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TUMOR NECROSIS FACTOR-α AND MONOCYTE CHEMOATTRACTANT PROTEIN-1 AS BIOMARKERS IN CHRONIC MYELOID LEUKEMIA

Sanja Veličković[1,2], Jelena Milenković[3], Dijana Stojanović[3], Filip Veličković[4,5], Lazar Kostić[2]

Chronic myeloproliferative neoplasms (MPN) are specific clonal hematopoietic stem-cell disorders that continuously and without interruption activate the physiologic signal-transduction pathways necessary for normal and adequate hematopoiesis. A marked proinflammatory milieu in chronic myeloid leukemia (CML) is led by many interleukins. This study aimed to evaluate differences in plasma levels of tumor necrosis factor- α (TNF-α) and monocyte chemoattractant protein (MCP-1) between chronic-phase CML patients and healthy individuals as controls.

The study included 50 consecutive patients diagnosed with CML in the chronic phase and under the standard tyrosine kinase inhibitor (TKI) treatment, as well as 20 healthy controls. Blood concentrations of tumor necrosis factor-α (TNF-α) and monocyte Chemoattractant protein-1 (MCP-1) were measured by the enzyme-linked immunosorbent assay method. The levels of MCP-1 were higher in patients than in controls (334.37 vs. 172.18 pg/ml, p = 0.006), while no difference was determined for TNF-α.

There is great importance of MCP-1 and IL-6 as novel, strong, and predictive plasma biomarkers for treatment-free remission in CML. Additional and future research in this field will be of special and great importance in understanding the pathophysiology and treatment of myeloproliferative diseases.

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Key words: *myeloproliferative neoplasms, inflammation, tumor necrosis factor-α, monocyte chemoattractant protein-1, tyrosine kinase inhibitors*

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Introduction

Chronic myeloproliferative neoplasms (MPN) are specific clonal hematopoietic stem-cell disorders that continuously and without interruption activate the physiological signal-transduction pathways necessary for normal and adequate hematopoiesis. Chronic myeloid leukemia (CML), defined by the Philadelphia (Ph) chromosome, along with three Ph-negative neoplasms—polycythemia vera, essential thrombocythemia, and primary myelofibrosis—belongs to the group of four classic MPN (1). A seemingly benign disease, can turn into a more aggressive form, like acute myeloid leukemia, at any time. Chronic myeloid leukemia is a myeloid neoplasm with the reciprocal chromosome translocation between chromosomes 9 and 22, and resulting BCR-ABL1 fusion gene. It is a relatively rare type of myeloproliferative neoplasm, with an incidence of 0.7–1.0/100,000. CML is a hematologic disorder with three phases. Most patients are in the chronic phase of disease, presenting with a high white cell count, splenomegaly, abdominal pain, fatigue and symptoms of hyperviscosity. A smaller percentage of patients, 4–5% present in an accelerated phase CML. This transitional stage of disease is associated with additional genetic mutations and instability, along with an increase in blasts and immature cells in peripheral blood, resulting in treatment resistance. And finally, the smallest number of patients, approximately 1–2%, is in the blast phase of CML. This phase has a poor prognosis and involves transformation into acute leukemia, which may be myeloid, lymphoid or mixed phenotype (2).

Inflammation has played a very important part in all levels of tumorogenesis, from the creation of conditions that influence genetic mutations and making an inflammatory

microenvironment, which supports the development and proliferation of mutated cells. Activation of signaling pathways occurs due to the formation of oncoprotein BCR-ABL, including the RAS-RAF-MEK-ERK pathway, the phosphoinositide 3-kinase (PI3K)-AKT pathway, and the STAT5 pathway. Current research shows a connection between inflammation and the development of malignant processes, including myeloproliferative neoplasms. Cytokines, growth factors, and other modulatory mediators are pivotal for cooperation between the leukemia clone and the tumor microenvironment in the bone marrow (3, 4). Accordingly, several interleukins (IL) are determined in this communication, and make a proinflammatory milieu in CML, led by IL-1β, IL-6, IL-2R, tumor necrosis factor- α (TNF-α), chemokines IL-8, monocyte chemoattractant protein (MCP-1), interferon-induced protein 10, and growth factors (transforming growth factor (TGF)-β, platelet-derived GF, vascular endothelial GF), etc. (5, 6).

Tumor growth occurs due to numerous mutations in the genetic material, driven by the inflammatory process. Chronic inflammation, which constitutively affects the development of mutations in the genetic material of cells, is responsible for the process of tumorogenesis. Chronic inflammation can be caused by various infections, different types of irritants or autoimmune diseases, and all of them severely contribute to tumor formation by making an inflammatory microenvironment conducive to genetic mutations and inadequate cell proliferation and development. Inflammation, as an early stage of tumorogenesis, is strongly linked to neoangiogenesis, fast tumor growth, dissemination of tumor, environmental and general immunosuppression, and finally, unstable genetic material of cells (7, 8).

This study aimed to evaluate differences in plasma levels of TNF-α and MCP-1 between chronic-phase CML patients and healthy individuals as controls.

Materials and Methods

This prospective cross-sectional case-control investigation encompassed 50 consecutive patients diagnosed with CML in the chronic phase of the disease, and recruited at the Clinic of Hematology, Allergology and Clinical Immunology, University Clinical Center Niš, Serbia, in January and February 2023. Clinical, molecular, and biochemical data were prospectively and retrospectively procured from the patients' medical history and through clinical examinations. All included patients were diagnosed and classified according to the current WHO classification and were followed by hematological and cytological assessments per guidelines and measurement of BCR-ABL1 transcript levels using real-time quantitative polymerase chain reaction standardized to the international reporting scale (2, 9).

The patients selected for the study received at least 3 months of tyrosine kinase inhibitor (TKI) treatment. The control group consisted of 20 healthy, age-matched, and community-based adult volunteers who agreed to participate in the investigation. The collected EDTA blood samples were analyzed and examined at the Faculty of Medicine, University of Niš, Serbia. Plasma samples, from patients and controls, were safely stored at -80 °C until a final assessment and measurement of biomarkers by quantitative sandwich technique, enzyme-linked immunosorbent assay (ELISA) kits. The levels of TNF-α and MCP-1 plasma concentrations were estimated using adequate ELISA kits, the Quantikine® R&D Systems (Inc., Minneapolis, MN, USA) following the producer's protocol for each biomarker analyzed. Complete blood count (CBC) parameters—erythrocyte, leukocyte, platelet counts, and hemoglobin concentrations—were evaluated using the COULTER® AcT Diff Analyzer (Beckman Coulter Corporation, Hialeah, FL, USA) before their participation in the study.

Ethical Statement

The study was conducted according to the World Medical Association Code of Ethics (Declaration of Helsinki) for experiments on human subjects. The study was approved by the Faculty's Ethical Board of the Faculty of Medicine, University of Niš, Serbia (Decision No. 12-1693-1/2-4 of February 24, 2025), and all the participants gave and signed informed consent.

Statistical Analysis

Statistical analysis was performed using SPSS 25.0 software (SPSS Inc., Chicago, IL, USA). Data are reviewed as percentages and mean ± standard deviation (SD). The independent t-test was used, based on the normal distribution of the samples (Shapiro–Wilk test), all tests were two-tailed. The Pearson correlation coefficient test was also used. Significance was assumed at a value of $p < 0.05$.

Results

The average age of study subjects was 58.46 ± 14.20 years for the patient group and 56.02 ± 10.13 for the group of healthy subjects ($p > 0.05$). There were 46% of males and 54% of females in the patient group. All patients were under treatment with TKI, and in complete hematological and cytogenetic remission, with the major molecular response (BCR-ABL1 values of ≤ 0.1% IS) present in 78% (n = 39) at the last medical examination.

About half of them received TKI imatinib (52%) or nilotinib (48%). Mean plasma levels of MCP-1 were 334.37 ± 165.15 pg/ml in patients

vs. 172.18 ± 56.37 pg/ml in controls, p = 0.006 (95%CI [49.097–275.284]) (Figure 1a).

Conversely, no significant difference was determined in plasma concentrations of TNF-α between the patient group, 371.19 ± 210.33 pg/ml, and the control group, 214.32 ± 214.32 pg/ml, for p = 0.315 (Figure 1b).

There was no correlation between the measured cytokines.

The average values of all parameters of the CBC and leukocyte differential formula were in the normal range.

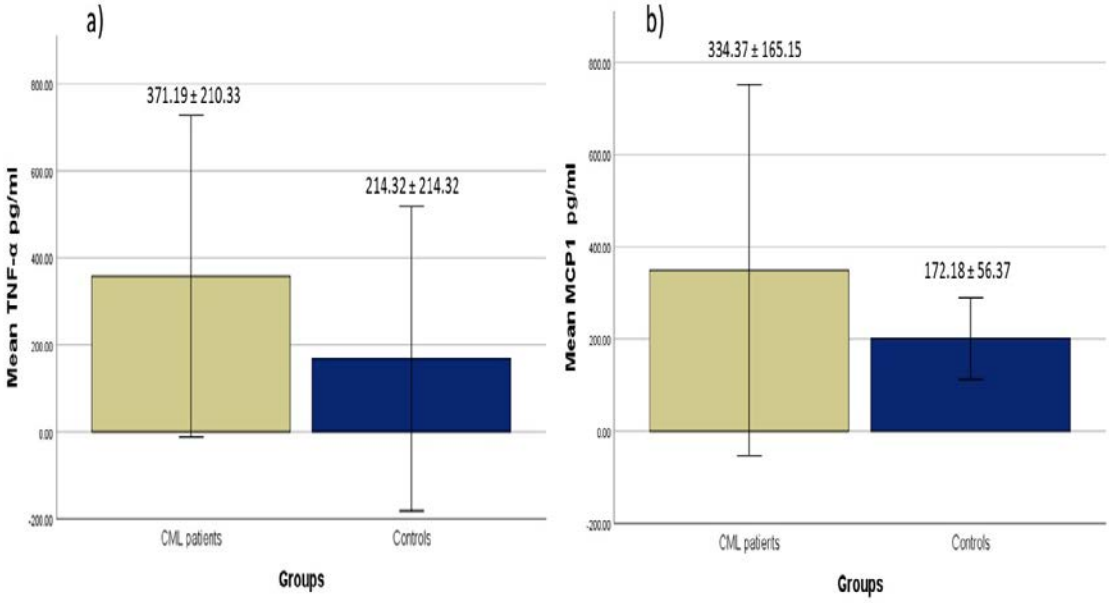

Figure 1. Plasma concentration of a) TNF-α, b) MCP-1 in patients with CML and control groups

Discussion

Myeloproliferative neoplasms are heavily supported by a chronic inflammation that transforms the bone marrow niche into a malignant clone-supporting environment. Patients suffering from myeloproliferative diseases often show elevated levels of proinflammatory cytokines (10). Chronic inflammation and autoimmune disorders affect tumor cells by contributing different levels of inflammatory cytokines such as TNF-α, IL-6, IL-8 and MCP-1 that improve cell proliferation, inhibit apoptosis, change the pathophysiology of cells, stimulate neoangiogenesis and stimulate cell migration, which are actually the processes of tumorogenesis. On the other hand, cancer cells themselves can be a source of inflammatory mediators that modify the microenvironment that may lead to cancer progression (11).

