and the Society for Vascular Medicine. J Vasc Surg Venous Lymphat Disord 2024; 12(1): 101670. Erratum in: J Vasc Surg Venous Lymphat Disord 2024; 12(5): 101923.
25. *Farah MH, Nayfeh T, Urtecho M, Hasan B, Amin M, Sen I,* et al. A systematic review supporting the Society for Vascular Surgery, the American Venous Forum, and the American Vein and Lymphatic Society guidelines on the management of varicose veins. J Vasc Surg Venous Lymphat Disord 2022; 10(5): 1155–71.
26. *Barros FS, Storino J, Cardoso da Silva NA, Fernandes FF, Silva MB, Bassetti Soares A.* A comprehensive ultrasound approach to lower limb varicose veins and abdominal-pelvic connections. J Vasc Surg Venous Lymphat Disord 2024; 12(3): 101851.
27. *Wang JC, Gu J, Li Y, Ma Q, Feng J, Lu S*. Transforming growth factor-β1 and inducible nitric oxide synthase signaling were involved in effects of prostaglandin E on progression of lower limb varicose veins. J Vasc Surg Venous Lymphat Disord 2021; 9(6): 1535–44.
28. *El-Sharkawy YH*. Development of a custom optical imaging system for non-invasive monitoring and delineation of lower limb varicose veins using hyperspectral imaging and quantitative phase analysis. Photodiagnosis Photodyn Ther 2023; 44: 103808.
29. *Adler C, Mousa A, Rhee A, Patel MD*. Varicose Veins of the Lower Extremity: Doppler US Evaluation Protocols, Patterns, and Pitfalls. Radiographics 2022; 42(7): 2184–200.

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CASE REPORTS

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Floating right atrial thrombus associated with submassive pulmonary embolism

Flotirajući tromb desne pretkomore udružen sa submasivnom embolijom pluća

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Abstract

Introduction. Massive free-floating thrombi in the right heart are rarely seen in everyday clinical practice and are most often the result of embolization by thrombotic masses from the veins of the lower extremities. Thrombi in the right heart represent a great risk for life-threatening submassive or massive pulmonary embolism, making early diagnosis and adequate treatment crucial. **Case report.** We present the case of a 56-year-old male patient who developed submassive pulmonary embolism as a consequence of a large floating thrombus in the right heart, rapidly diagnosed by echocardiography. On admission, the patient was hemodynamically stable with a blood pressure of 140/100 mmHg. The initial risk assessment was performed using the Pulmonary Embolism Severity Index score, which was 86, that placed the patient in class III, indicating a moderate risk of mortality within 30 days of the event. Given the patient's hemodynamic stability and the massive size of the right heart thrombus, thrombolytic therapy was avoided due to the risk of dispersal of thrombotic masses, additional embolization, and hemodynamic load of the pulmonary circulation. Catheter-based procedures were not feasible as such interventions are not performed at the treating institution. Due to all of the above, it was decided that surgical thrombectomy was the most appropriate and safest solution for the patient. A surgical thrombectomy was successfully performed. The postoperative course was uneventful, and the patient recovered quickly. **Conclusion.** Massive pulmonary embolism concomitant with right heart thrombosis is associated with high mortality. This case highlights the importance of rapid diagnosis, a multidisciplinary approach, timely decision-making, and individualized treatment, which reduce mortality in these patients.

Key words:
diagnosis; echocardiography; heart atria; pulmonary embolism; thrombectomy; thrombosis.

Apstrakt

Uvod. Masivni flotirajući trombovi u desnom srcu retko se viđaju u svakodnevnoj kliničkoj praksi, a najčešće su rezultat embolizacije trombotičnim masama iz vena donjih ekstremiteta. Trombovi u desnom srcu predstavljaju veliki rizik za nastanak submasivne ili masivne embolije pluća, zbog čega je važno rano ih dijagnostikovati i adekvatno lečiti. **Prikaz bolesnika.** Prikazan je 56-godišnji bolesnik kod kojeg je nastala submasivna plućna embolija na terenu velikog flotirajućeg tromba u desnom srcu koji je brzo dijagnostikovan ehokardiografijom. Bolesnik je na prijemu bio hemodinamski stabilan sa vrednostima krvnog pritiska 140/100 mmHg. Inicijalna procena rizika učinjena je pomoću skora *Pulmonary Embolism Severity Index* koji je iznosio 86 što je bolesnika svrstalo u klasu III, odnosno umereni rizik od mortaliteta unutar 30 dana od događaja. S obzirom na to da je bolesnik bio hemodinamski stabilan, a imajući u vidu masivnost tromba u desnim srčanim šupljinama, nije primenjena trombolitička terapija zbog rizika od rasipanja trombnih masa, dodatne embolizacije i hemodinamskog opterećenja plućne cirkulacije. Nije bilo moguće razmatrati katetersku proceduru s obzirom na to da se takva vrsta procedure ne radi u centru gde je bolesnik lečen. Zbog svega prethodno navedenog, odlučeno je da je hirurška trombektomija najprihvatljivije i po bolesnika najbezbednije rešenje. Hirurška trombektomija je uspešno obavljena. Postoperativni tok protekao je bez komplikacija i bolesnik se brzo oporavio. **Zaključak.** Masivna plućna embolija udružena sa trombozom desnog srca povezana je sa visokim mortalitetom. Ovaj slučaj ističe važnost brze dijagnoze, multidisciplinarnog pristupa, pravovremenog donošenja odluke i individualizovanog lečenja, čime se smanjuje smrtnost ovih bolesnika.

Ključne reči:
dijagnoza; ehokardiografija; srce, pretkomora; pluća, embolija; trombektomija; tromboza.

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Introduction

Free-floating thrombus (FFT) masses in the right heart in patients without predisposing conditions, such as structural heart disease or atrial fibrillation, are of rare occurrence. A mobile clot within the right heart poses a high risk for further embolization. In almost all cases, there is an associated pulmonary embolism (PE) [1]. They most often occur as a result of traveling thrombi from the deep veins of the lower extremities. Right heart FFT masses have a high mortality rate and therefore represent a real emergency and require prompt treatment [2]. The lack of randomized control trials makes the management of right heart thrombi in transit controversial. There are several treatment modalities, which include systemic anticoagulation, thrombolysis (systemic or catheter-directed), catheter-based thrombectomy, and surgical thrombectomy [1].

We present a case of a patient with submassive PE concomitant with a large thrombotic mass in the right heart, which was surgically removed. This case emphasizes the importance of a multidisciplinary approach to treating right heart thrombi and highlights the need for individualized treatment strategies in these patients.

Case report

A 56-year-old male patient was admitted to the Cardiology Clinic, Clinical Center of Montenegro, Podgorica, Montenegro, because of chest pain, difficulty breathing, weakness, and dizziness. He had no previous cardiac conditions. He denies any other medical conditions, and he was not taking any regular medications. His physical examination was within normal limits. The patient was hemodynamically stable on admission. His blood pressure was 140/100 mmHg with a regular heart rate of 120 beats *per* minute, and oxygen saturation of 94% on room air. Electrocardiographically mild ST depressions in leads V4-V6, as well as a deep S wave in lead D1, were recorded. Laboratory findings showed markedly elevated values of D-dimer (35.2 μg/mL; normal range < 0.5 μg/mL) and troponin (1,112 ng/L; normal range < 15 ng/L). The platelet count, prothrombin time, and partial thromboplastin time were within the normal range. Additionally, other laboratory results, including complete blood count, urea, and creatinine, were within normal limits.

An emergent computed tomography (CT) scan of the pulmonary arteries was performed, which showed filling defects in the terminal branches of both pulmonary arteries as well as in all lobar and segmental branches bilaterally, corresponding to thrombi, i.e., PE (Figure 1). The initial risk assessment was done using the Pulmonary Embolism Severity Index score, which was 86, that placed the patient in class III, with a moderate risk of mortality within 30 days of the event.

Upon admission, echocardiography was performed, and it revealed a large floating mass in the right atrium, measuring up to 43 mm in diameter. The mass prolapsed into the right ventricle and, based on its characteristics, mostly resembled a large thrombotic mass (Figure 2).

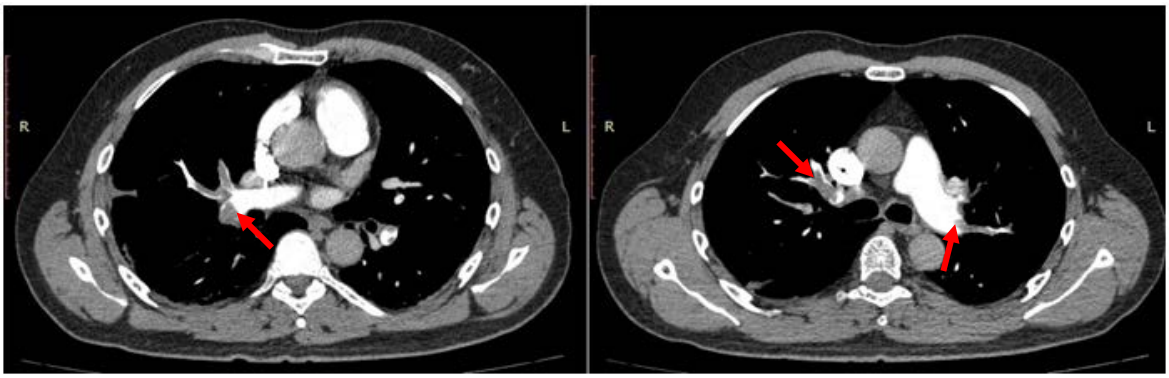

Fig. 1 – Computed tomography scan showing the presence of thrombi in the main pulmonary branches (red arrows).

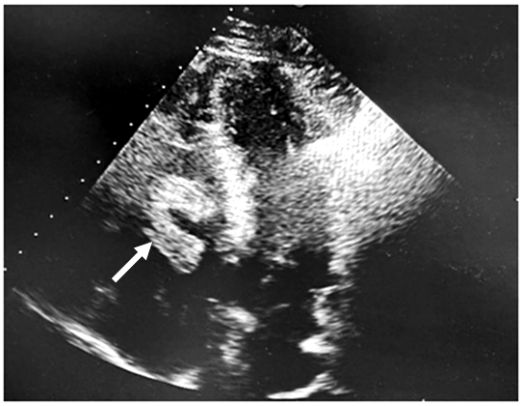

Fig. 2 – Transthoracic echocardiogram showing a large "worm-like" thrombus (white arrow) in the right atrium passing through the tricuspid valve to the right ventricle.