Tyrosine kinase inhibitors are a type of targeted therapy in CML, which attack specific types of tumor cells while causing less damage to normal cells. In CML, TKIs target the abnormal BCR-ABL1 protein that causes uncontrolled CML cell growth and block its function, causing the destruction of CML cells. It is possible that TKI-induced suppression of the feed-forward circles between STATs, IL-6, and NF-κB. For example, TKI was shown to downregulate IL-6 and IL-8 in primary CML cells in vitro, and imatinib mesylate inhibits TNF-α production by myeloid cells *in vitro* (12, 13). TNF-α and MCP-1 are well-known cytokines associated with CML. TNF-α levels were similar between CML patients on TKI treatment and healthy controls in our study, while MCP-1 showed significantly raised concentrations in CML patients. These results are in accordance with other studies with a similar patient population (CML on TKI with achieved MMR) (14, 15).

The pleiotropic function of TNF-α is reflected in the upregulation of multiple pro-inflammatory proteins via the canonical NF-κB and MAPK pathways. The CML stem/progenitor cells produce TNF-α at higher levels compared to their normal counterparts, which provides survival signals through NF-κB/TNF-α feedback loop and, importantly, TNF-α production is not BCR-ABL kinase-dependent (13, 16). Consistently, TNF-α is being activated in TKI-persisting quiescent LSC, and its plasma levels correlate with MPN progression (17). There is a lot of information in the literature on the nature of CML LSC, which are independent of the function of BCR-ABL1, but unfortunately, their eradication has remained largely elusive. It is considered that CML stem/progenitor cells are the source of resistance to therapy and relapse. This is precisely why it is

essential to examine the milieu of these cells as well as the production of cytokines and chemokines around them. Leukemia stem/progenitor cells show a distinct upregulation of inflammatory cytokines, which is associated with resistance to chemotherapy.

The process of onco-inflammation is now well known. In various tumors, the participation of cytokines, chemokines and cancer matrix clearly indicates the process of tumorigenesis, but in hematological malignancies, it has long been unknown.

Monocyte chemoattractant protein-1, as one of the first discovered human chemokines, is part of the CC-motif chemokine family, a very big group which includes cell signaling molecules and related receptors. MCP-1 is strong chemotactic factor for monocytes. It is secreted by a lot of dissimilar cell species, such as endothelial, epithelial, immune system cells, smooth muscle, tumor cells, mesangial, monocytic, CNS and fibroblastic cells. MCP-1 can be produced continuously or induced behind to oxidative stress, some activity of cytokines or after responding to a certain factor (17).

The primary role of the MCP-1 chemokine is the regulation of migration and infiltration of monocytes/macrophages. MCP-1 also signals through the NF-κB pathway and is one of the key elements at the intersection of the pathway signaling in MPNs (15, 18). It can arrest the activation of primitive normal progenitor cells, while not affecting the cycling of primitive CML progenitors (16). A recent study identified MCP-1 and IL-6 as novel, strong, and predictive plasma biomarkers for treatment-free remission in CML, among 20 cytokines tested. MCP-1 and IL-6 levels were markedly increased in CML patients in treatment-free remission compared to others. The low MCP-1/IL-6 levels harmed relapse-free survival and showed a significant prediction (8-fold higher risk) of relapse compared to high MCP-1 levels. The results are supposed to arise from a distinct TKI-associated mechanism that is not directed at killing LSC (18). The evidence aligns with our results of increased MCP-1 levels in CML patients who are diagnosed in the stable chronic stage of the disease.

Conclusion

A chronic inflammation of the bone marrow heavily supports myeloproliferative neoplasms. Many proinflammatory cytokines have been investigated. There is great importance of MCP-1 and IL-6 as novel, strong, and predictive plasma biomarkers for treatment-free remission in CML. Additional and future research in this field will be of special and great importance in understanding the pathophysiology, proinflammatory cytokines, and treatment of CML.

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References

1. Fisher DAC, Fowles JS, Zhou A, Oh ST. Inflammatory pathophysiology as a contributor to myeloproliferative neoplasms. Front Immunol 2021;12 683401. [CrossRef] [PubMed]
2. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia 2022;36(7):1703–19. [CrossRef] [PubMed]
3. Masselli E, Pozzi G, Gobbi G, Merighi S, Gessi S, Vitale M, et al. Cytokine profiling in myeloproliferative neoplasms: Overview on phenotype correlation, outcome prediction, and role of genetic variants. Cells 2020;9(9):2136. [CrossRef] [PubMed]
4. Kleppe M, Kwak M, Koppikar P, Riester M, Keller M, Bastian L, et al. JAK–STAT pathway activation in malignant and nonmalignant cells contributes to MPN pathogenesis and therapeutic response. Cancer Discov 2015;5(3):316–31. [CrossRef] [PubMed]
5. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nature Reviews Cancer 2009;9(11):798–809. [CrossRef] [PubMed]
6. Camacho V, Kuznetsova V, Welner RS. Inflammatory cytokines shape an altered immune response during myeloid malignancies. Front Immunol 2021;12:772408. [CrossRef] [PubMed]
7. Nishida A, Andoh A. The role of inflammation in cancer: Mechanisms of tumor initiation, progression, and metastasis. Cells 2025;14(7):488. [CrossRef] [PubMed]
8. Hoermann G, Greiner G, Valent P. Cytokine regulation of microenvironmental cells in myeloproliferative neoplasms. Mediators Inflamm. 2015;2015:869242. [CrossRef] [PubMed]
9. Branford S. Why is it critical to achieve a deep molecular response in chronic myeloid leukemia? Haematologica 2020;105(12):2730–7. [CrossRef] [PubMed]
10. Wang Y, Zuo X. Cytokines frequently implicated in myeloproliferative neoplasms. Cytokine X 2019;1(1):100005. [CrossRef] [PubMed]
11. Anand M, Chodda SK, Parikh PM, Nadkarni JS. Abnormal levels of proinflammatory cytokines in serum and monocyte cultures from patients with chronic myeloid leukemia in different stages, and their role in prognosis. Hematol Oncol 1998;16(4):143–54. [CrossRef] [PubMed]
12. Copland M. Treatment of blast phase chronic myeloid leukaemia: A rare and challenging entity. Br J Haematol 2022;199(5):665–78. [CrossRef] [PubMed]
13. Wolf AM, Wolf D, Rumpold H, Ludwiczek S, Enrich B, Gastl G, et al. The kinase inhibitor imatinib mesylate inhibits TNF-α production in vitro and prevents TNF-dependent acute hepatic inflammation. Proc Natl Acad Sci U S A 2005;102(38):13622–7. [CrossRef] [PubMed]
14. Nievergall E, Reynolds J, Kok CH, Watkins DB, Biondo M, Busfield SJ, et al. TGF-α and IL-6 plasma levels selectively identify CML patients who fail to achieve an early molecular response or progress in the first year of therapy. Leukemia 2016;30(6):1263–72. [CrossRef] [PubMed]
15. Pavlovsky C, Cordoba BV, Sanchez MB, Moiraghi B, Varela A, Custidiano R, et al. Elevated plasma levels of IL-6 and MCP-1 selectively identify CML patients who better sustain molecular remission after TKI withdrawal. J Hematol Oncol 2023;16(1):43. [CrossRef] [PubMed]
16. Cashman JD, Eaves CJ, Sarris AH, Eaves AC (1998) MCP-1, not MIP-1alpha, is the endogenous chemokine that cooperates with TGF-beta to inhibit the cycling of primitive normal but not leukemic (CML) progenitors in long-term human marrow cultures. Blood 1998;92(7):2338-44. [CrossRef] [PubMed]
17. Mulholland BS, Forwood MR, Morrison NA. Monocyte chemoattractant protein-1 (MCP-1/CCL2) drives activation of bone remodelling and skeletal metastasis. Curr Osteoporos Rep 2019;17(6):538–47. [CrossRef] [PubMed]
18. Gallipoli P, Pellicano F, Morrison H, Laidlaw K, Allan EK, Bhatia R, et al. Autocrine TNF-α production supports CML stem and progenitor cell survival and enhances their proliferation. Blood 2013;122(19):3335–9. [CrossRef] [PubMed]

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FAKTOR NEKROZE TUMORA-α I MONOCITNI HEMOATRAKTANTNI PROTEIN-1 KAO BIOMARKERI U HRONIČNOJ MIJELOIDNOJ LEUKEMIJI

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Hronične mijeloproliferativne neoplazme (engl. *chronic myeloproliferative neoplasms* – CMPNs) predstavljaju specifične klonske poremećaje hematopoetskih matičnih ćelija koji kontinuirano i bez prekida aktiviraju fiziološke puteve prenosa signala neophodne za normalnu i adekvatnu hematopoezu. Izražen proinflamatorni milje u hroničnoj mijeloidnoj leukemiji (engl. *chronic myeloid leukemia* – CML) predvode mnogi interleukini. Cilj ovog rada bio je da se procene razlike između nivoa faktora nekroze tumora-α (engl. *tumor necrosis factor* – TNF-α) i monocitnog hemoatraktantnog proteina-1 (engl. *monocyte chemoattractant protein* – MCP-1) u plazmi pacijenata u hroničnoj fazi hronične mijeloidne leukemije i zdravih osoba kao kontrolne grupe. Studija je obuhvatila pedeset pacijenata sa dijagnozom CML-a u hroničnoj fazi i na standardnoj terapiji inhibitorima tirozin kinaze (engl. *tyrosine kinase inhibitors* – TKIs) i dvadeset zdravih osoba u kontrolnoj grupi. Koncentracije TNF-α i MCP-1 u krvi merene su metodom ELISA (engl. *enzyme-linked immunosorbent assay*). Nivoi MCP-1 bili su viši kod pacijenata nego kod zdravih osoba (334,37 naspram 172,18 pg/ml; p = 0,006). S druge strane, nije utvrđena razlika kada je ispitivan TNF-α. Velik je značaj MCP-1 i interleukina-6 (IL-6) kao novih, jakih i prediktivnih biomarkera u plazmi za remisiju CML-a bez lečenja. Dodatna i buduća istraživanja u ovoj oblasti biće posebno važna za razumevanje patofiziologije i lečenja mijeloproliferativnih bolesti.

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***Ključne reči**: mijeloproliferativne neoplazme, zapaljenje, faktor nekroze tumora-α, monocitni hemoatraktantni protein-1, inhibitori tirozin kinaze*

Original article

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COMPARISON OF THE McGRATH VIDEOLARYNGOSCOPE AND THE MACINTOSH LARYNGOSCOPE FOR ENDOTRACHEAL INTUBATION IN SURGICAL PATIENTS WITHOUT PREDICTORS OF A DIFFICULT AIRWAY

Vesna Dinić[1], Jelena Živadinović[1], Ljubomir Dinić[2,3], Milena Vasilijić[1], Nada Pejčić[1], Miloš Stepanović[1]

The study analyzed and compared the effectiveness of the McGrath MAC videolaryngoscope and the Macintosh laryngoscope for endotracheal intubation in patients without predictors of a difficult airway undergoing elective surgery.

The study included 60 patients randomly divided into two groups, each consisting of 30 patients: the McGrath (MG) and Macintosh (MAC). The primary objective of our study was to determine and compare the duration of intubation and laryngeal view according to Cormack–Lehane grade (CL). Secondary objectives were the comparison of duration of laryngoscopy, first attempt intubation success rate, number of intubation attempts, adverse events (mucosal trauma, desaturation SpO2 < 90%, dental damage), number of failed attempts, Backward Upward Rightward Pressure (BURP) maneuver and the use of bougie.

The results of the study showed that the CL grade I was significantly more frequent in the MG group compared to the MAC group (80% vs. 40%, p = 0.004). Duration of laryngoscopy was significantly shorter in the MG group in comparison to the MAC group (7.87 ± 1.70 vs. 6.00 ± 0.95, p < 0.001). However, there was no statistically significant difference between the groups in duration of intubation (26.03 ± 1.32 s vs. 28.30 ± 5.66 s in the MG and MAC, respectively, p = 0.057). Successful intubation on the first attempt was in 96.7% of patients in the MG group and in 86.7% of patients in the MAC group (p = 0.350). During laryngoscopy the BURP the maneuver was significantly more frequent in the MAC group (p = 0.024). Regarding complications, there were no significant differences between the groups.

In patients without predictors of a difficult airway undergoing elective surgery, the McGrath significantly improves glottis view, reduces the duration of laryngoscopy, and the requirement for the BURP maneuver. Regarding intubation time and the rate of successful intubation on the first attempt, the Macintosh and McGrath are comparable.

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Key words: *McGrath, Macintosh, videolaryngoscopy, elective surgery*

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Introduction

Direct laryngoscopy is considered the gold standard in airway management (1). From its introduction, the Macintosh laryngoscope is the most commonly used device for direct laryngoscopy (2). For optimal glottis visualization using Macintosh, it is essential to align the oral, pharyngeal, and laryngeal axes (3). In cases when it is impossible, anesthesiologists face difficult laryngoscopy and intubation. Failed intubation is associated with a high rate of morbidity and mortality. In patients with a difficult airway, repeated attempts of laryngoscopy and intubation lead to swelling, bleeding, or tissue trauma, which additionally makes each subsequent attempt of endotracheal tube (ETT) placement more difficult.

Videolaryngoscopes (VLs) are designed to overcome these three-axes problems, as achieving an optimal glottis view during video laryngoscopy does not require the alignment of oral, pharyngeal, and laryngeal axes. They enable an anesthesiologist's indirect view of the glottis using a camera on the distal end of the blade, whereas the picture is seen on the LCD screen. Videolaryngoscopes are used in different clinical scenarios. Numerous studies have analyzed the performances of various types of VLs in emergency, trauma, difficult airway, and ICU settings, and have shown that video laryngoscopy

improves intubation success (4-6). Among VLs, there are differences in the design, and therefore, in their performance and outcome.

The McGrath videolaryngoscope is a small portable device with two types of blades: hyperangulated—McGrath X-blade and a Macintosh–type blade (C-MAC blade). Most of the studies that analyzed the efficacy of McGrath in difficult airway showed a higher success rate of tracheal intubation (7–9). In clinical practice, VLs are mostly used in the management of difficult airways and are recommended as the first rescue device in difficult intubation (10). Based on previous data of its efficacy in a difficult airway, the question arises whether the use of McGrath could further improve airway management not only in difficult but also in normal airways (11).

The study compared the effectiveness of the McGrath MAC videolaryngoscope and the Macintosh laryngoscope for endotracheal intubation in patients without predictors of difficult airways undergoing elective surgery.

Materials and Methods

Our randomized controlled study included 60 patients of both sexes, scheduled for elective surgical procedures under general endotracheal anesthesia. Inclusion criteria were age over 18 years, the American Society of Anesthesiologists (ASA) I–III grade, BMI ≤ 30 kg/m^2, elective surgery. Exclusion criteria were pregnant women, ASA IV, BMI > 30 kg/m^2, patients with difficult or anticipated difficult airway (at least one of the criteria: Mallampati classes III and IV, restrected neck movements, interincisor gap < 3 cm, thyreomental distance < 6.5 cm, sternomental distance < 12.5 cm, already known history of difficult intubation), double-lumen tube intubation, neurosurgical procedures.

After institutional ethics committee approval, the research was conducted at the Clinic of Anesthesia and Intensive Therapy, University Clinical Center Niš, in the period from January to February 2025. Written informed consent was obtained from all patients before recruitment. Patients were randomly divided into two groups with a randomization ratio of 1:1. The MAC group included patients intubated using the Macintosh laryngoscope (blade size 4), while patients in the MG group were intubated using the McGrath MAC videolaryngoscope (McGrath® MAC, Aircraft Medical Ltd., 10 Edinburgh, United Kingdom; McGrath MAC blade size 4). Intubations were performed by two anesthesiologists with more than 200 intubations using Macintosh and 50 intubations using McGrath MAC. All patients underwent a preanesthetic check before surgery. Standard monitoring was used intraoperatively (ECG, SpO2, heart rate, and noninvasive blood pressure). Preoxygenation was performed by oxygen mask at a flow of 5 l/min. General anesthesia was induced with midazolam 0.05 mg/kg IV, fentanyl 3μg/kg IV, and propofol 2 mg/kg IV. After checking mask ventilation, rocuronium-bromide was given at a dose of 0.6 mg/kg IV, and after achieving muscle relaxation, intubation was performed using either the Macintosh laryngoscope or the McGrath videolaryngoscope. After performing laryngoscopy anesthesiologist graded the glottis view according to Cormack–Lehane grade (CL) (12).An anti-fog solution was used for the camera before intubation with the McGrath. The choice of the size of ETT was at the discretion of the anesthesiologist. The malleable ETT stylet was used during intubation in both groups. Duration of laryngoscopy was defined as the time from inserting the blade between the teeth until optimal glottis visualization. Duration of intubation was defined as the time from inserting the blade between the teeth until confirmation of the correct ETT placement by two capnographic curves and chest auscultation. First attempt intubation success was defined as correct placement of ETT the on first attempt, but without removing the laryngoscope from oral cavity. Each removal of the blade out of oral cavity was considered as another attempt of intubation. In case of two consecutive unsuccessful intubation attempts, the patient was ventilated by mask, and further, it was proceeded according to the protocol of the University Clinical Center, Clinical of Anesthesiology Reanimatology and Intersive Therapy. Time was measured by stopwatch and was recorded by the resident who was blinded to the study protocol. If intubation couldn't be performed after the second attempt, it was considered a failed intubation. Additional maneuvers during intubation, such as applying Backward, Upward, Rightward Pressure (BURP) manuver on the larynx and the use of a bougie were recorded. Also, adverse events including desaturation (SpO2 < 90%), mucosal trauma, bleeding, dental damage, and esophageal intubation were recorded.

The primary objective of our study was to compare the duration of intubation and laryngeal view by Cormack–Lehane grade. Secondary outcomes included first-attempt intubation success rate, duration of laryngoscopy, number of intubation attempts, adverse events (mucosal trauma, desaturation SpO2 < 90%, dental damage), number of failed attempts, BURP maneuver, and the use of bougie.

Statistical analysis of data was conducted using the Statistical Package for the Social Sciences version 16 program (SPSS Inc., Chicago, IL, USA). Data are presented as arithmetic mean ± SD, as well as absolute and relative numbers. Comparison of variables was performed using the t-test or Mann–Whitney test, depending on data distribution normality. Categorical variables comparison was performed using the Chi-Square test and Fisher's Exoct test. A statistical difference was considered significant at $p < 0.05$.

Results

The study included 60 patients divided into two groups, each consisting of 30 patients. There was no statistically significant difference in age, gender, ASA classification, BMI, and Mallampati score between the groups (Table 1).

Duration of laryngoscopy was significantly shorter in the MG group compared with the MAC group (7.87 ± 1.70 vs. 6.00 ± 0.95, p < 0.001). During laryngoscopy, CL grade I was significantly more frequent in the MG group compared to the MAC group (80% vs. 40%, p = 0.004) (Figure 1).

Table 1. Patients' characteristics

Characteristics	MAC group (n = 30)	MG group (n = 30)	p-value
Age (mean ± SD) years	54.93 ± 11.60	53.80 ± 10.68	0.695[2]
Male n (%) Female n (%)	14 (46.7) 16 (53.3)	15 (50) 15 (50)	1.000[1]
ASA n (%) I II III	 9 (30.0) 20 (71.4) 3 (10.7)	 7 (23.3) 16 (53.3) 8 (26.7)	0.254[1]
BMI (Mean ± SD kg/m²)	25.29 ± 2.89	25.46 ± 3.12	0.555[2]
Mallampati (n %) I II	 9 (30.0) 21 (70)	 7 (23.3) 23 (76.6)	0.559[1]