The peak pulmonary artery pressure was 45 mmHg. Due to dilatation of the right ventricle, paradoxical movements of the septum were registered. A Doppler of the lower extremities was performed, which showed signs of chronic venous thrombosis with recanalization in the area of the distal third of the saphenous vein, superficial femoral vein, and popliteal vein. Doppler sonographic findings of the arterial blood vessels of the lower extremities were normal. Although the patient remained hemodynamically stable, evidence of right ventricular dysfunction confirmed by laboratory markers (elevated troponin) and imaging studies was consistent with a diagnosis of submassive PE. Since there was a high risk that the administration of thrombolytic therapy would lead to the lysis of thrombus and the dispersion of thrombotic masses into the pulmonary circulation, which was already burdened with numerous thrombotic masses seen on the previous CT scan of the pulmonary arteries, and since the patient was hemodynamically stable and without comorbidities, a decision was made to perform a surgical intervention. After consultation with a cardiac surgeon, a surgical thrombectomy was performed, and a large thrombus was removed from the right atrium, being 50 mm in its largest diameter (Figure 3).

The surgical procedure as well as the postoperative course were without complications. The removed thrombotic mass was sent for pathohistological analysis, and the results confirmed that it was a fibrinous thrombus (Figure 4).

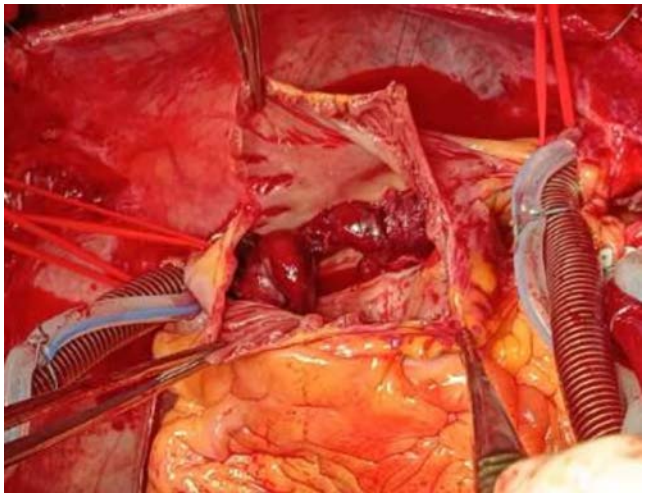

Fig. 3 – Intraoperative finding of a large thrombotic mass in the right atrium.

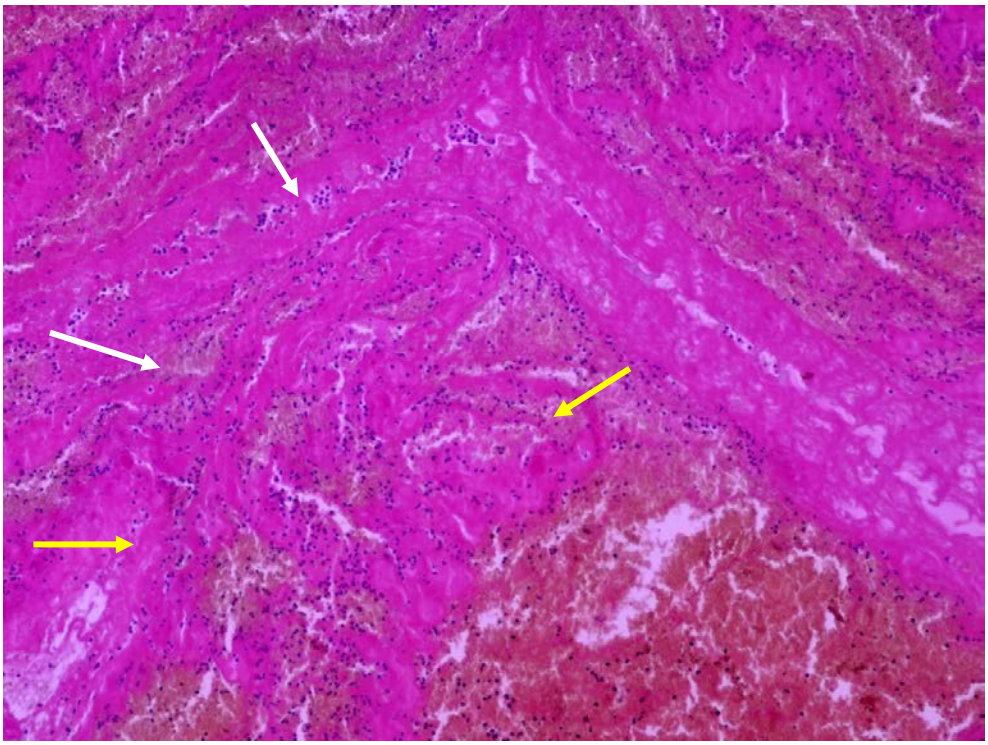

Fig. 4 – Pathohistological finding of a thrombus composed predominantly of fibrin (white arrows), which is permeated with erythrocytes and granulocytes (yellow arrows) (hematoxylin and eosin staining, ×100).

Additional diagnostics did not indicate the potential existence of malignancy. Screening for thrombophilia was negative. At discharge, the patient was hemodynamically stable, without complaints, and the echocardiographic findings were normal. Standard therapy for PE was prescribed, including subcutaneous enoxaparin sodium administered twice daily. Six months after the operation, the patient remains well, and follow-up echocardiographic findings were normal. The successful outcome in this case can be attributed to rapid diagnosis using transthoracic echocardiography and CT, prompt decision-making to proceed with surgical intervention, and efficient postoperative care.

Discussion

The presence of massive FFT masses in the right heart is not often seen in everyday clinical practice. It is most often the result of embolization by thrombotic masses from the veins of the lower extremities. Mortality in these patients is very high (27–50%), especially if the right heart thrombus is associated with PE [1–4]. In a study by Chartier et al. [1], nearly all patients with large right heart thrombi were either in cardiogenic shock or classified as New York Heart Association class IV. However, those who were not in cardiogenic shock had a higher survival rate. Our patient was hemodynamically stable throughout the entire hospitalization. That is why urgent diagnosis and prompt treatment are necessary. Echocardiography is the most important diagnostic tool when it comes to thrombosis of the right heart. The main role of echocardiography is certainly the visualization of thrombotic masses. Thrombi of the right heart can be morphologically presented in three forms, type A, which has a worm-like shape, type B, which is similar to the left heart thrombi, which means that it has a broad base and is mostly adhesive to the wall of the ventricles or atria, and type C, which has the characteristics of both of the previously mentioned types [5, 6]. Type A is very mobile and its presence usually indicates the origin of the thrombus from the deep veins of the legs, while type B mostly occurs *in situ* [5]. They can easily mimic myxoma on echocardiography. When there is ambiguity, transesophageal echocardiography can be helpful [7]. Echocardiography revealed a type A, or "worm-like", thrombus configuration, most likely resulting from blood flow dynamics and the thrombus's migration path to the heart. This indicates that the thrombus did not originate *in situ* in the right heart. In addition to the visualization of thrombus, echocardiographic signs of right ventricular strain, pulmonary hypertension, and paradoxical septal movements are most often seen [1, 6, 8]. In our patient, all the mentioned signs were present. As already mentioned, FFT masses in the right heart are an emergency condition with a high mortality rate, and therefore, prompt treatment is necessary [9]. Despite advances in early diagnosis, the management of PE complicated by free-floating right heart thrombus remains very debatable due to the lack of consensus and evidence-based guidelines. Treatment can be surgical, pharmacological (thrombolytic or anticoagulant therapy), and interventional (percutaneous techniques) [8]. Some studies suggest that the best outcomes are with thrombolytic therapy [2, 10, 11]. In a study by Rose et al. [2] involving 177 patients with right heart thrombus, the mortality rate was lower in patients who received thrombolytic therapy (11.3%) compared to those who underwent surgical thrombectomy (23.8%). Thrombolytic therapy has its advantages as it quickly leads to the lysis of the thrombus, thus improving right heart function in a short time. Additionally, at the same time, it acts on thrombi in the lungs and, if present, in the deep veins of the lower extremities [12]. Ferrari et al. [13] showed that after thrombolysis, 50% of the clots disappeared within 2 hrs. The risk of clot fragmentation and subsequent cardiogenic shock following thrombolysis of a large right heart thrombus remains unclear [14]. Although some case reports describe successful outcomes with anticoagulant therapy alone, most researchers indicate that anticoagulation is insufficient because the thrombus may not break down, and the probability of embolization is higher [15]. Percutaneous catheter-based treatments are considered an alternative to thrombolysis in patients with PE who have contraindications to thrombolytic therapy or remain hemodynamically unstable even after the application of fibrinolysis [8]. However, there are no clear guidelines when it comes to the use of catheter-based procedures in the treatment of FFT in the right heart. Percutaneous mechanical thrombectomy is increasingly being employed as an alternative for managing right heart thrombotic masses. Other techniques, such as fragmentation of blood clots with aspiration and intrapulmonary administration of fibrinolytics, have also been utilized [16]. Torbicki et al. [17] compared mortality in patients treated with anticoagulant therapy, thrombolysis, and surgical procedures, and reported that the mortality rate is highest among patients treated with anticoagulant therapy (60%), followed by thrombolysis (40%), and surgical interventions (27%), which suggested that the surgical approach is the most effective. Surgical thrombectomy is a treatment option that has good outcomes and is mainly used in hemodynamically unstable patients [18–20]. Chartier et al. [1] reported in their study that mortality is not directly correlated with the type of treatment, but they recommend surgical treatment if there are no contraindications. Delay in thrombus removal surgery can result in early mortality [21]. Our patient was hemodynamically stable throughout the entire hospitalization and had no comorbidities, and after consultation with a cardiac surgeon, we decided to proceed with surgical thrombectomy. Surgical thrombectomy has the advantage of allowing complete removal of the thrombus with minimal risk of potential fragmentation, dispersal, and embolization. However, it cannot resolve thrombi distally in the pulmonary circulation, so additional pharmacological treatment is necessary [22]. Several potential complications due to surgical thrombectomy are described in the literature, among them the most significant are postoperative bleeding, cardiac tamponade, and sternal wound infection [23]. Surgical thrombectomy carries the risk of inherent delay of a few hours due to patient preparation, but when the patient is hemodynamically stable, this is acceptable.

Conclusion

The presence of massive free-floating thrombotic masses in the right heart is a rare phenomenon and mostly arises as a consequence of traveling thrombi from the veins of the lower extremities and usually coexists with an already massive pulmonary embolism. Due to the high mortality rate, it represents an emergent, life-threatening condition that must be quickly diagnosed and adequately treated in order to increase the probability of survival.