ASA—American Society of Anesthesiologists, BMI—body mass index

p < 0.05 statistically significant, [1]Chi-Square test, [2]t-test

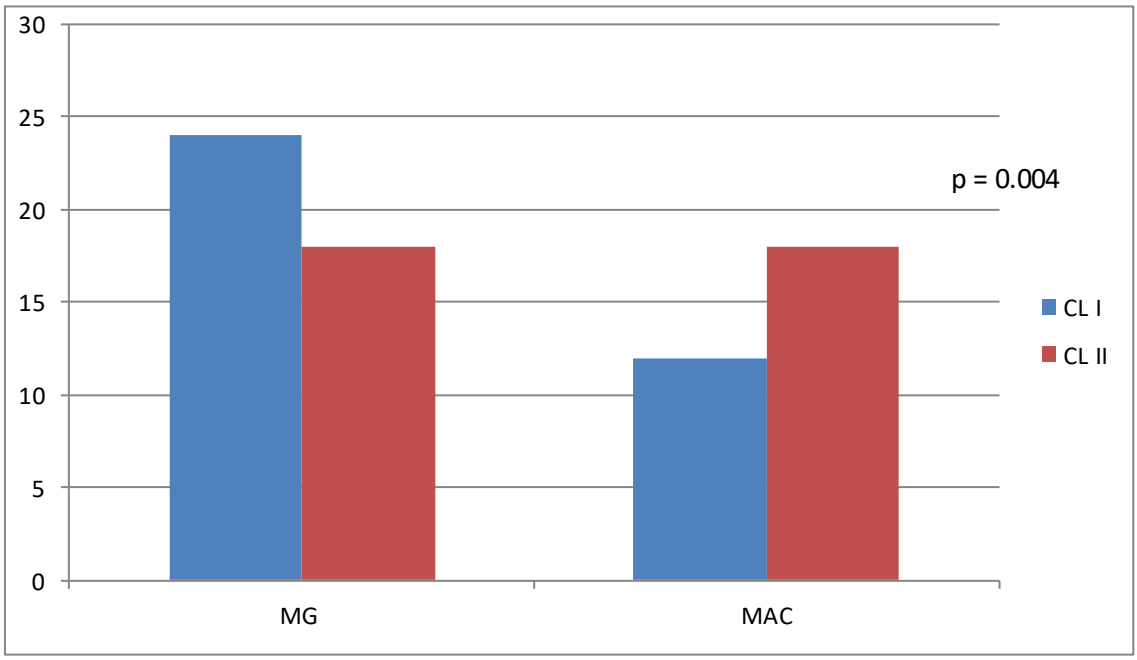

Figure 1. Comparison of Cormack–Lehane grade between the McGrath and Macintosh groups

Although the mean duration of intubation was shorter in the MG group compared to the MAC group, the difference between the groups was not statistically significant (26.03 ± 1.32 s vs. 28.30 ± 5.66 s; p = 0.057). The maximal intubation time with McGrath was 29 s, and with Macintosh 47 s.

In comparison with the Macintosh, the McGrath videolaryngoscope was associated with a higher rate of successful intubations on first attempt: 96.7% (29/30) vs. 86.7% (26/30), respectively. However, the difference was statistically comparable between the groups (p = 0.350). Both groups experienced no more than two intubation attempts. However, the MG group had fewer second attempts compared to the MAC group (1 vs. 4). There were no failed intubations in either group. Backward, Upward, Rightward maneuver was more frequent in the MAC group compared to the MG group (20% vs. 0%, p = 0.024). Bougie was not used in either group. Desaturation (SpO2 < 90%) was not observed in either group of patients (Table 2).

With the exception of mucosal trauma no other complications were observed in either group. Mucosal trauma was present in 2 patients in the MAC group, while in the MG group no patient underwent mucosal injury. There was no significant difference in complication rates between the two devices (Table 2). There were no esophageal intubations in either group.

Table 2. Comparison of outcome parameters

Parameters	MAC group (n = 30)	MG group (n = 30)	p-value
T laryngoscopy mean ± SD	7.87 ± 1.70	6.00 ± 0.95	< 0.001[2*]
T intubation mean ± SD	28.30 ± 5.66	26.03 ± 1.32	0.057[2]
CL grade CL I CL II	 12 (40%) 18 (60%)	 24 (80%) 6 (20%)	0.004[1*]
First attempt intubation	26 (86.7%)	29 (96.7%)	0.350[1]
Second attempt intubation	4 (13.3%)	1 (3.3%)	0.605[3]
Failed intubation	0 (0.0%)	0 (0%)	NA
ELM	6 (20%)	0 (0%)	0.024[*]
Bougie	0 (0.0%)	0 (0%)	NA
Desaturation (SpO2 < 100%)	0 (0.0%)	0 (0.0%)	NA
Esophageal intubation	0 (0%)	0 (0%)	NA
Dental damage	0 (0%)	0 (0%)	NA
Mucosal trauma	2 (6.7%)	0 (0.0%)	0.492[1]
Bleeding	0 (0%)	0 (0%)	NA

[*]p < 0.05 statistically significant, [1]Chi-Square test, [2]T-test, [3]Fisher's test, NA/not applicable,

Discussion

Our study showed that the duration of laryngoscopy was significantly shorter in the MG group compared with the MAC group (p < 0.001). McGrath was associated with a significantly higher rate of CL I grade. The explanation is in the design of McGrath. The camera on the distal tip of the blade enables better glottis view and therefore shortens laryngoscopy time. Our results are supported by previous researches (13, 14).

However, despite the fact that McGrath improved the glottis view, the duration of intubation between the MG and MAC groups was comparable (p = 0.057). The results of our study suggest that improved CL grade does not necessarily mean faster intubation, even though CL grade I is considered one of the predictors of "easy" intubation. There are several reasons for this. First of all, indirect laryngoscopy requires hand–eye coordination. Second, intubation using McGrath might take more time because it requires maneuvering of the ETT that should pass a steep angle to enter the glottis (15). To improve intubation and facilitate the placement of ETT through the glottis in our study, we used a malleable stylet, which might be the reason for shorter mean intubation time with McGrath compared with Macintosh (26.03 vs. 28.30, respectively), but still without statistical significance. Our results are in line with the results obtained by Kaur et al. They reported shorter mean intubation time with McGrath compared to Macintosh, but the difference was not statistically significant although McGrath improved CL grade. Also, there was no difference in the first attempt success intubation rate (16).

Data regarding intubation time for Macintosh and McGrath are conflicting. In a study that

compared Macintosh, McGrath, and C-MAC, Abhaynkar et al. reported superior glottis view with McGrath, but significantly longer time of both laryngoscopy and intubation with McGrath ($p < 0.0001$), which is opposite to our results. The authors reported fogging of the camera as one of the main reasons for prolonged intubation (17). In our study anti-fog solution was used prior to videolaryngoscopy, and fogging was present in 1 out of 30 cases.

Walker et al. compared Macintosh with the McGrath in patients with normal airways and reported significantly longer intubation time in McGrath group (18). The results of our study are not consistent with those of Walker et al., given that intubations in their study were performed by inexperienced anesthetists. Contrary to our results, are results of Hoshijima et al. They reported significantly longer intubation time with McGrath (19). Similar results were reported by Sansone et al. (20), who compared McGrath with Macintosh and found that intubation time with McGrath was longer, but without statistical significance. However, most of the providers in these studies had more clinical experience with the Macintosh than with McGrath videolaryngoscope, which could be an explanation for longer intubation time during videolaryngoscopy. Bakshi et al. concluded that expertise with direct laryngoscopy does not necessarily mean expertise with VLs (10). Different skills are necessary to efficiently perform both videolaryngoscopy and direct laryngoscopy. All this emphasize the importance of clinicians' experience and skills in using VLs and Macintosh as well as its impact on intubation success, complications, and duration of the procedure.

In our study, 96.7% of patients (29/30) were successfully intubated on the first attempt using McGrath and 86.7% (26/30) using Macintosh. Although the difference was not statistically significant, its clinical significance should not be ignored. The results of our study are in line with the results of previous studies (17, 18, 21). However, in a recent study with a large number of patients with normal airway Kriege et al. have reported significantly higher first-pass intubation success rate with McGrath compared to Macintosh (22). The difference between our results and those obtained in their study can be explained by the small sample size of our study.

Regarding the use of optimization maneuvers, the BURP maneuver was not performed during videolaryngoscopy, but it was significantly more frequent in the Macintosh group, which is supported by the fact that during direct laryngoscopy, manipulation is needed to align all three axes to visualize the glottis. Our results suggest that McGrath improves intubation conditions by superior glottis view, shortens laryngoscopy time, and lessens the requirement for BURP. All this should contribute to fewer intubation attempts and therefore lead to fewer complications.

The importance of limiting the number of intubation attempts was highlighted in the recent ASA guideline (23). Multiple attempts of intubation are associated with tissue trauma and desaturation. In the present study, most patients were intubated on the first attempt, and a few on the second. There was no desaturation in either group. We did not find significant differences in adverse events between the groups. It has been shown that “during direct laryngoscopy in difficult airway, complications are 45 times more common than in those with predicted easy airways” (24). Given that the current study included patients with predicted non-difficult airways, the only reported trauma was oral mucosal injury that happened in 2 patients intubated with a Macintosh, whereas in the MG group, none of the patients underwent any trauma. The injuries occurred as a result of the pressure of the blade on the gingiva. Regarding trauma, current research did not find statistically significant differences between Macintosh and McGrath, which is in line with previous research (15, 16). There is a report about palatal perforation with McGrath, probably due to “blind period of intubation” (25). In the present study, no serious trauma occurred.

Our study had some limitations. First of all, the anesthesiologists who performed intubations were not blinded to the devices they used. Furthermore, this was a small sample study.

Conclusion

In patients without predictors of difficult airway, McGrath significantly improves glottis view, reduces the duration of laryngoscopy, and lessens the requirement for optimization maneuvers such as BURP. Regarding intubation time, rate of successful intubation on first attempt and complications Macintosh and McGrath are comparable.

References

1. Geun Joo Choi. The golden era of videolaryngoscopy: costs we should consider. Korean J anesthesiol 2022; 75(4):293-4. [CrossRef] [PubMed]
2. Xue FS, Yang BQ, Liu YY, Li HX, Yang GZ. Current evidences for the use of UEscope in airway management. Chin Med J 2017;130(15): 1867-75. [CrossRef] [PubMed]
3. Bannister F, MacBeth R. Direct laryngoscopy and tracheal intubation. Lancet 1944; 244: 651–4. [CrossRef]
4. Su YC, Chen CC, Lee YK, Lee JY, Lin KJ. Comparison of videolaryngoscopes with direct laryngoscopy for tracheal intubation: A meta-analysis of randomised trials. Eur J Anaesthesiol 2011; 28(11):788–95. [CrossRef] [PubMed]
5. Köhl V, Wünsch VA, Müller MC, Sasu PB, Dohrmann T, Peters T, et al. Hyperangulated vs. Macintosh videolaryngoscopy in adults with anticipated difficult airway management: a randomised controlled trial. Anaesthesia 2024; 79(9):957-66. [CrossRef] [PubMed]
6. Kreutziger J, Hornung S, Harrer C, Urschl W, Doppler R, Voelckel WG, et al. Comparing the McGrath Mac Videolaryngoscope and Direct Laryngoscopy for Prehospital Emergency Intubation in Air Rescue Patients: A Multicenter, Randomized, Controlled Trial. Crit Care Med 2019; 47(10):1362-70. [CrossRef] [PubMed]
7. Taylor AM, Peck M, Launcelott S, Hung OR, Law JA, MacQuarrie K, et al. The McGrath® Series 5 videolaryngoscope vs the Macintosh laryngoscope: a randomised, controlled trial in patients with a simulated difficult airway. Anesthesia 2013; 68(2):142-7. [CrossRef] [PubMed]
8. Shippey B, Ray D, McKeown D. Use of the McGrath videolaryngoscope in the management of difficult and failed tracheal intubation. Br J Anaesth 2008; 100(1):116–9. [CrossRef] [PubMed]
9. OLaery AM, Sandison MR, Myneni N, Cirilla DJ, Roberts KW, Deane GD. Preliminary evaluation of a novel videolaryngoscope, the Mc Grtah series 5 in the management of difficult and challenging endotracheal intubation. J Clin Anesth 2008; 20(4):320-1. [CrossRef] [PubMed]
10. Bakshi SG, Vanjari VS, Divatia JV. A prospective, randomised, clinical study to compare the use of McGrath(®), Truview(®) and Macintosh laryngoscopes for endotracheal intubation by novice and experienced Anaesthesiologists. Indian J Anaesth 2015; 59(7):421–7. [CrossRef] [PubMed]
11. CC Becerra Gómez, Ángel Rojal M. Should videolaryngoscopy be routinely used for airway management? An approach from different scenarios in medical practice. Rev colomb anestesiol 2024; 52(1):4. [CrossRef]
12. Glosser L. Assessment of endotracheal tube intubation. Review of existing scales. Disaster Emerg Med J 2017;2: 91-3. [CrossRef]
13. Sato Boku A, Sobue K, Kako E, Tachi N, Okumura Y, Kanazawa M, et al. The usefulness of the McGrath MAC laryngoscope in comparison with Airwayscope and Macintosh laryngoscope during routine nasotracheal intubation: a randomaized controlled trial. BMC Anesthesiol 2017;17(1):160. [CrossRef] [PubMed]
14. Noppens RR, Mobus S, Heid F, Schmidtmann I, Werner C, Piepho T. Evaluation of the McGrath Series 5 videolaryngoscope after failed direct laryngoscopy. Anaesthesia 2010; 65(7):716–20. [CrossRef] [PubMed]
15. Bhola R, Bhalla S, Gupta R, Singh I, Kumar S. Tracheal intubation in patients with cervical spine immobilization: A comparison of McGrath videolaryngoscope and Truview EVO2 laryngoscope. Indian J Anesth 2014; 58(3):269-74. [CrossRef] [PubMed]
16. Kaur G, Gupta S, Mehta N, Dhingra JS. Comparative Evaluation of McGrath MAC, Truview Videolaryngoscopes and Macintosh Laryngoscope for Endotracheal Intubation in Patients Undergoing Surgery under General Anaesthesia. Anesth Essays Res 2020; 14(1):20-4. [CrossRef] [PubMed]
17. Abhyankar P, Sabharwal N, Gupta A, Das AK. Comparative evaluation of C-MAC and McGrath MAC videolaryngoscopes with Macintosh direct laryngoscope for endotracheal intubation in adult patients undergoing elective surgeries. J Anaesthesiol Clin Pharmacol 2023; 39(3):422-8. [CrossRef] [PubMed]
18. Walker L, Brampton W, Halai M, Hoy C, Lee E, Scott I, et al. Randomized controlled trial of intubation with the McGrath Series 5 videolaryngoscope by inexperienced anaesthetists. Br J Anaesth 2009; 103(3):440-5. [CrossRef] [PubMed]
19. Hoshijima H, Mihara T, Maruyama K, Denawa Y, Takahashi M, Shiga T, et al. McGrath videolaryngoscope versus Macintosh laryngoscope for tracheal intubation: A systematic review and meta-analysis with trial sequential analysis. J Clin Anesth 2018; 46:25–32. [CrossRef] [PubMed]
20. Sansone P, Giaccari LG, Bonomo A, Gargano F, Aurilio C, Coppolino F, et al. Comparison of McGrath Videolaryngoscope versus Macintosh Laryngoscope in Tracheal Intubation: An Updated Systematic Review. Journal of Clinical Medicine 2023; 12(19):6168. [CrossRef] [PubMed]
21. Liu ZJ, Yi J, Guo WJ, Ma C, Huang YG. Comparison of McGrath Series 3 and Macintosh Laryngoscopes for Tracheal Intubation in Patients With Normal Airway by Inexperienced Anesthetists: A Randomized Study. Medicine (Baltimore) 2016; 95 (2):e2514. [CrossRef] [PubMed]
22. Kriege M, Noppens RR, Turkstra T, Payne S, Kunitz O, Tzanova I, et al. A multicentre randomised controlled trial of the McGrath™ Mac videolaryngoscope versus conventional laryngoscopy. Anaesthesia 2023;78 (6):722-9. [CrossRef] [PubMed]
23. Apfelbaum JL, Hagberg CA, Connis RT, Abdelmalak BB, Agarkar M, Dutton RP, et al. 2022 American Society of Anesthesiologists Practice Guidelines for Management of the Difficult Airway.

Anesthesiology 2022; 136 (1): 31-81. [CrossRef] [PubMed]

24. Cumberworth A, Lewith H, Sud A, Jefferson H, Athanassoglou V, Pandit JJ. Major complications of airway management: a prospective multicentre observational study. Anaesthesia 2022; 77 (6):640-8. [CrossRef] [PubMed]

25. Williams D, Ball DR. Palatal perforation associated with McGrath videolaryngoscope. Anaesthesia 2009;64 (10):1144-5. [CrossRef] [PubMed]

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POREĐENJE McGRATH VIDEO-LARINGOSKOPA I MACINTOSH LARINGOSKOPA U ENDOTRAHEALNOJ INTUBACIJI HIRURŠKIH PACIJENATA BEZ PREDIKTORA OTEŽANOG DISAJNOG PUTA

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U ovom istraživanju smo analizirali i upoređivali efikasnost upotrebe McGrath video-laringoskopa i Macintosh laringoskopa u endotrahealnoj intubaciji pacijenata bez prediktora otežanog disajnog puta koji se podvrgavaju elektivnim hirurškim procedurama.

Istraživanje je obuhvatilo šezdeset pacijenata, koji su podeljeni u dve grupe sačinjene od trideset pacijenata: McGrath (MC) grupu i Macintosh (MAC) grupu. Glavni cilj istraživanja bilo je poređenje trajanja intubacije i vizuelizacije glotisa na osnovu Cormack–Lehane (CL) skale. Sporedni cilj bilo je poređenje trajanja laringoskopije, procenta uspešnih intubacija iz prvog pokušaja, broja pokušaja intubacije, neželjenih događaja (trauma tkiva, desaturacija < 90%, lomljenje zuba), broja neuspešnih intubacija, BURP (engl. *Backward, Upward, Rightward Pressure* – BURP) manevara i upotreba bužija.

Rezultati su pokazali značajno češći CL gradus I u MG grupi nego u MAC grupi (80% prema 40%; p = 0,004). Trajanje laringoskopije bilo je značajno kraće u MG grupi nego u MAC grupi (7,87 ± 1,70 prema 6,00 ± 0,95; p < 0,001). Međutim, nije bilo statistički značajne razlike u trajanju intubacije između grupa (26,03 s ± 1,32 s prema 28,30 s ± 5,66 s u MG grupi i MAC grupi; p = 0,057). Iz prvog pokušaja uspešno je intubirano 96,7% pacijenata u MG grupi i 86,7% pacijenata u MAC grupi (p = 0,350). BURP manevar bilo je značajno češće u MAC grupi (p = 0,024) u toku laringoskopije. Nisu zabeležene značajna razlike među grupama kada je reč o komplikacijama.

Kod pacijenta bez prediktora otežanog disajnog puta koji se podvrgavaju elektivnim hirurškim procedurama McGrath video-laringoskop značajno poboljšava vizuelizaciju glotisa, skraćuje trajanje laringoskopije i smanjuje potrebu za izvođenje BURP manevre. Ne postoji značajna razlika u trajanju intubacije, stopi uspešnih intubacija iz prvog pokušaja i komplikacija prilikom korišćenja McGrath video-laringoskopa i Macintosh laringoskopa.

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***Ključne reči**: McGrath, Macintosh, video-laringoskopija, elektivna hirurgija*

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HEALTHCARE-ASSOCIATED INFECTIONS AND MICROBIOLOGICAL ANALYSIS AT THE CLINIC FOR ANESTHESIA AND INTENSIVE THERAPY IN A TERTIARY HEALTH INSTITUTION DURING A ONE-YEAR PERIOD

Milena Stojanović

The term Healthcare-Associated Infections (HAI) or Nosocomial Infections (NI) refers to infections that occur in patients during their stay and treatment in medical conditions, but were not present at the time of admission and that manifest at least 48 h after hospital admission. HAIs are more frequent in developing countries (5.7–19.1%) than in developed ones (3.5–12%). Antimicrobial resistance is one of the global health problems with a significant burden, and it is an inevitable consequence of using antimicrobial drugs. The unjustified use of antibiotics has contributed to antimicrobial resistance and changes in the causative pathogens responsible for HAIs. These types of infections prolong the length of hospital stay, lead to long-term disability, and increase morbidity and mortality. The most common types of HAIs are: Ventilator-Associated Pneumonia (VAP), Central Line-Associated Bloodstream Infections (CLABSI), Catheter-Associated Urinary Tract Infections (CAUT) and Surgical Site Infections (SSI).

This study aimed to determine the most common bacteria causes of HAIs as well as the distribution according to the type of isolated bacteria from different types of samples.

The retrospective study was conducted at the Clinic for Anesthesia and Intensive Therapy, University Clinical Center Niš, during the period from January to December 2024. The patient samples were obtained, namely: blood, groin swab, oral cavity swab, wound swab, rectum swab, armpit swab, urine, aspirate from the endotracheal tube, nose swab, drain content, abdominal cavity content, tip of the central venous catheter, tip of the drain and aspirate from bronchia. The material was sent to the local microbiological laboratory with the aim of obtaining a biogram and antibiogram.

Depending on the type of bacteria, the distribution was as follows: *Klebsiella* spp. (20.15%), *Enterococcus faecalis* (19.26%), *Acinetobacter* spp. (14.41%), *Pseudomonasaeruginosa* (7.9%), *Escherichia coli* (7.17%), *Staphylococcus epidermidis* (4.99%), *Proteus mirabilis* (3.96%), *Enterococcus faecium* (2.94%), *Enterobacter* spp. (2.94%) and *Staphylococcus* spp. (2.19%). *Klebsiella* spp. was the dominant bacterium isolated from oral cavity swab (3.28%), *Enterococcus faecium* from groin swab (5.60%), *Acinetobacter* spp. from endotracheal aspirate (1.91%), *Pseudomonas aeruginosa* from wound swab (2.25%) and *Escherichia coli* from rectal swab (1.84%).