REFERENCES

1. *Chartier L, Béra J, Delomez M, Asseman P, Beregi JP, Bauchart JJ,* et al. Free-floating thrombi in the right heart: diagnosis, management, and prognostic indexes in 38 consecutive patients. Circulation 1999; 99(21): 2779–83.
2. *Rose PS, Punjabi NM, Pearse DB.* Treatment of right heart thromboemboli. Chest 2002; 121(3): 806–14.
3. *Jenab Y, Ariannejad H, Rabbani Z, Soveyzi F, Hosseinsabet A, Shirani S.* Pulmonary Embolism and Right Heart Thrombi: A Single-Center Experience. J Tehran Heart Cent 2021; 16(1): 26–30.
4. *De Vrey EA, Bax JJ, Poldermans D, van der Wall EE, Holman ER.* Mobile right heart thrombus and massive pulmonary embolism. Eur J Echocardiogr 2007; 8(3): 229–31.
5. *The European Working Group on echocardiography, Kronik G.* The European Cooperative Study on the clinical significance of right heart thrombi. Eur Heart J 1989; 10(12): 1046–59.
6. *Naeem K.* Floating thrombus in the right heart associated with pulmonary embolism: The role of echocardiography. Pak J Med Sci 2015; 31(1): 233–5.
7. *Nixdorff U, Erbel R, Drexler M, Meyer J.* Detection of thromboembolus of the right pulmonary artery by transesophageal two-dimensional echocardiography. Am J Cardiol 1988; 61(6): 488–9.
8. *Konstantinides SV, Meyer G, Becattini C, Bueno H, Geersing GJ, Harjola VP,* et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). Eur Heart J 2020; 41(4): 543–603.
9. *Chapoutot L, Nazeyrollas P, Metz D, Maes D, Maillier B, Jennesseaux C,* et al. Floating right heart thrombi and pulmonary embolism: diagnosis, outcome and therapeutic management. Cardiology 1996; 87(2): 169–74.
10. *Dzudovic B, Obradovic S, Rusovic S, Gligic B, Rafajlovski S, Romanovic R,* et al. Therapeutic approach in patients with a floating thrombus in the right heart. J Emerg Med 2013; 44(2): e199–205.
11. *Charif F, Mansour MJ, Hamdan R, Najjar C, Nassar P, Issa M,* et al. Free-Floating Right Heart Thrombus with Acute Massive Pulmonary Embolism: A Case Report and Review of the Literature. J Cardiovasc Echogr 2018; 28(2): 146–9.
12. *Levine MN.* Thrombolytic therapy in acute pulmonary embolism. Can J Cardiol 1993; 9(2): 158–9.
13. *Ferrari E, Benhamon M, Berthier F, Baudouy M.* Mobile thrombi of right heart in pulmonary embolism: delayed disappearance after thrombolysis treatment. Chest 2005; 127(3): 1051–3.
14. Ruiz-Bailén M, López-Caler C, Castillo-Rivera A, Rucabado-Aguilar L, Ramos Cuadra JA, Lara Toral J, et al. Giant right atrial thrombi treated with thrombolysis. Can J Cardiol 2008; 24(4): 312–4.
15. *Temtanakitpaisan Y, Mahatanan R, Rishikof DC, Young DZ.* Use of heparin alone in treating pulmonary emboli found in association with in-transit right-heart thrombi in a nonagenarian. Tex Heart Inst J 2013; 40(4): 487–8.
16. *Sekhri V, Mehta N, Rawat N, Lehrman SG, Aronow WS.* Management of massive and nonmassive pulmonary embolism. Arch Med Sci 2012; 8(6): 957–69.
17. *Torbicki A, Galié N, Covezzoli A, Rossi E, De Rosa M, Goldhaber SZ; ICOPER Study Group.* Right heart thrombi in pulmonary embolism: results from the International Cooperative Pulmonary Embolism Registry. J Am Coll Cardiol 2003; 41(12): 2245–51.
18. *Lohrmann GM, Peters F, van Riet S, Essop MR.* Double trouble--a case report of mobile right atrial thrombus in the setting of acute pulmonary thromboembolism. Heart Lung Circ 2014; 23(10): e214–6.
19. *Cuccia C, Campana M, Centurini PB, Bolognesi R, Costa F, Zogno M.* A rare case of massive pulmonary embolism and in transit cardiac thrombosis. G Ital Cardiol 1998; 28(9): 1028–31. (Italian)
20. *Fischer JI, Huis in 't Veld MA, Orland M, Harvey P, Panebianco NL, Dean AJ.* Diagnosis of near-fatal pulmonary embolus-in-transit with focused echocardiography. J Emerg Med 2013; 45(2): 232–5.
21. *Verma R, Duncanson ER, Bajpai A, Skeik N, Shafi S.* Right atrial thrombus arising from the junction of the right atrium and the inferior vena cava. Cardiovasc Pathol 2014; 23(5): 317–8.
22. *Hisatomi K, Yamada T, Onohara D.* Surgical embolectomy of a floating right heart thrombus and acute massive pulmonary embolism: report of a case. Ann Thorac Cardiovasc Surg 2013; 19(4): 316–9.
23. *Moriarty JM, Edwards M, Plotnik AN.* Intervention in Massive Pulmonary Embolus: Catheter Thrombectomy/Thromboaspiration versus Systemic Lysis versus Surgical Thrombectomy. Semin Interv Radiol 2018; 35(2): 108–15.

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Application of single-pass albumin dialysis in the acute phase of amanitin syndrome caused by mushroom poisoning

Primena jednoprotočne albuminske dijalize u akutnoj fazi amanitinskog sindroma uzrokovanog trovanjem pečurkama

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Abstract

Introduction. The frequency of mushroom poisoning is increasing both in the world and in our country. The most poisonous type of mushroom in our region is *Amanita phalloides*, which causes amanitin syndrome in poisoned patients, and it is considered responsible for the majority of deaths of patients intoxicated by mushrooms. The liver is one of the primary target organs of amatoxin toxicity. Clinical symptoms and signs of amatoxin poisoning manifest themselves in different ways and can range from simple gastrointestinal disorders to fatal outcomes. Currently, there is no knowledge in the literature about the existence of a specific antidote that should be used in the acute phase of amanitin syndrome. The basis of therapy is symptomatic and supportive therapy. Albumin dialysis is an extracorporeal, non-biological mechanism of liver function support used in liver failure of various etiologies. **Case report.** The initial treatment of a patient treated with the clinical picture of alimentary intoxication with mushrooms and amanitine syndrome included symptomatic and supportive therapy. Due to elevated values of liver enzymes, as well as ammonia and present oliguria, a multidisciplinary analysis determined that single-pass albumin dialysis (SPAD) should be applied as a treatment measure in this patient to support hepatic and renal function. Following a temporary improvement, the general condition worsened upon continuing the treatment. That was manifested by a worsening of the state of consciousness and, consequently, the respiratory function. Further treatment included mechanical ventilation and repeated SPAD procedure. This eventually led to positive outcomes, including improved consciousness, better respiratory function, and normalization of laboratory indicators of liver and kidney function. **Conclusion.** Considering that liver function is compromised in amanitin syndrome, SPAD is a good choice in the treatment of a patient with a severe clinical picture of mushroom poisoning. The presented patient is the first mushroom-intoxicated patient with this severity of the clinical picture, treated at our institution, in whom no fatal outcome was recorded. Based on further tests or analyses and more clinical experience that will be gained with the further use of SPAD, it is necessary to create a clear protocol for the use of this method during the treatment of patients intoxicated by mushrooms.

Key words:
amanitins; dialysis; hepatic encephalopathy; hepatic insufficiency; mushroom poisoning.

Apstrakt

Uvod. Učestalost trovanja pečurkama je u porastu kako u svetu tako i u našoj zemlji. Najotrovnija vrsta pečuraka na našim prostorima je Zelena pupavka (*Amanita phalloides*), koja prouzrokuje amanitinski sindrom kod otrovanih pacijenata i smatra se odgovornom za većinu smrtnih slučajeva kod pacijenata otrovanih pečurkama. Jetra je jedan od primarnih ciljnih organa toksičnog delovanja amatoksina. Klinički simptomi i znaci trovanja amatoksinom se različito manifestuju, od jednostavnih gastrointestinalnih poremećaja do smrtnog ishoda. Trenutno ne postoje literaturna saznanja o postojanju specifičnog antidota koji bi trebalo da se primenjuje u akutnoj fazi amanitinskog sindroma. Osnovu terapije čine simptomatska i suportivna terapija. Albuminska dijaliza je ekstrakorporalni, nebiološki mehanizam potpore

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funkcije jetre koji se koristi kod insuficijencije jetre različitih etiologija. **Prikaz bolesnika.** Inicijalni tretman pacijenta lečenog pod kliničkom slikom alimentarne intoksikacije pečurkama i amanitinskog sindroma uključio je simptomatsku i suportivnu terapiju. Zbog povišenih vrednosti enzima jetre, kao i amonijaka i prisutne oligurije, multidisciplinarnom analizom donesena je odluka da se u lečenju ovog pacijenta primeni jednoprotočna albuminska dijaliza (JPAD) kao terapijska mera potpore funkcijama jetre i bubrega. Posle prolaznog poboljšanja, u nastavku lečenja, dolazi do pogoršanja opšteg stanja. To se manifestovalo pogoršanjem stanja svesti i, posledično, respiratorne funkcije. Dalje lečenje uključivalo je mehaničku ventilaciju i ponovljene JPAD procedure. Ovo je na kraju dovelo do pozitivnih ishoda, uključujući poboljšanje svesti, bolju respiratornu funkciju i normalizaciju laboratorijskih pokazatelja funkcija jetre i bubrega. **Zaključak.** S obzirom da je funkcija jetre kompromitovana u amanitinskom sindromu, JPAD predstavlja dobar izbor u lečenju pacijenata sa teškom kliničkom slikom trovanja pečurkama. Prikazani pacijent je prvi pacijent otrovan pečurkama sa ovim stepenom težine kliničke slike, lečen u našoj ustanovi, kod koga nije zabeležen smrtni ishod. Na osnovu daljih ispitivanja i analiza i većeg kliničkog iskustva koje će se steći daljom upotrebom JPAD-a, potrebno je napraviti jasan protokol za upotrebu ove metode u toku lečenja pacijenata otrovanih pečurkama.

Ključne reči:
amanitin; dijaliza; hepatička encefalopatija; jetra, insuficijencija; trovanje pečurkama.

Introduction

Mushrooms are certainly an indispensable food item in the varied nutrition of modern humans [1]. With a greater prevalence in human nutrition, the risk of mushroom intoxication is inevitably increased [2].

Toxic substances from fungi cannot yet be reliably identified in a laboratory. Therefore, in practice, analyses that prove the presence of toxins in the organism of the poisoned person are generally not used for the diagnosis of mushroom intoxication. Instead, diagnosis is based on certain symptoms and signs, and patients are clinically classified into specific toxic syndromes.

The most poisonous mushroom species in our region is *Amanita* (*A.*) *phalloides*, which causes amanitin syndrome in humans. This species is considered responsible for most fatal outcomes in mushroom-intoxicated patients [3, 4]. According to data from the National Center for Poison Control, Belgrade, Serbia, three to eight fatal outcomes are recorded every year due to poisoning with this species of mushroom [5].