Healthcare-associated infections have a significant negative impact on hospitalized patients. They lead to permanent disability, prolonged patients' length of stay and increased mortality as well as expenditure and waste of medical resources. The predominance of microorganisms depends both on the hospital conditions, as well as on the patients and antibiotics prescribed by physicians. It is recommended to establish a precise schedule for antibiotic use in each region based on the most common pathogens and antibiotic resistance patterns, and surveillance programs as important tools which would be helpful to clinicians to choose the most appropriate antimicrobial therapy for hospitalized patients.

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Introduction

The term Healthcare-Associated Infections (HAI) or Nosocomial Infections (NI) refers to infections that occur in patients during their stay and treatment in medical conditions, but were not present at the time of admission, and they should

manifest at least 48h after hospital admission (1–3). The term HAIs refers not only to hospital infections, but also to infections acquired in any healthcare facilities, public and private (nursing homes, ambulatory, and rehabilitation centers) which provide healthcare or diagnostic services to patients. According to World Health Organization (WHO) data, about 5–15% of hospitalized patients develop HAIs (4), and the results of the EPIC II study showed that HAIs can be expected in even 51% of patients in intensive care units (ICUs) (5). A lack of data results in an inadequate and insufficiently developed system of supervision and control.

HAIs are more frequent in developing countries (5.7–19.1%) than in developed ones (3.5–12%) (6). Among newborns, the occurrence of ICU-related infections is 3–20 times higher. In developing countries two-thirds of operated patients develop HAI, which is nine times higher than in developed regions (7). Literature review estimates that 10–70% of HAIs are preventable with appropriate infection control (8).

The most common risk factors for HAIs include mechanical ventilation, critical care units, wound and burn units, dialysis unit, operation theatres, delivery rooms, neonates, prolonged hospital stay, utilization of invasive life-prolonging procedures including venous and arterial catheterization, urinary catheterization, invasive intracranial pressure monitoring, prior and prolonged antibiotic therapy, colonization of neighboring patients and health care personnel, weakened immune system of the patient, chronic and debilitating disease and diabetes (9). In addition to the host's susceptibility, it is important to know the biological characteristics of the infectious agent (10).

Aim

This study aimed to determine the most common bacterial causes of HAIs as well as the distribution according to the type of isolated bacteria from different types of samples.

Materials and Methods

The retrospective study was conducted at the Clinic for Anesthesia and Intensive Therapy, Clinical Center Niš, Serbia, during the period from the beginning of 2024 until the end of the same year. The patient samples were obtained, including blood, groin swab, oral cavity swab, wound swab, rectum swab, armpit swab, urine, aspirate from the endotracheal tube, nose swab, drain content, abdominal cavity content, tip of the central venous catheter, tip of the drain, and aspirate from the bronchia. The material was sent to the local microbiological laboratory.

The data are presented as absolute numbers and percentages, which are then compared with data from the literature.

Results

The total number of samples was 1464. Of them, 232 were blood samples, 179 groin swabs, 166 oral cavity swabs, 132 wound swabs, 122 rectal swabs, 108 armpit swabs, 106 urine samples, 94 endotracheal aspirate samples, 63 nose swabs, 56 aspirates, 43 drain contents, 15 abdominal cavity contents, 15 tips of the central venous catheters, 11 tips of the drains, 10 aspirates from bronchia and 112 the other samples, (unclassified) (Figure 1).

Depending on the type of bacteria, the distribution was as follows: *Klebsiella* spp. (20.15%), *Enterococcus faecalis* (19.26%), *Acinetobacter* spp. (14.41%), *Pseudomonas aeruginosa* (7.9%), *Escherichia coli* (7.17%), *Staphylococcus epidermidis* (4.99%), *Proteus mirabilis* (3.96%), *Enterococcus faecium* (2.94%), *Enterobacter* spp. (2.94%) and *Staphylococcus* spp. (2.19%) (Figure 2).

Klebsiella spp. was the dominant bacterium isolated from oral cavity swab (3.28%), *Enterococcus faecium* from groin swab (5.60%), *Acinetobacter* spp. from endotracheal aspirate (1.91%), *Pseudomonas aeruginosa* from wound swab (2.25%) and *Escherichia coli* from rectal swab (1.84%) (Table 1).

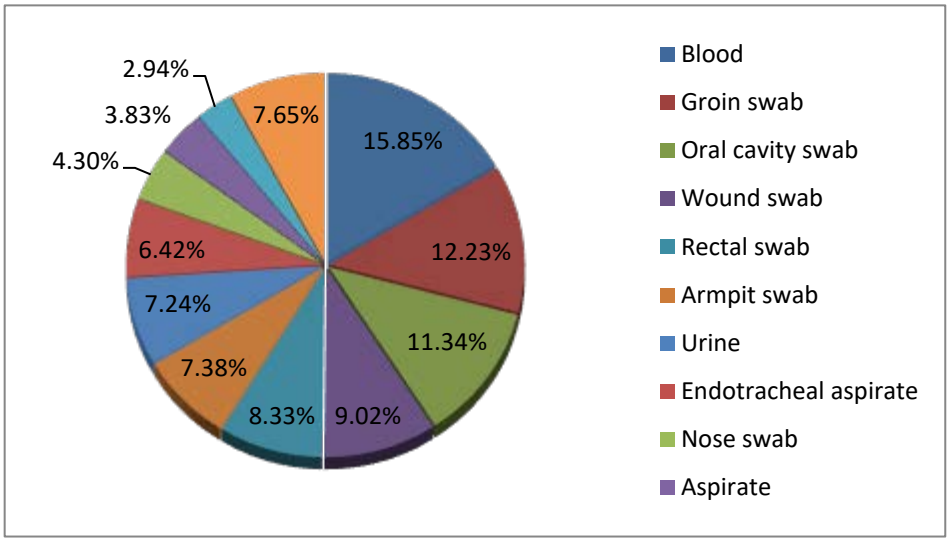

Figure 1. Distribution according to sample type

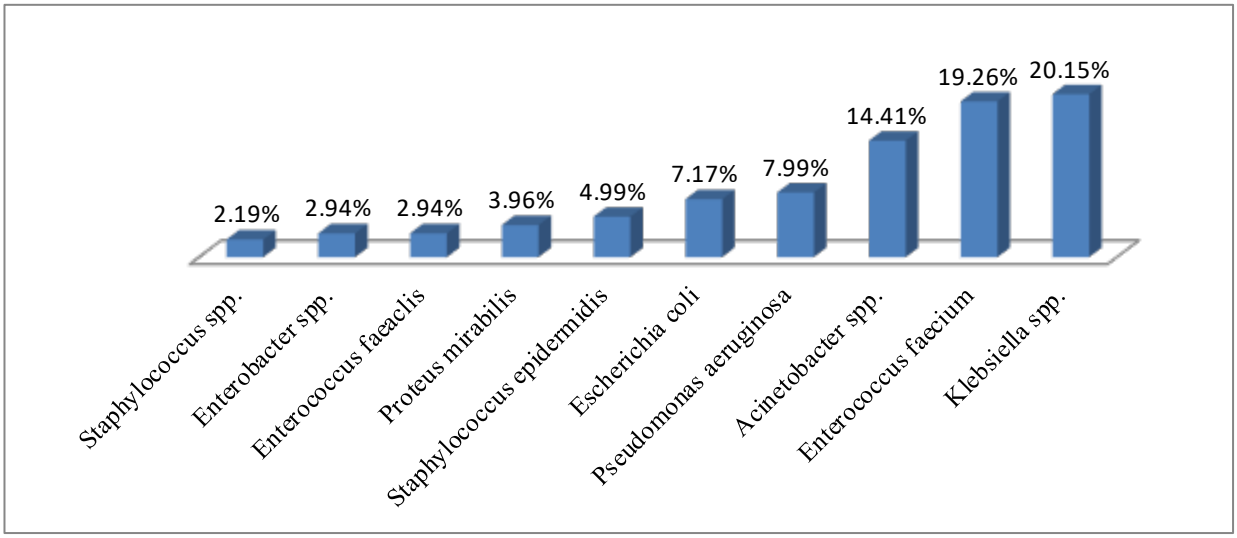

Figure 2. Distribution of bacteria by species

Table 1. Distribution according to the type of the sample and isolated bacteria

			Klebsiella spp.		*E. faecium*		*Acinetobacter* spp.		*Pseudomonas aeruginosa*		*Escherichia coli*	
Sample type	Number of samples	Percent of samples	No.	%	No.	%	No.	%	No.	%	No.	%
Blood	232	15.85%	3	0.20%	13	0.89%	6	0.41%	6	0.41%	9	0.61%
Groin swab	179	12.23%	30	2.05%	82	5.60%	18	1.23%	9	0.61%	18	1.23%
Oral cavity swab	166	11.34%	48	3.28%	51	3.48%	22	1.50%	13	0.89%	17	1.16%

Wound swab	132	9.02%	24	1.64%	7	0.48%	25	0.71%	33	2.25%	5	0.34%
Rectal swab	122	8.33%	16	1.09%	52	3.55%	7	0.48%	4	0.27%	27	1.84%
Armpit swab	108	7.38%	19	1.30%	48	3.28%	20	1.37%	4	0.27%	3	0.20%
Urine	106	7.24%	43	2.94%	2	0.14%	13	0.89%	3	0.20%	13	0.89%
Endotracheal aspirate	94	6.42%	25	1.71%	1	0.07%	28	1.91%	11	0.75%	2	0.14%
Nose swab	63	4.30%	12	0.82%	14	0.96%	17	1.16%	5	0.34%	3	0.20%
Aspirate	56	3.83%	12	0.82%			22	1.50%	8	0.55%	2	0.14%
Drain content	43	2.94%	18	1.23%	3	0.20%	2	0.14%	1	0.07%		
Abdominal cavity content	15	1.02%	2	0.14%					2	0.14%	3	0.20%
Central venous catheter tip	15	1.02%	6	0.41%	3	0.20%	2	0.14%	1	0.07%		
Drain tip	11	0.75%	2	0.14%			2	0.14%	1	0.07%	1	0.07%
Bronchial aspirate	10	0.68%	5	0.14%			2	0.14%				
Other	112	7.65%	39	0.25%	6	0.41%	22	1.50%	13	0.89%	1	0.07%
Total	1464	100%	**295**	**20.15%**	**282**	**19.26 %**	**211**	**14.41 %**	**117**	**7.99%**	**105**	**7.17 %**

Discussion

Antimicrobial resistance is one of the global health problems with a significant burden, and it is an inevitable consequence of using antimicrobial drugs. Unjustified use of antibiotics has led to antimicrobial resistance and changes in the causative pathogens responsible for HAIs. These types of infections prolong the length of hospital stay, lead to long-term disability, and increase morbidity and mortality. All this has a negative impact on healthcare costs, and annual costs are estimated to be about 7 billion euros in Europe and 6.5 billion dollars in the United States (11). Some examples are methicillin-resistant *Staphylococcus aureus* (MRSA), which is still an important challenge for many countries. In addition to MRSA, multidrug-resistant *Escherichia coli*, vancomycin-resistant enterococci (VRE), and carbapenem-resistant Enterobacteriaceae are becoming a problem of public health importance (10).

Antibiotic resistance is responsible for the death of a child every five minutes in the Southeast Asian region. Medicines that were used to treat deadly diseases are now losing their potential, precisely because of the development of resistance (12). Self-medication, inadequate dosage, prolonged use, and lack of standards among healthcare institutions are the main reasons. Resistance threatens effective control against bacteria caused by urinary tract infections, pneumonia and bloodstream infections. The most common among them are MRSA and multidrug-resistant gram-negative bacteria (13). In the region of Southeast Asia are strains of *E. coli* and *Klebsiella pneumoniae* resistant to third-generation cephalosporines, and more than a quarter of infections caused by *S. aureus* are resistant to methicillin (14).

The sources of HAI infections are: 1) endogenous—the patient's microflora, and most frequently, 2) exogenous. Bacteria are the most common cause of HAIs (90%). Some of them are part of the patient's normal flora and lead to infection only when the patient's immune system declines. About 5% of HAIs are caused by viruses (15). Among them, the most common are influenza, HIV, rotavirus, herpes simplex virus and hepatitis. Among the fungi, the most

common causative agents are *Aspergillus* spp. and *Candida albicans* (16). The main routes of transmission include contact (direct and indirect), airborne, common vehicle (through contaminated items like food, water, medications, equipment) and vector-borne (mosquitoes, flies, rats) (17).

Nowadays, according to WHO, the most common causes of HAI are *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumonia, Clostridium difficile, E. coli* and *Staphylococcus aureus* (18). These bacteria developed additional mechanisms against currently available antibiotics (19). That is why the development and introduction of new antibiotics into clinical practice is urgently necessary.

According to the current guidelines, when the patient is at risk of infection with resistant organisms, it is advisable to use broad-spectrum combination therapy with more than two antibiotics as the initial empirical therapy (20).

Results showed that the total number of samples was 1,464. Of them, 232 (15.85%) were blood samples, 179 groin swabs (12.23%), 166 oral cavity swabs (11.34%), 132 wound swabs (9.02%), 122 rectal swabs (8.33%), 108 armpit swabs (7.38%), 106 urine samples (7.24%), 94 endotracheal aspirate samples (6.42%), 63 nose swabs (4.30%), 56 aspirates (3.83%), 43 drain contents (2.94%), 15 abdominal cavity contents (1.02%), 15 tips of the central venous catheter (1.02%), 11 tips of the drain (0.75%), 10 aspirates from bronchi (0.68%) and 112 (7.65%) the other samples (unclassified)(Figure 1). Depending on the type of bacteria, the distribution was as follows: *Klebsiella* spp. (20.15%), *Enterococcus faecalis* (19.26%), *Acinetobacter* spp. (14.41%), *Pseudomonas aeruginosa* (7.9%), *Escherichia coli* (7.17%), *Staphylococcus epidermidis* (4.99%), *Proteus mirabilis* (3.96%), *Enterococcus faecium* (2.94%), *Enterobacter* spp. (2.94%) and *Staphylococcus* spp. (2.19%) (Figure 2).

Table 1 shows the distribution of bacteria according to the type of biological material from which they were isolated. *Klebsiella* spp. was the dominant bacterium isolated from oral cavity swab (3.28%), *Enterococcus faecium* in groin swab (5.60%), *Acinetobacter* spp. in endotracheal aspirate (1.91%), *Pseudomonas aeruginosa* in wound swab (2.25%) and *Escherichia coli* in rectal swab (1.84%).

The most common types of HAIs are Ventilator-Associated Pneumonia (VAP), Central Line-Associated Bloodstream Infections (CLABSI), Catheter-Associated Urinary Tract Infections (CAUT) and Surgical Site Infections (SSI).

Central line-associated bloodstream infections incidence is about 12–25% (21). Catheters can cause serious and deadly infections, resulting in compromised health and increased care costs. They are usually caused by gram-positive organisms, including coagulase-negative *Staphylococcus*, *Staphylococcus aureus* and Enterococci (22).

According to the Liao et al. study (23) of 584 blood samples, a total of 267 (45.7%) were caused by fermentative gram-negative organisms, 137 (23.5%) by non-fermentative gram-negative organisms, and 134 (22.9%) by gram-positive organisms. Fungi were isolated in samples (6.8%). The most common bacteria causing bloodstream infections among gram-negative strains were *Klebsiella* spp. (14%), *E. coli* (14%), *Enterococcus* spp. (11%), *Acinetobacter* spp. (10%), *Enterobacter* spp. (9%), *S. aureus* (9%) and *Pseudomonas* spp. (8%). In our study, the main cause was gram-positive bacteria, namely *Enterococcus faecium.*

In 1990, the predominant bacteria causing bloodstream infections were gram-positive, namely *Staphylococcus aureus.* Later, in 2000, it was *Acinetobacter baumannii*, and in recent years, it has been gram-negative Enterobactriales (24).

Some of the mortality risk factors according to the above-mentioned study are: low body weight, comorbidity with malignancy and liver cirrhosis, high C- reactive protein level, high Charlson Comorbidity Index, and internal medicine and hematology/oncology patients.

According to European national prevalence studies, the rate of nosocomial urinary tract infections (UTI) is 23–49% (25). They are mainly caused by the patients' microflora, where the catheters serve as a conduit for entry of bacteria and imperfect drainage, and retained urine provides the stability to bacterial residence. A common cause of nosocomial urinary tract infections are *E. coli, Pseudomonas aeruginosa, Klebsiella* spp., *Proteus mirabilis, Staphylococcus epidermidis*, Enterococci and *Candida* spp. (25, 26).

Asymptomatic bacteriuria in catheterized patients, according to most clinicians, should not be treated. As long as the presence of a catheter is temporary, this condition is transient and will resolve spontaneously. In justified cases, it is recommended to use silver-coated catheters. The most significant preventive measure is to avoid the use of the indwelling catheters whenever possible. The study of Jain P et al. (27) showed that the unjustified use of urinary catheters was in 21% of cases.

The study conducted by Nouri et al. (28) showed that among gram-negative bacteria in the urine samples, *E. coli* was the most common, accounting for 832 cases (59.6%). Other notable findings included *K. pneumoniae* with 139 cases (9.9%), *P. aeruginosa* with 71 cases (5.09%), *Enterobacter* spp. with 31 cases (2.2%), *Acinetobacter baumannii* with 30 cases (2.15%) and *Klebsiella oxytoca* with 18 cases (1.2%). The most frequent pathogens identified in tracheal aspirates were *K. pneumoniae* (164 cases; 27.8%), *Acinetobacter baumannii* (118 cases; 20.06%), *Pseudomonas aeruginosa* (97 cases;

16.4%) and *E. coli* (57 cases; 9.6%). In our study, *Klebsiella* spp. was the most prevalent bacterium.

The frequency of surgical site infections in surgical patients is 2–5%, mainly caused by endogenous microflora of the patients. Among bacteria, the most often isolated is *Staphylococcus aureus,* commonly found amongst the normal flora of the skin (29, 30), followed by *Candida* spp*., E. coli, P. aeruginosa and S. epidermidis* (31).

Nosocomial pneumonia, which develops at least 48 h after intubation, is defined as ventilator-associated pneumonia. The prevalence is 10–65%, and the mortality rate is about 20% (32, 33). The most common risk factors are elderly men, smokers, coexisting lung diseases and admission because of respiratory problems (34). The most common pathogens are gram-negative bacteria such as *Acinetobacter sp., Pseudomonas aerugnosa, Klebsiella pneumonia* and gram-positive cocci like *Staphylococcus aureus*, especially MRSA (35). In the study conducted by George et al. (35, 36), the most common isolate was *Acinetobacter* spp*.* (37.5%). Our findings are consistent with the aforementioned study.

Infection Control Programs and Prevention of HAIs

The standard procedure performed on patients suspected of having infections in the ICU implies: 1) Gram staining results, usually available after 24 h; 2) organism identification from the culture, typically returned after 48 h (16); 3) the full antibiogram results, which actually present a susceptibility test for various antimicrobials available after approximately 72 h. The antibiogram will give a definitive answer (37).

Some of the most important preventive measures include early detection of a colonized patient, eradication of the source of reservoir, isolation of the infected or colonized patient, emphasis on hand hygiene before and after patient handling, use of disposable gloves, prohibition of unjustified antibiotic prescription/use, establishment and implementation of the surveillance system for antimicrobial resistance, education of hospital staff and community (38).

The Centers for Disease Control and Prevention estimates that about 100 million antibiotics are prescribed annually, of which about 50% are unnecessary (39). Antibiotic selection should be based on the patient's tolerance, in addition to the nature of the disease and the pathogen. The goal of antimicrobial therapy is to choose an antibiotic that is selective for the suspected pathogen, has a low probability of causing resistance, and has no adverse reactions (16).

Adequate surveillance of HA involves data collection from various sources: information should include administrative data, demographic risk factors, patient medical history, diagnostic tests and data validation.

Surveillance led by the WHO can serve as a guide to help healthcare institutions introduce their own system of surveillance and control of HA infections. Adequate training of hospital staff is of great importance in the prevention of HA infections. Also, decontamination of the patient by treating the gut and skin has been proven to be effective.

Recently, a strong focus has been placed on the prevention and control of these types of infections, as well as on the non-negligible financial aspect.

To reduce the costs, it is important: 1) for healthcare facilities to follow strict infection control protocols, 2) to improve surveillance of HAIs, 3) to educate the staff to follow the preventive practice, and 4) to implement standard precautions such as hand hygiene practice at the bedside (40).

Conclusion

Healthcare-associated infections have a significant negative impact on hospitalized patients. They lead to permanent disability, prolong patients' length of stay and increase mortality and both expenditure and waste of medical resources. The bacterial types causing nosocomial infections differ from country to country, even among the institutions in the same region. The predominance of microorganisms depends on the hospital conditions, patients, and antibiotics prescribed by physicians. Bacteria develop effective defense mechanisms and develop resistance to the most commonly prescribed antibiotics. Thus, temporary restriction in the use of certain antibiotics is one of the solutions to reduce resistance, but also giving antibiotics only when needed. Further, it is recommended to establish a precise schedule for antibiotic use in each region based on its antibiotic resistance pattern. An increase in antimicrobial resistance requires the need for surveillance programs as important tools, which would be helpful to clinicians to choose the most appropriate antimicrobial therapy for hospitalized patients.