The most toxic substance found in *A. phalloides* is amatoxin. The mortality rate after ingesting *A. phalloides* ranges from 25% to 50% [6]. The lethal dose of amanita toxin is 0.1 mg/kg of body weight, and, therefore, severe poisoning can occur with only 5 to 7 mg of amanita toxin, which is the dose that can be found in a single mushroom [6]. Amatoxin has high thermal stability; it is very soluble in water, resistant to enzymatic degradation, and as such is very toxic (it cannot be destroyed by boiling or drying) [7]. Moreover, amatoxins are not subject to enzymatic degradation or the action of acids, so they are not inactivated after entering the gastrointestinal tract. Amatoxin is easily absorbed from the gastrointestinal system, does not bind to albumin, is quickly eliminated from the blood, within 48 hrs it is distributed to the liver and kidneys [8]. The liver is the primary target organ of amatoxin toxicity, and the hepatocellular effect is the most severe manifestation of *A. phalloides* poisoning. This toxin is not metabolized in the body and is excreted from the body mainly through urine and a small amount through bile [9]. The mortality rate in these patients is correlated with the degree of liver damage, the ability of the remaining healthy part of the liver to regenerate, as well as the degree of expression of complications that may develop during intoxication. In addition to hepatotoxicity, amatoxin also exhibits nephrotoxicity, probably due to the dominant route of toxin elimination through the kidneys [10].

Clinical symptoms and signs of amatoxin poisoning manifest themselves differently and can range from simple gastroenterological disorders to fatal outcomes. Clinical symptoms are primarily caused by toxic damage to the liver and, to some extent, the kidneys [11].

Currently, there are no clear guidelines in the literature for treating the acute phase of amanitine syndrome caused by mushroom ingestion [12]. Symptomatic and supportive therapy is aimed primarily at complaints related to the gastrointestinal tract, but also at alleviating symptoms and signs resulting from hepatic encephalopathy [12]. It is recommended to use activated charcoal in the first few hrs after ingestion, as well as adequate rehydration, primarily due to large gastrointestinal losses. Due to the reduced synthetic function of the liver, these patients are prone to bleeding, so blood and blood derivatives are often represented in the treatment of these patients [13]. Since the urinary tract is the main route for eliminating these toxins, forced diuresis is recommended (100–200 ml/hr). This approach is especially important because amanitin also damages renal function in affected patients. After applying conservative measures, in case of inadequate response, the possibility of hemodialysis should be considered, especially in patients with uremic encephalopathy, hyperkalemia, acidosis, and fluid overload in the form of pulmonary edema.

Based on previous retrospective studies [13, 14], certain trends can be observed in the survival of patients treated with different substances as potential antidotes. Based on the mortality rate, the authors of these studies concluded that the substance silibinin, alone or in combination with N-acetylcysteine, achieved the best effect in terms of reducing the amatoxin impact. However, further research is underway, which should result in a substance that is a specific antidote for amatoxin.

Intravenous administration of albumin is beneficial in multiple ways for patients with cirrhosis and hepatic insufficiency, achieving its effects through several different mechanisms. Namely, albumin is an essential molecule involved in detoxification by binding different substances, and is therefore very important in various liver diseases. This characteristic of albumin is the basis for the use of albumin as a binding and filtration molecule in albumin-based dialysis devices, which recommends these devices for patients with liver failure [15].

Albumin dialysis is an extracorporeal non-biological mechanism of liver support, and is chosen therapy for patients with liver failure of various etiologies [16]. There are several commercial types of albumin dialysis, such as the molecular adsorbent recycling system, single-pass albumin dialysis (SPAD), or the Prometheus system. SPAD is the simplest form of albumin dialysis and is based on the general principles of hemodialysis and hemofiltration. The SPAD procedure is performed on a standard machine for continuous veno-venous hemodiafiltration, where the patient's blood passes through a standard filter impermeable to albumin and is filtered, but with the use of dialysate containing albumin in a concentration of 2–5%. This removes protein-bound molecules that are small enough to pass through the membrane pores, as well as water-soluble toxins that also pass through the membrane [17].

In the clinical picture of patients with liver failure, due to compromised detoxification, synthetic, metabolic, and regulatory functions of the liver are often present with life-threatening disorders such as acute renal insufficiency due to hepatorenal syndrome, hepatic encephalopathy, cerebral edema, hemorrhage, clinically significant hypotension, and infection progressing to multiorgan dysfunction. One of the primary mechanisms of the origin of these disorders is the accumulation of various toxins that the insufficiently functioning liver is unable to remove. The effects of these substances, such as ammonia, bilirubin, bile acids, nitrogen oxides, various free radicals, and cytokines, lead to the listed life-threatening conditions [16, 18].

Regarding the rapid clearance of amatoxin from the blood, it has been questioned whether extracorporeal purification techniques, such as hemodialysis, albumin dialysis, and plasmapheresis, are useful for patients with amanitin syndrome at all. However, it has been practically proven that the use of albumin dialysis for patients with liver failure caused by mushroom poisoning increases the incidence of survival [15]. The reason for this is that, besides amatoxin, albumin dialysis can also filter out other substances that are in excess in the blood, primarily due to compromised liver function, such as ammonia.

Considering the limited amount of data available in the literature regarding extracorporeal support, particularly SPAD albumin dialysis, in conditions caused by mushroom intoxication, the aim of this case report was to highlight the potential beneficial effects of this procedure on patient survival and treatment outcomes. The result of this case report indicates the potential of albumin dialysis in treating the condition, as well as the importance of a multidisciplinary approach.

Case report

A case of mushroom poisoning of eight people was registered in the vicinity of Šabac, Serbia, at the beginning of October 2022. The onset of the disease was characterized by non-specific symptoms of the gastrointestinal tract in the form of nausea, vomiting, and abdominal pain, and all the poisoned persons reported themselves to the local Healthcare Center for treatment. After it was found out that all eight patients had consumed mushrooms bought at the city market and from the same seller, alimentary intoxication with mushrooms was suspected. After the initial assessment and triage, three patients with the most severe clinical picture were referred to the National Poison Control Center, Clinic for Emergency and Clinical Toxicology of the Military Medical Academy in Belgrade. One female patient, out of the three referred patients, was admitted in hemorrhagic shock. She was quickly subjected to cardiopulmonary resuscitation measures, and after two days, a fatal outcome was recorded. The second patient had less expressed symptoms and signs and was discharged after a few days in good general condition. The third patient, 48 years old, was admitted to the Clinic for Emergency and Clinical Toxicology of the Military Medical Academy, Belgrade, with non-specific gastrointestinal symptoms in the form of nausea, vomiting, abdominal pain, and watery diarrhea.

On admission to the Intensive Care Unit (ICU) of the Clinic for Emergency and Clinical Toxicology, the patient was adynamic, sweaty, subicteric, tachycardic, and hypotensive. He had a painfully sensitive epigastrium, which forced him into a specific position in bed. Despite this, he was breathing spontaneously with good respiratory parameters. The first results of blood analysis and biochemical parameters showed high values of liver enzymes, bilirubin, and ammonia (Figures 1–3).

According to the anamnestic data, clinical picture, and laboratory values of liver enzymes as well as nitrogenous substances, it was concluded that the patient had alimentary intoxication complicated by acute hepatic insufficiency and acute renal insufficiency manifested by oliguria (Figure 4).

The initial treatment of the patient included the application of the standard protocol used in alimentary intoxication with mushrooms, primarily through intensive parenteral infusion therapy and hepatoprotective application of N-acetylcysteine according to a 21-hr protocol with extended application, as well as silibinin from the second day in a dose of 500 mg every 4 hrs. On the second and third day of hospitalization, the patient underwent extracorporeal detoxification by plasmapheresis with albumin exchange. Since the values of liver enzymes as well as ammonia remained high with the presence of oliguria and consequently high values of nitrogenous substances in biochemical analyses, it was decided on the fourth and fifth day to apply albumin dialysis type SPAD for the first time for patients poisoned by mushrooms. The SPAD procedure was performed at the ICU of the Clinic for Anesthesiology and Intensive Therapy, Belgrade. After this treatment, both the patient's general condition and laboratory parameters, especially liver and kidney

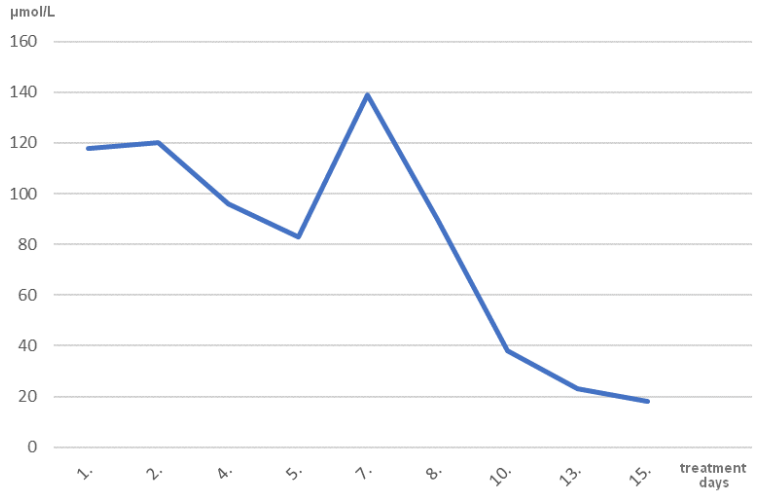

Fig. 1 – Ammonia concentration in blood during treatment.
***Note*: Ammonia reference physiological values are 11-32 µmol/L.**

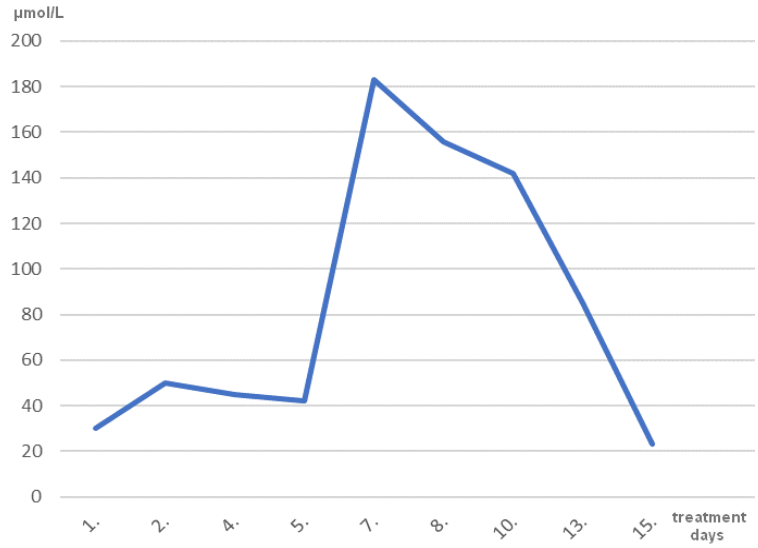

Fig. 2 – Blood bilirubin concentration during treatment.
***Note*: Bilirubin reference physiological values are 0-21 µmol/L.**

Fig. 3 – Concentration of liver enzymes in blood during treatment.
AST – aspartat aminotransferaza; ALT– alanin aminotransferaza; LDH – laktat dehidrogenaza.
***Note*: AST, ALT, and LDH reference physiological values are 0-37 U/L, 10-49 U/L, and 120-246 U/L respectively.**

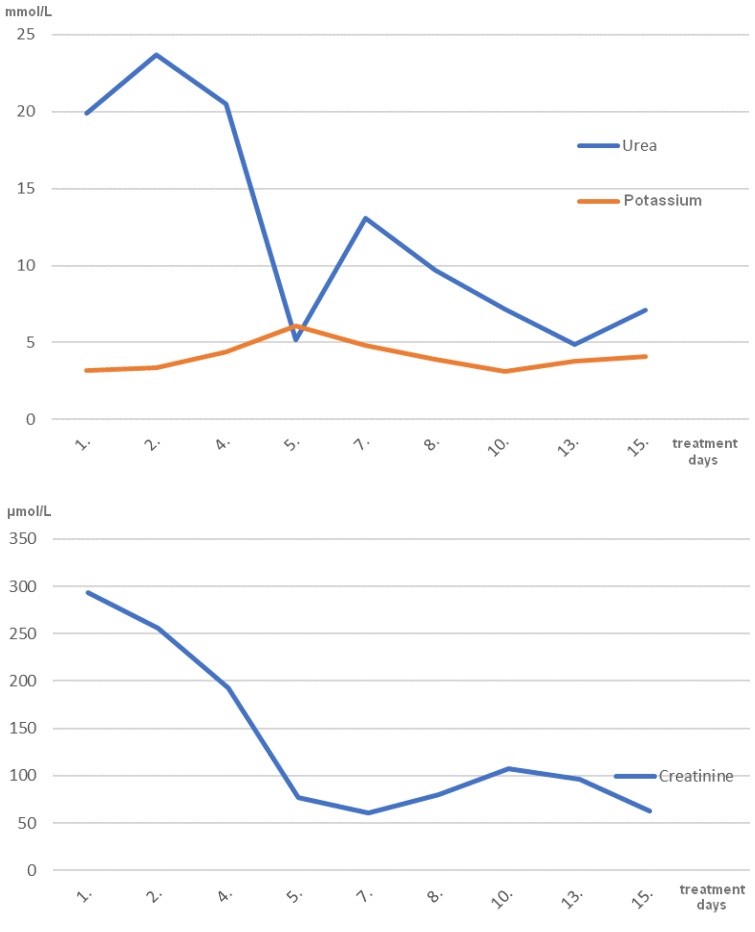

Fig. 4 – Concentration of laboratory parameters of renal functions in blood during treatment: A) urea, kalium, and B) creatinine.
***Note*: Urea, potassium, and creatinine reference physiological values are 2.5-7.5 mmol/L, 3.5-5.1 mmol/L, and 44-88 µmol/L respectively.**

function, were improved. Due to the still present oliguria, renal support in the form of hemodialysis was continued.

Still, the improvement of the condition was only transient. On the seventh day of hospitalization, the patient's general condition worsened again. This was manifested by a deterioration of consciousness, and for the first time, respiratory function disorders appeared, including hyperventilation, decreased partial pressure of oxygen in arterial blood, and reduced blood oxygen saturation. Therefore, on the seventh day of hospitalization, the patient had to be intubated and subjected to mechanical ventilation measures. As expected, in the laboratory biochemical analyses, an increase in the values of hepatic enzymes and ammonia concentration was observed, which is why this deterioration of the state of consciousness can be characterized as a consequence of hepatic encephalopathy. Furthermore, all biochemical parameters, which indicate the quality of the synthetic role of the liver, show a transient drop in values from the seventh day, which remains for the next few days. Due to the deterioration of the condition, it was decided to repeat the SPAD on the eighth, ninth, and tenth day.

Following these procedures, there was an improvement in the general condition, as well as in the values of the monitored biochemical parameters. After eight days, the patient was weaned from mechanical ventilation, with spontaneous breathing, and his respiratory parameters were within physiological limits. Since confusion and disorientation continued occasionally, therapy for hepatic encephalopathy aimed at fixing free ammonia (lactulose, Hepa-Merz®, and Normix®) was continued for the next ten days after consulting a gastroenterologist.

From the second to the fourth day, the patient developed manifest gastrointestinal bleeding, presenting as hematemesis and hematochezia. The condition was treated conservatively with proton pump inhibitors and procoagulant drugs, along with the replacement of blood and blood products. The assumption is that the bleeding has occurred primarily due to a reduced synthetic function of the liver, and due to the risk of invasiveness of the procedure itself and the creation of major bleeding, the endoscopic procedure was not indicated.

In the further course of treatment, the clinical condition stabilized, and the liver and kidney function parameters normalized. Physical therapy was initiated, first with exercises performed in bed, followed by verticalization of the patient.

The entire treatment of this patient, except for the duration of the SPAD procedure, was carried out at the ICU of the Clinic for Emergency and Clinical Toxicology and involved a multidisciplinary approach by doctors of various specialties.

Discussion

Poisoning with amatoxin, the dominant toxin of the mushroom *A. phalloides*, has a high mortality rate, and the primary cause of this is liver lesions, which occur in large numbers of patients poisoned by this toxin [18]. The degree of survival is correlated with the degree of liver damage, the ability of the remaining part of the liver to regenerate, and the degree of expression of complications that may develop during intoxication. As already mentioned, an effective antidote for this toxin has not yet been identified, so the treatment strategy is based on symptomatic and supportive therapy. In the initial phase of treatment, according to existing recommendations, silibinin and N-acetylcysteine were used. However, analyses and studies revealed that these treatments did not yield the expected results, and the mortality rate remained high [19]. Symptomatic and supportive therapy was represented in the treatment, primarily through aggressive hydration and forced diuresis, electrolyte replacement, and, due to gastrointestinal bleeding, the patient received replacement blood and blood derivatives.

Following the existing protocol for the treatment of this type of intoxication, the patient underwent plasmapheresis as a type of extracorporeal blood purification technique [20–22]. Plasmapheresis as a method involves taking venous blood, separating plasma from blood cells (by centrifugation or membrane filtration), and reinfusing blood cells with autologous plasma or an adequate substitute (most often 5% albumin solution or fresh frozen plasma). Although plasmapheresis is often used in various intoxications, clear evidence of the benefit of this procedure in mushroom intoxication is still lacking [22]. Additional difficulties in analyzing the success of the procedure exist because plasmapheresis is rarely used alone in the treatment of intoxication, but is almost always combined with the use of antidotes or some other type of extracorporeal filtration such as hemodialysis. Plasmapheresis reduces the concentration of amatoxin in the blood by removing the toxin bound to the plasma protein. The results obtained in the filtration of toxins by plasmapheresis are best when the toxin does not have a fast metabolism, when it has a high affinity for protein as well as a small volume of distribution. Since amatoxin metabolizes very quickly, the role of plasmapheresis in the treatment of amatoxin intoxication is debatable. Study results are conflicting, with some suggesting an effect on mortality and others finding no impact [23, 24].

For the first time in our institution, and according to the available data from the literature, most likely for the first time in our country, SPAD was applied to this patient in the treatment of conditions caused by amanitin intoxication, i.e., mushroom poisoning.

Based on the laboratory results (elevated ammonia and liver enzyme levels in the blood) and the clinical picture, it was concluded that the patient had developed signs of hepatic insufficiency and encephalopathy. These were manifested by deterioration of consciousness and, consequently, impairment of other vital functions, especially breathing and hemodynamics. Therefore, the decision was made to treat the patient with SPAD as a form of extracorporeal support of liver function. Furthermore, this method supported the function of the kidneys, since the clinical picture was characterized by oliguria. Previous experiences in the application of different types of extracorporeal filtration in liver failure of various etiologies point out that albumin dialysis has given good results in supporting, above all, the detoxification role of the liver [25]. SPAD was applied to the patient on the fourth and fifth day of treatment in our institution, after which there was an improvement in both the clinical picture and the monitored values of biochemical parameters, primarily liver function. The improvement was only temporary. After the patient's condition worsened again—primarily with deterioration of consciousness and respiratory function—biochemical parameters also indicated impaired detoxification and synthetic function of the liver. For this reason, SPAD had to be repeated three more times, on the eighth, ninth, and tenth day of hospitalization. After these treatments, a definite improvement in the patient's condition was observed.

Obviously, it is necessary to have more experience and perform more procedures to reach the correct recommendation on when to include extracorporeal filtration in the treatment of these patients, as well as the extent and type of this procedure that is best for the patient. Besides, this is the first patient who had a severe clinical picture of hepatic encephalopathy with a consequent disturbance of the state of consciousness as well as the need for mechanical ventilation for adequate respiratory exchange, for whom no fatal outcome was recorded in the end. The assumption is that one of the reasons for this is the decision to include the support of hepatic function with extracorporeal circulation in the treatment for the first time. Further experiences will show the credibility of this assumption as well as determine the right place and the right way to include albumin dialysis as an extracorporeal type of liver function support, which is particularly compromised in patients intoxicated by mushrooms.

Conclusion

Based on the available data from the professional literature, it can be noted that the pathophysiology of amanitin syndrome in alimentary intoxication with mushrooms has not yet been sufficiently studied and explained. This fact may also be a partial explanation for the continued absence of an effective antidote in the treatment of amanitin syndrome. Therefore, the treatment of this syndrome is a constant challenge primarily for toxicologists, but also for anesthesiologists-intensivists, nephrologists, and gastroenterologists-hepatologists. In that regard, until a specific and effective antidote for mushroom toxins, especially amanitin, becomes available to modern medicine, albumin dialysis can serve as a useful supportive measure. It is particularly valuable in the advanced stages of intoxication when the clinical picture includes symptoms and signs of hepatic insufficiency, and also for intoxicated patients with renal insufficiency.

R E F E R E N C E S

1. *Ng TB, Cheung RCF, Wong JH, Chan YS, Dan X, Pan W,* et al. Fungal proteinaceous compounds with multiple biological activities. Appl Microbiol Biotechnol 2016; 100(15): 6601–17.
2. *Eren SH, Demirel Y, Ugurlu S, Korkmaz I, Aktas C, Güven FM.* Mushroom poisoning: retrospective analysis of 294 cases. Clinics (Sao Paulo) 2010; 65(5): 491–6.
3. *Alves A, Gouveia Ferreira M, Paulo J, França A, Carvalho A.* Mushroom poisoning with Amanita phalloides—a report of four cases. Eur J Intern 2001; 12(1): 64–6.
4. *Bonnet MS, Basson PW.* The toxicology of Amanita phalloides. Homeopathy 2002; 91(4): 249–54.
5. *Režić S, Vučinić J, Jović-Stošić V, Kilibarda V, Jaćević M, Marković M,* et al. Poison Control Center Yearbook. Beograd: Vojnomedicinska akademija; 2022. pp. 16–57.
6. *Jander S, Bischoff J, Woodcock BG.* Plasmapheresis in the treatment of Amanita phalloides poisoning: II. A review and recommendations. Ther Apher 2000; 4(4): 308–12.
7. *Wieland T, Faulstich H.* Amatoxins, phallotoxins, phallolysin, and antamanide: the biologically active components of poisonous Amanita mushrooms. CRC Crit Rev Biochem 1978; 5(3): 185–260.
8. *Jaeger A, Jehl F, Flesch F, Sauder P, Kopferschmitt J.* Kinetics of amatoxins in human poisoning: therapeutic implications. J Toxicol Clin Toxicol 1993; 31(1): 63–80.
9. *Karlson-Stiber C, Persson H.* Cytotoxic fungi--an overview. Toxicon 2003; 42(4): 339–49.
10. *Splendiani G, Mazzarella V, Zazzaro D, Dipietrantonio P, Vega A, Cipriani S,* et al. Clinical experience in treatment of Amanita phalloides poisoning. G Ital Nefrol 2002; 19(1): 31–6. (Italian)
11. *Wieland T.* The toxic peptides from Amanita mushrooms. Int J Pept Protein Res 1983; 22(3): 257–76.
12. *Le Daré B, Ferron PJ, Gicquel T.* Toxic Effects of Amanitins: Repurposing Toxicities toward New Therapeutics. Toxins (Basel) 2021; 13(6): 417.
13. *Enjalbert F, Rapior S, Nouguier-Soulé J, Guillon S, Amouroux N, Cabot C.* Treatment of amatoxin poisoning: 20-year retrospective analysis. J Toxicol Clin Toxicol 2002; 40(6): 715–57.
14. *Poucheret P, Fons F, Doré JC, Michelot D, Rapior S.* Amatoxin poisoning treatment decision-making: pharmaco-therapeutic clinical strategy assessment using multidimensional multivariate statistic analysis. Toxicon 2010; 55(7): 1338–45.
15. *Sen S, Williams R, Jalan R.* Emerging indications for albumin dialysis. Am J Gastroenterol 2005; 100(2): 468–75.
16. *Tsipotis E, Shuja A, Jaber BL.* Albumin Dialysis for Liver Failure: A Systematic Review. Adv Chronic Kidney Dis 2015; 22(5): 382–90.
17. *Gadour E, Kaballo MA, Shrwani K, Hassan Z, Kotb A, Aljuraysan A,* et al. Safety and efficacy of Single-Pass Albumin Dialysis (SPAD), Prometheus, and Molecular Adsorbent Recycling System (MARS) liver haemodialysis vs. Standard Medical Therapy (SMT): meta-analysis and systematic review. Prz Gastroenterol 2024; 19(2): 101–11.
18. *Garcia J, Costa VM, Carvalho A, Baptista P, de Pinho PG, de Lourdes Bastos M,* et al. Amanita phalloides poisoning: Mechanisms of toxicity and treatment. Food Chem Toxicol 2015; 86: 41–55.
19. *Ganzert M, Felgenhauer N, Schuster T, Eyer F, Gourdin C, Zilker T.* Amanita poisoning-comparison of silibinin with a combination of silibinin and penicillin. Dtsch Med Wochenschr 2008; 133(44): 2261–7. (German)
20. *Jones JS, Dougherty J.* Current status of plasmapheresis in toxicology. Ann Emerg Med 1986; 15(4): 474–82.
21. *Szczepiorkowski ZM, Winters JL, Bandarenko N, Kim HC, Linenberger ML, Marques MB,* et al. Guidelines on the use of therapeutic apheresis in clinical practice–evidence-based approach from the Apheresis Applications Committee of the American Society for Apheresis. J Clin Apher 2010; 25(3): 83–177.
22. *Nenov VD, Marinov P, Sabeva J, Nenov DS.* Current applications of plasmapheresis in clinical toxicology. Nephrol Dial Transplant 2003; 18 Suppl 5: v56–8.
23. *Karvellas CJ, Tillman H, Leung AA, Lee WM, Schilsky ML, Hameed B,* et al. Acute liver injury and acute liver failure from mushroom poisoning in North America. Liver Int 2016; 36(7): 1043–50.
24. *Giordano C, Rivas J, Zervos X.* An Update on Treatment of Drug-Induced Liver Injury. J Clin Transl Hepatol 2014; 2(2): 74–9.
25. *Rifai K.* Extracorporeal albumin dialysis. Hepatol Res 2008; 38 Suppl 1: S41–5.

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Zdravko Nižetić in Heidelberg in 1934: a herald of cadaver cornea transplantation

Zdravko Nižetić u Hajdelbergu 1934: vesnik transplantacije rožnjače umrlog davaoca

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Key words:
cadaver; corneal transplantation; history of medicine; history, 19th century; history, 20th century; ophthalmologic surgical procedures.

Ključne reči:
kadaver; rožnjača, transplantacija; istorija medicine; istorija, XIX vek; istorija, XX vek; hirurgija, oftalmološka, procedure.

Introduction

The lack of appropriate human donor corneas from enucleated pathologic living human eyes was pointed out by Arthur von Hippel as early as 1878: "Unfortunately, until recently, I was unable to find simultaneously both an eye suitable for keratoplasty, and the other, with a clear cornea, intended for enucleation. The conditions are not as favorable elsewhere as in London, where a colossal amount of material is concentrated in the hands of a few surgeons" [1].

The donor material from stillborn infants was also scarce. Among thirty cases of keratoplasty presented by Fuchs in 1894, only four corneas were obtained from this source [2].

First of the milestones along the road leading to the solution to this problem was Magitot's experimentation during 1911 and 1912 which resulted in three achievements: a medium of hemolyzed blood for tissue preservation, a successful transplantation of a living human donor cornea after being kept in such a fluid, and a retained transparency of that transplant during a long follow-up [3]. The next milestone was the result of the steadfast work of Vladimir Filatov of Odessa, who sought a massive source of donor corneas as an answer to the growing need for sight restoration, and found it in cadavers. Obviously inspired by Magitot's results of tissue preservation [3], Elschnig's large series of penetrating keratoplasties [4], and encouraged by simultaneous attempts at cadaver blood transfusion [5–7], Filatov and his associates performed 455 keratoplasties from 1922 to 1938, out of which they made use of the preserved cadaver donor corneas in 264 cases [8]. Such a convincing number influenced legislators to facilitate the process of organ and tissue donation and conceived the foundation of eye banks by Paton, McLean and Brackinridge in the following decade [9, 10].

Filatov's achievement was followed by Zdravko Nižetić, who performed the first keratoplasty in Yugoslavia in 1933 and made a series of 90 transplantations during the following six years, with 88.2% of cadaver donor corneal transplantations out of 51 keratoplasties performed from 1937 to 1939 [11]. This pioneering work secured him a place in the history of ophthalmology [12, 13].

The purpose of this paper was to find out whether the report presented by Nižetić at the meeting of the German Ophthalmological Society in Heidelberg on August 6, 1934 [14], was the first announcement of a successful use of cadaver cornea as a material for transplantation in Western Europe.

Sources

The timeline of events leading to the successful use of corneas from deceased adult human donors as a material for transplantation was documented in the memoirs of Zdravko Nižetić, as well as in editions of the daily newspaper *Vreme*, which covered this topic during 1933 and 1934. The memoirs offer insight into the life and deeds of Zdravko Nižetić in the form of sixty handwritten pages covering the period from June 7, 1919, to April 6, 1941. They were found in the personal archive of Dr. Ljiljana Marjanović (born Nižetić), and published in 2010 [15]. The *Vreme* newspaper, founded on

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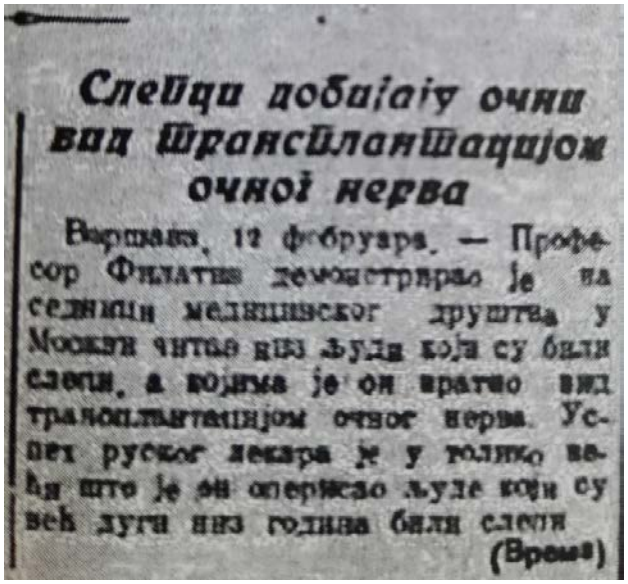
the initiative of King Alexander I as a competitor to *Politika* [16], regularly reported on progress in medicine.

The statements from these sources were considered subjective. They were, therefore, compared to such objective sources as the Report from the Meeting in Heidelberg [14], and scientific papers and books written by Filatov, Nižetić, and other authors [8, 11, 12].

Memories of the first keratoplasty

Nižetić remembers in his memoirs: "…Five years later, I began to publish again, and one of my papers appeared in the leading German ophthalmology journal, *Klinische Monatsblätter für Augenheilkunde*, in 1933. Also, my wishes were fulfilled, and my attempts at corneal transplantation were realized at that time. I often thought of this operation while I worked in Niš, but it was impossible to find both a donor and a recipient simultaneously… One day, the newspaper *Vreme* reported on a successful transplantation of a cadaver eye by a Russian scientist, Filatov." [15].

Indeed, a short note entitled "Blind men regain vision after transplantation of the optic nerve" appeared in *Vreme* on February 13, 1933. The whole text reads: "Warsaw, February 12. At the medical society session in Moscow, Professor Filatov presented a whole range of previously blind people who regained vision after undergoing transplantation of the optic nerve. The success of the Russian physician is all the greater because he operated on people who had been blind for many years [17]" (Figure 1).


Слепци добијају очни вид трансплантацијом очног нерва

Варшава, 12 фебруара. — Професор Филатов демонстрирао је на седници медицинског друштва у Москви читав низ људи који су били слепи, а којима је он вратио вид трансплантацијом очног нерва. Успех руског лекара је у толико већи што је он оперисао људе који су већ дуги низ година били слепи

(Време)

Fig. 1 – News about Vladimir Filatov's success published in *Vreme* on February 13, 1933.

It was hidden on page 7, between other curiosities from the world, such as "A lion eating in a restaurant," "Two swimmers devoured by a shark," and "A fight with axes and pistols" [17].

Obviously puzzled by the news, Nižetić continues: "I started to think about it, knowing that the transplantation of the whole eye was impossible. A tormenting idea immediately came to my mind that these might have been cases of corneal grafting, and I decided to try it. Both Professor Šahović and Professor Milovanović were ready to make the material available to me" [15].

It is hard to know exactly how many patients Filatov presented at the session mentioned in *Vreme*. One can only guess that it was a series of 95 keratoplasties, or a part of it, which were about to appear in his later publications. The number of recipients of preserved cadaver donor corneas must have been much smaller, as he began applying this method in May 1931 [18]. Moreover, his follow-up usually lasted for over a year.

Nižetić, on the other hand, probably counted on cadaver corneas from the beginning, at least in some cases. This statement is supported by his saying that both the head of pathology and the head of forensic medicine were ready to make "the material" available to him, and what else could that material be but cadaver corneas? On an unknown date in 1933, he performed his first keratoplasty and left a poetic description of his endless happiness on the first postoperative day, "when the graft appeared shining like a drop of oil" [15]. At that time, he was the chief of the Eye Department of the State General Hospital in Belgrade, Yugoslavia, by decree of His Majesty King Alexander I (No. 15302) issued on August 15, 1932 [15].

The first in Europe?

Nižetić soon reached tremolo with his pace of transplantations and publications. His modification of Filatov's technique of keratoplasty appeared in a respectable German journal of ophthalmology at the beginning of 1934 [19], although Filatov did not consider it a novelty [20]. Seven months later, he attended the 50th Congress of German Society of Ophthalmology in Heidelberg, where he "was able to present a case of a successful corneal transplantation with the material from cadaver, performed in Europe, in fact on the continent outside the Soviet Union, as Filatov's work had been strictly within the borders of Russia and unknown to Europe" [15].

Reading the names of the speakers at the afternoon session of this congress, held on August 6, 1934, is like reading a textbook of ophthalmology full of eponyms: Thiel, Comberg, Eugen von Hippel, Arruga, Meesmann, Wessely, Junius, Bücklers, Franceschetti, Marchesani, Bielschowsky. Among those few without such an aura, one finds Zdravko Nižetić from Belgrade, Yugoslavia. His oral presentation "Keratoplasty with a corneal material taken from a cadaver eye" was a case report of a cosmetic keratoplasty for unilateral adherent leucoma after scarlet fever in a fifteen-year-old girl, with a clear transplant after a four-month follow-up. The donor was a girl who had died 20 hrs earlier. In addition, Nižetić mentioned two recent keratoplasties with a follow-up of only two weeks, where the donor corneas had been taken 16 hrs post mortem from a three-year-old child who had died from enteritis [14]. He used his own modification of Filatov's technique, with a temporary conjunctival flap over the graft and the lens protection with a knife inserted into the anterior chamber before trephination [19].

The printed version of his presentation at this meeting has 109 lines of text. It took Nižetić 40 lines to report his

cases, while almost the same space, 36 lines, was needed for the description of Filatov's results of 96 cases of the cadaver cornea transplantations, two of them with a long (9–13 months) follow-up. The rest was spent on the introduction and conclusion, which ends with the statement that the results presented by both Filatov and Nižetić indicated that cadaver corneas were suitable for transplantation [14].

Was this presentation novel to Western Europe, as suggested by Nižetić in his memoirs? His statement looks rather clumsy because he clearly avoids the word "first", either because he was aware of the previous occasional attempts at cadaver cornea transplantation, or because he was trying not to sound presumptuous [15]. Indeed, Filatov listed those who published abroad, such as Fuchs and Magitot, alongside Shimanovsky, Saveliev, and Komarovich, whose papers appeared in Russian, as authors who had already tried keratoplasty using material taken from cadavers. All their occasional cases ended in failure, except for one reported by Magitot, who had used a cornea from an embryo [20]. On the other hand, Nižetić successfully used the cornea of a deceased adult donor to obtain a clear graft in a cosmetic keratoplasty, with a rather short, four-month follow-up [19].

Was Filatov's work really unknown to Europe at that moment? At least five of his papers on corneal transplantation were published in German journals from 1924 to 1933, and many of his reports in Russian appeared abroad as reviews from the foreign literature [11, 20]. Therefore, his work on keratoplasty with a graft from a living donor was well known. However, his first paper on cadaver cornea as a material for transplantation published abroad was the one which appeared in a French journal in September 1934 [21], one month after Nižetić had presented his case in Heidelberg [14]. Further, Filatov's most cited reports on his large series of patients treated in this way are those published in the Archives of Ophthalmology in 1935 [22] and in the Lancet in 1937 [18], a year and four years later. Therefore, it seems that Nižetić's statement was true – he really had the advantage of one month over the first subsequent communication on that matter published in the West.

On the other hand, how could he write an extensive description of Filatov's experience with this method in the introduction of his presentation at Heidelberg if it was unknown in Europe? The answer to this question may be found both in his memoirs [15] and the newspaper *Vreme* [23].

On May 5, 1934, journalist Radmilo Milenković published a long interview with Dr. Nižetić in *Vreme*. Its title, "In Belgrade, surgeons will soon treat blind people by transferring eyes from – a dead person" [23], was a promise of a giant step forward (Figure 2).

Субота, 5 мај 1934

Време

СТАРИ РЕВОЛУЦИОНАР НА СЛОБОДИ

Амнестија генерала Санхурха била је разлог оставке г. Леруа

Шпански генерал Хозе Санхурхо, вођа у шпанској револуцији 14 августа 1932, пуштен је на слободу. Као што је познато генерал Санхурхо издржао је своју казну у затвору тврђаве Санта Каталина у Кадизу. Овај закон о амнестији био је разлог да претседник владе г. Леру поднесе оставку на свој положај. На нашој слици види се генерал Санхурхо, кога при изласку из затвора дочекују његове присталице.

ДА БИ СПАСЛА СТОКУ ...

Старица налази смрт у запаљеној колиби

Зајечар, 4 маја. — Даница удова Миливоја Живановића из Рготине, стара 50 година, пошла је јуче у свој виноград на рад. Виноград је удаљен око пет километара од села. У винограду се налазила и једна колиба, која је служила за чување стоке. Ту је Даница затворила две краве, а пред вратима је наложила ватру да би сачувала стоку од голубачке мушице, која се појавила у великом броју у овим крајевима. Пошто је наложила ватру она је отишла да ради на растојању од близу 300 метара од колибе.

Међутим, тек што је почела да ради приметила је како се из колибе диже густ дим. Сва успламирена потрчала је према колиби, где је стала запањена пошто је већ сва била у пламену. Несрећна жена избезумљено је гле-

ЈЕДАН НАУЧНИ НАПОР КОЈИ СЕ ВРШИ У НАШИМ ЛЕКАРСКИМ КРУГОВИМА

У Београду ће ускоро хирурзи лечити слепе људе преносом очију са — мртвог човека

И после 36 часова „смрти" око поново може да функционише кад се прекалеми

После успелог конгреса југословенског хируршког друштва у Београду, на коме су приказани последњи успеси хирургије и демонстрирани начини лакших и тежих случајева, интересантно је, поред многих случајева који указују на гигантске кораке наше хирургије, и медицине уопште, указати на један редак пример.

Пре стотину и више година покушало се први пут са трансплантацијом рожњаче

Први је о трансплантацији рожњаче писао Рајсингер још 1818 године, када су и учињени први покушаји у тада још младој хирургији, неспремној за тако велике подухвате. До седамдесетих година прошлог века та идеја није имала практичан карактер, већ је остајала у теоретисању. Али, кад је Хипел доцније пронашао свој механички трепан, покушало се трансплантацију применити и на човеку, али материјал узет од животиње са намером да се пресади на човечију рожњачу није се показао као нешто што би се доцније могло примењивати, већ је тај покушај забележен у аналима медицине као један Хипелов неуспех. То је декуражирало лекаре све до краја деветнаестог и почетка двадесетог столећа. Године 1905 професор Цирм заинтересовао је цео свет, офталмологе поготову, кад му је успела трансплантација људске рожњаче на леукоматозно око. Резултат свег његовог рада над пацијентом трајао је три године, управо до смрти пацијента. Трансплантат је одржао прозирност леукоматозног ока. То је био успех коме су следовали опити других познатих научника, који су имали сличног успеха, а то су били Плангe, Ленлаји и Шимановски. Тек после светског рата професор Елшниг успео је да даде право значење трансплантацији, у чему је постигао огромне успехе, који су претстављали давање вида и распознавање предмета до потпуног и нормалног вида.

Одговор на то питање, и раздешњење целе операције ока, или пак главног, калемљења, дао нам је г. др. Нижетић, који од дана када је „Време" објавило једну вест о трансплантацији ока, стоји у сталном контакту са чувеним руским професором г. др. Филатовом из Одесе, који је у 98 случајева у првој серији калемљења ока дао здраву базу за даљи рад.

— Обавештени сте врло добро, каже др. Нижетић.

Не само да се ради о преносу накалемљивању целог ока него и о преносу људске рожњаче, за учене било из којих разлога. Преношење ока је успевало али није било тако јасног успеха по вид. Срасло је, али функција није била васпостављена, док се сада више ради на преносу рожњаче и то са великим успехом.

Г. др. Нижетић

Трансплатација би отишла до данас много даље, али појединачни случајеви, који су успевали и чији је успех био кратког века, декуражирали су многе офталмологе-хирурге, који су сматрали да је то неблагодарна ствар, и да се не исплате ни рад ни труд. Зато, да би питање добило завршну форму и тачне резултате, треба рећи отворено да онај који је осуђен на вечиту таму, у жељи да дође до вида ништа не жртвује при покушају операције, а у многоме доприносе и себи и другима. Најмањи вид, најмања сенка светлости коју би угледао за њега би значила добитак. При томе је дужност лекара да покуша у случајевима који могу указати пут ка успеху, не бојећи се за своју репутацију, на успех или неуспех, јер мора се једино увек имати у виду: губитак вида била је и јесте једна врло страшна ствар.

Како се врши пренос ока са мртвог на живог човека

— Да ли се радом и искуствима стекло известно убеђење које обећава успешну интервенцију у том погледу?

— Усавршавањем хируршке технике и стриктном асепсом, као и искуствима оних протагониста наше струке који су је стекли на великом материјалу, постављајући тачне индикације, утврђујући изгледе и могућности операције, знатно су се побољшали изгледи на успех кератопластике. Али, тешкоћа лежи у томе што се кератопластика није могла развијати и усавршити колико је корисно по њеним наменама због недостатка материјала за пресађивање, јер само велики заводи могу пружити могућности за пресађивање, па и онда би било мало тешкоћа, јер свако око не одговара за пренос; потребно је отклонити све што садржи инфекцију. У томе баш и јесте успех др. Филатова из Одесе. Његови експерименти, завршени са успехом а објављени почетком ове године, запањују, јер је он утврдио да се за кератопласа — леша! Јасно је да неће нико уступити своје око, здраво, али можда и непогодно да се пресади ономе коме треба. Велика је разлика између услуга при трансфузијама крви и калемљења ока. Др. Филатова је трансплантат са леша послужио као и са живог човека, па чак кад је трансплантат узет 36 часова после смрти; он се одржао са успехом за време седам месеци, колико је прошло од дана операције до дана кад је професор Филатов публиковао своје опсервације.

„Ова открића и постигнути успеси створили су једну нову еру у области кератопластике на ... можемо надати да ће ова врста операција себи створити нове путеве.

„Операција овакве врсте захтева много труда и рада. Професор Филатов много је радио и успео да чак створи извесне принципе по којима врши операције, и врло вешто оперише Грефовим ножем.

Пред својим успесима у Београду

„Професор Филатов, пре него што приступи трепанацији, објашњава даље г. др. Нижетић, Грефовим ножем отвори предњу комору и шири тај отвор на управном месту у величини од 4—6 м. При том ширењу неизбежно је ... течност из предње коморе исцури. После кроз два отвора, један од ... а други од 6 мм., провлаче се нарочито направљене плочице од белокости, које на крају имају рупице; то се фиксира концем. ... дан од тих конаца држи асистент а други оператор левом руком. Филатов тек сада приступа трепанацији, истренира отвор и ставља трансплантат у рупу, па ... спреда фиксира са кесељицом к... нуктиве. Кад је фиксирао кесељицу он отклони конце и извади плочицу из предње коморе. Тад ... рад завршен. Међутим ми смо ... нашим одељењима модификовали целу операцију на сасвим други начин, али у многоме је узето од Филатова; само су три његова акта операције у нашем случају сведена на један, и ми радимо ... једним ножем нарочито конструисаним. У ствари он је продужен ланцета, али оштра са обе стране. Овај начин има предности у томе што не исцури течност из предње коморе, пре трепанације, а то ... врло важан моменат.

„Створи ли се могућност за рад нашим офталмолозима-хирурзима, није искључено да у најкраће време забележимо један овакав успех у аналима светске науке, успех који ће бити изведен од наших лекара, и у нашим клиникама". Др. Нижетић верује да ће се ... успеха убрзо доћи.

РАДМИЛО МИЛЕНКОВИЋ

Fig. 2 – Interview with Zdravko Nižetić in *Vreme*, May 5, 1934.

One single sentence from this interview gave us a clue for the solution of our puzzle: "A clarification… was given to us by Dr. Nižetić, who has been in continuous contact with the famous Russian Professor Dr. Filatov from Odessa since the day when the news about transplantation of the eye appeared in *Vreme* [23]." This means that Nižetić contacted Filatov soon after February 13, 1933.

Now, when the date has been established, one needs to know about the content of their correspondence, and goes back to the memoirs to find out that "… in those years, I used to frequent the University Hospital in Munich almost yearly, and visit its rich library. I wrote to Filatov from there, and we started a continuous correspondence. He sent me all his papers, and I sent him mine" [15].

Among these papers of Filatov's must have been the progenitor of all his later scientific communications, "Cadaver cornea as a material for keratoplasty," from the *Soviet Vestnik of Ophthalmology*, published at the beginning of 1934 [24]. Nižetić was obviously acquainted with it at the time of his interview, May 5, 1934, when he said: "The success of Dr. Filatov from Odessa lies in the fact that his astonishing experiments, completed and published at the beginning of this year, proved that keratoplasty could be done with a graft taken from – a corpse!" [23].

Therefore, we may conclude that Nižetić, while presenting his own work, was the first European promoter and follower of Filatov's ideas and surgical technique of cadaver cornea transplantation, shown in a large series of successful cases. The promotion of both the pupil and the distant mentor reached its peak in May 1935, when Nižetić received an invitation from the University Clinic in Munich, to perform a keratoplasty, which he did "in front of two professors and all doctors, after obtaining the tissue for transplantation from a deceased adult female donor in Schwabing Hospital morgue" [15] (Schwabing is a borough of Munich, author's remark). The statement of European priority in publication on cadaver cornea transplantation, no matter how much true *sensu stricto,* would seem presumptuous had Nižetić ever missed the opportunity to mention his role model even at the height of his career [11].

The timeline of discoveries, their practical applications, and publications that originated in minds and hands of Filatov and Nižetić have, at least in part, been influenced not only by the course of history of medicine and socio-economic regeneration after the war and revolution, but also by personal virtues and events from biographies of these two outstanding figures, as shown in the following croquis.

Nižetić and Filatov as antipodes

All that Nižetić is famous for has been created by a burst of his activity during a period of six years. It took him just eighteen months to grasp a new idea from a newspaper notice and to turn it into a capital presentation of a new approach to transplantation [11]. On the other hand, Filatov's inventive work continued steadily for decades, some years being more productive and more successful than others, with a painstaking refinement of details, and a long follow-up [20]. Nižetić was a cosmopolitan and a man of the 20th century, somewhat hectic and eager attendant of congresses abroad, while Filatov had reached his adulthood in the 19th century and was more rooted in his homeland and Orthodox religion. Both of them had an excellent medical education, but they lived in different social environments and had different abilities to adapt to their milieu. Both of them were imprisoned, one in Austria for sympathizing the nationalist Slavic movement at the beginning of World War I, the other during Stalin's purges; both witnessed building of their institutes *de novo*, but with different luck in running them: Nižetić was replaced, and Filatov remained at his position for life, even after a relocation of the Institute to the trans-Urals city of Tashkent during World War II [20].

Highly decorated academician Filatov lived to his old days with honors and left a myriad of famous pupils and followers [20]. Nižetić got his professorship in Zagreb in 1947, the last year of his short life [15]. His only follower, Dr. Đorđe Lukić, left Belgrade for South America soon afterward (personal communication with professors Z. Kecmanović and S. Dergenc). Patients who needed keratoplasty were sent from Yugoslavia to Odessa, where masterful surgical skills and human comfort were offered by Dr. Filatov [25]. The art of corneal transplantation returned to Belgrade more than a decade later, after a new generation of ophthalmologists had arrived from their observerships in France under Paufique in Lyon, and under Sourdille in Nantes. Together, they performed 15 keratoplasties *per* year, equaling the number set by Nižetić almost thirty years before [26].

Conclusion

The case of cadaver cornea transplantation performed by Zdravko Nižetić and presented by him at the Congress of the German Ophthalmological Society in Heidelberg in 1934 has been mentioned in our literature on the history of ophthalmology in Serbia only as a title. Our paper uses the report from this meeting to describe his presentation in detail and to determine the time sequence of its appearance by comparison to the dates of publication of Filatov's papers. It can be safely concluded that Nižetić was the first in Western Europe to promote the successful transplantation of the cornea taken from an adult deceased person with the use of Filatov's method.

R E F E R E N C E S

1. *Hippel von A.* On Transplantation of the Cornea. Gräfes Arch Ophthalmol 1878; 24(2): 235–56. (German)
2. *Fuchs E.* On Keratoplasty. Wien Klin Wochenschr 1894; 7(45): 843–5. (German)
3. *Magitot A.* Transplantation of the human cornea previously preserved in an antiseptic fluid. JAMA 1912; 59(1): 18–21.
4. *Elschnig A, Vorisek EA.* Keratoplasty. Arch Ophthalmol 1930; 4(2): 165–73.
5. *Shamov WN.* The transfusion of stored cadaver blood. Lancet 1937; 230(5945): 306–9.
6. *Judine S.* Transfusion of cadaver blood to a human. Paris: Masson et Cie; 1933. (French)
7. *Yudin SS.* Transfusion of cadaver blood. JAMA 1936; 106 (12): 997–9.
8. *Sitchevska O.* Corneal transplantation with fresh, preserved and fixed material: experimental and clinical observation. Arch Ophthalmol 1944; 3(1): 118–9.
9. *McLean JM.* Corneal transplantation; technique. Am J Ophthalmol 1948; 31(11): 1370–4.
10. *Paton RT.* Eye-bank program. Am J Ophthalmol 1956; 41(3): 419–24.
11. *Nižetić Z.* On the development and the present state of corneal transplantation. Bücherei des Augenarztes. Stuttgart: Ferdinand Enke Verlag; 1940. (German)
12. *Castroviejo R.* Atlas of keratectomy and keratoplasty. Philadelphia-London: WB Saunders Company; 1966. p. 40.
13. *Wayenborgh JP.* IBBO: International Biography and Bibliography of Ophthalmologists and Vision Scientists A-Z. Julius Hirschberg, The Monographs, Vol 7, Part 1-2. Ostend: Wayenborgh Publishing; 2002. p. 490.
14. *Nižetić Z.* Keratoplasty with corneal material obtained from a cadaver eye. In: *Wagenmann A*, editor. Report on the Fiftieth Meeting of the German Ophthalmological Society in Heidelberg 1934. Berlin: Springer; 1934. pp. 341–4. (German)
15. *Kecmanović Z, Paunović D.* Professor dr Zdravko Nižetić 1895-1947. Beograd, Zrenjanin: Udruženje oftalmologa Srbije, Art-Projekt; 2010. p. 182. (Serbian)
16. *Dragović V.* Serbian press between two wars. Book 1. Basis for bibliography of Serbian periodicals: 1915-1945. Belgrade: SANU; 1956. pp. 54. (Serbian)
17. *Vreme.* Blind men regain vision after transplantation of the optic nerve. Vreme 1933; February 13, year XIII, number 3992, page 7, article 27. (Serbian)
18. *Filatov VP.* Transplantation of the cornea from preserved cadavers' eyes. Lancet 1937; 229(5937): 1395–7.
19. *Nižetić Z.* A modification of technique of the total penetrating keratoplasty after Filatov. Klin Mbl Augenheilk 1934; 93(1): 89–91. (German)
20. *Filatov VP.* Optical transplantation of the cornea and tissue therapy. Moscow: Narkomzdrav, State Publishers of Medical Literature "Medgiz"; 1945. (Russian) Reviewed by *Sitchevska O.* Arch Ophthalmol 1947; 37(5): 698–700.
21. *Filatov VP.* Cadaver cornea as a material for transplantation. Ann d'Oculist 1934; 171(Sept): 721–34. (French)
22. *Filatov VP, Sitchevska O.* Transplantation of the cornea. Arch Ophthalmol 1935; 13(3): 321–47.
23. *Milenković R.* In Belgrade, surgeons will soon treat blind people by transferring eyes from - a dead person. Vreme 1934 May 5; year XIV, number 4425, page 5, article 7. (Serbian)
24. *Filatov VP.* Cadaver cornea as a material for keratoplasty. Sov vestn oftalmol 1934; 4(2): 222–4. (Russian)
25. *Pinto G.* New Life of Andrija Mijušković. Borba 1946 July 23; year XI, number 175, page 4, article 65. (Serbian)
26. *Stanković M.* Vision saved by corneal grafting. Borba 1962 March 3; year XXVII, number 70, page 8, article 37. (Serbian)

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DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

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