References

1. Kollef MH, Torres A, Shorr AF, Martin-Loeches I, Micek ST. Nosocomial infection. Criti Care Med2021; 49:169–87. [CrossRef][PubMed]
2. Monegro AF, Muppidi V, Regunath H. Hospital Acquired Infections. Treasure Island, FL: Stat Pearls Publishing 2022.[PubMed]
3. Blot S, Ruppé E, Harbarth S, Asehnoune K, Poulakou G, Luyt CE, et al. Healthcare-associated infections in adult intensive care unit patients: Changes in epidemiology, diagnosis, prevention and contributions of new technologies. Intensive Crit Care Nurs 2022;70:103227. [CrossRef][PubMed]
4. WHO. The burden of health care-associated infection worldwide. 2016 [Online] Available from: http://www.who.int/gpsc/country_ work/burden_hcai/en/
5. Vincent JL, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA 2009; 302(21): 2323-9. [CrossRef][PubMed]
6. Khan HA, Baig FK, Mehboob R. Nosocomial infections: Epidemiology, prevention, control and surveillance. Asian Pac J Trop Biomed 2017; 7(5): 478–82.[CrossRef]
7. World Health Organization. Healthcare-associated infections: fact sheet, 2014. Available at: http://www.who.int/gpsc/country_work/gpsc_ccisc_ fact_sheet_en.pdf.
8. Harbarth S, Sax H, Gastmeier P. The preventable proportion of nosocomial infections: an overview of published reports. J Hosp Infect 2003; 54(4):258-66.[CrossRef][PubMed]
9. Joshi SG, Litake GM. Acinetobacter baumannii: An emerging pathogenic threat to public health. *World J Clin Infect Dis* 2013; 3(3): 25-36. [CrossRef] [PubMed]
10. Friedrich AW. Control of hospital acquired infections and antimicrobial resistance in Europe: the way to go Wien Med Wochenschr 2019; 169(suppl1):25– 30. [CrossRef][PubMed]
11. Klevens RM, Edwards JR, Richards CL Jr. Estimating health care-associated infections and deaths in US hospitals, 2002. Public Health Rep 2007;122(2):160-6. [CrossRef][PubMed]
12. Singh PK. Antibiotics, handle with care. Geneva: WHO; 2016. [Online] Available from: http://www.searo.who.int/mediacentre/ releases/2015/antibiotics-awareness-week-2015/en/
13. WHO. Antimicrobial resistance. Geneva: WHO; 2014. [Online] Available from: http://www.searo.who.int/thailand/factsheets/ fs0023/en/
14. WHO. WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health. Geneva: WHO; 2014.
15. Aitken CJD. Nosocomial spread of viral disease. Clin Microbiol Rev 2001; 14(3): 528-46.[CrossRef][PubMed]
16. Ducel JF, Nicolle L. Prevention of hospital-acquired infections. Geneva: WHO; 2002.
17. Prevention of hospital-acquired infections. A practical guide 2nd edition. World Health Organization Department of Communicable Disease, Surveillance and Response. 2002. Available at: http://www.who.int/csr/resources/publications/whoc dscsreph200212.pdf.
18. Asokan GV, Ramdahan T, Ahmed E, Sanad H. WHO global priority pathogens list: a bibliometric analysis of medline-pubmed for knowledge mobilization to infection prevention and control practices in bahrain. Oman Med J 2019;34 (3): 184–93.[CrossRef][PubMed]
19. Christaki E, Marcou M, Tofarides A. Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. J Mol Evol 2020; 88 (1): 26–40. [CrossRef][PubMed]
20. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005; 71:388–416. [CrossRef][PubMed]
21. Centers for Disease Control and Prevention (CDC).Vital signs: Central line–associated blood stream infections – United States, 2001, 2008, and 2009. Morb Mortal Wkly Rep 2011; 60(08): 243-8. [PubMed]
22. WHO. Preventing bloodstream infections from central line venous catheters. Geneva: WHO; 2016. [Online] Available from: http:// www.who.int/patientsafety/implementation/bsi/en/
23. Wei-Chih Liao, Wei-Sheng Chung, Ying-Chieh Lo, Wen-Hsin Shih , Chia-Hui Chou, et al. Changing epidemiology and prognosis of nosocomial bloodstream infection: A singlc enter retrospective study in Taiwan Journal of Microbiology, Immunology and Infection 2022;55(6): 1293-300. [CrossRef][PubMed]
24. Diekema DJ, Hsueh PR, Mendes RE, Pfaller MA, Rolston KV, Sader HS, Jones RN. The microbiology of bloodstream infection: 20- year trends from the SENTRY antimicrobial surveillance program. Antimicrob Agents Chemother 2019;63(7):19. e00355. [CrossRef][PubMed]
25. Gastmeier P, Kampf G, Wischnewski N, Schumacher M, Daschner F, Ruden H. Importance of the surveillance method various national prevalence studies on nosocomial infections and limits of comparison. Infect Control Hosp Epidemiol 1998; 19: 661–7.[CrossRef][PubMed]
26. CDC. Urinary tract infection (catheter-associated urinary tract infection [CAUTI] and non-catheter associated urinary tract infection [UTI]) and other urinary system infection [USI]) events. Atlanta, Georgia: CDC; 2016. [Online] Available from: http:// www.cdc.gov/nhsn/pdfs/pscmanual/7psccauticurrent.pdf
27. Jain P, Parada J, David A, Smith L. Overuse of the indwelling urinary tract catheter in hospitalized medical patients. Arch Intern Med 1995; 155: 1425–9.[CrossRef][PubMed]

28. Nouri F, Karami P, Zarei O, Kosari F, Alikhani MY, Zandkarimi E, ZarandiER, Taheri M. Prevalence of Common Nosocomial Infections and Evaluation of Antibiotic Resistance Patterns in Patients with Secondary Infections in Hamadan, Iran. Infect Drug Resist 2020; 15:13:2365-74. [CrossRef][PubMed]
29. Anderson DJ. Surgical site infections. Infect Dis Clin North Am 2011; 25(1): 135-53.[CrossRef][PubMed]
30. Owens CD. Surgical site infections: epidemiology, microbiology and prevention. J Hosp Infect 2008; 70(Suppl 2): 3-10. [CrossRef][PubMed]
31. Percival SP, Suleman L, Vuotto C. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. Journal of Medical Microbiology 2015; 64: 323–34. [CrossRef][PubMed]
32. Hunter JD. Ventilator associated pneumonia. BMJ 2012; 344: 40-4. [CrossRef][PubMed]
33. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections. Am J Infect Control 1988;16:128-40. [CrossRef][PubMed]
34. Kumar AV, Pillai VS, Dinesh KR, Karim S. The phenotypic detection of Carbapenemase in meropenem resistant Acinetobacter calcoaceticus baumannii complex in a tertiary care hospital in south India. J Clin Diagn Res. 2011;5(2):223-6.
35. George P, Sequiera, A. Antimicrobial sensitivity pattern among organisms isolated from the endotracheal aspirates of patients with ventilator associated pneumonia. J Clin Diag Res 2010;4: 3397-401.
36. Steven M, Koenig JDT. Ventilator-associated pneumonia: diagnosis, treatment, and prevention. Clin Microbiol Rev 2006; 19(4): 637-57.[CrossRef][PubMed]
37. Tabak YP, Vankeepuram L, YeG, Jeffers K, Gupta V, Murray PR. Blood culture turnaround time in U.S. acute care hospitals and implications for laboratory process optimization. J. Clin. Microbiol. 2018; 56: e00500–18. [CrossRef][PubMed]
38. Joshi SG, Litake GM. Acinetobacter baumannii: An emerging pathogenic threat to public health. *World J Clin Infect Dis* 2013; 3(3): 25-36. [CrossRef] [PubMed]
39. Dowell S, Marcy S, Phllips W, Gerber M, Scwartz B. Principles of judicious use of antimicrobial agents for pediatric upper respiratory tract infections. Pediatrics 1998;101(suppl):163-84. [CrossRef]
40. Custodio HT. Medscape: hospital-acquired infections, 2014. Available at: http://emedicine.medscape.com/article/967022-overview. Accessed 12 October 2014

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INTRAHOSPITALNA I MIKROBIOLOŠKA ANALIZA NA KLINICI ZA ANESTEZIOLOGIJU I INTENZIVNU TERAPIJU TERCIJARNE ZDRAVSTVENE USTANOVE U JEDNOGODIŠNJEM PERIODU

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Naziv intrahospitalne infekcije ili nozokomijalne infekcije podrazumeva infekcije koje se javljaju kod pacijenata u zdravstvenim ustanovama a nisu bile prisutne u vreme njihovog prijema u zdravstvenu ustanovu i manifestuju se u prvih četrdeset osam sati posle prijema. Češće su u zemljama u razvoju (od 5,7% do 19,1%) nego u razvijenim zemljama (od 3,5% do 12%). Antimikrobna rezistencija predstavlja globalni problem koji nastaje kao posledica upotrebe antimikrobnih lekova. Neopravdana upotreba antibiotika dovodi do razvoja bakterijske rezistencije i promene uzročnika infekcije odgovornih za intrahospitalne infekcije. Ove vrste infekcija produžavaju dužinu bolničkog lečenja, dovode do invaliditeta i povećavaju morbiditet i mortalitet. Najčešći tipovi intrahospitalnih infekcija jesu pneumonija povezana sa upotrebom respiratora, infekcije krvi izazvane upotrebom centralnog venskog katetera, urinarne infekcije uzrokovane kateterom i infekcije hirurških rana.

Cilj ove studije bio je da se odrede najčešći bakterijski uzročnici intrahospitalnih infekcija, kao i da se prikaže distribucija uzročnika prema vrsti biološkog materijala.

Ova retrospektivna studija se sprovodila na Klinci za anesteziologiju i intenzivnu terapiju Univerzitetskog kliničkog centra u Nišu od početka do kraja 2024. godine. Od pacijenata se uzimao biološki materijal: krv, bris prepona, bris usne duplje, bris rane, bris pazuha, bris čmara, urin, endotrahealni aspirat, bris nosa, obični aspirat, sadržaj drena, saržaj trbušne duplje, vrh centralnog venskog katetra, vrh drena, bronhoaspirat i drugo. Sav biološki materijal poslat je u mikrobiološku laboratoriju radi dobijanja biograma i antibiograma.

Pokazalo se da je ukupan broj materijala bio 1464: 232 (15,85%) uzorka krvi, 179 (12,23%) briseva prepona, 166 (11,34%) briseva usne duplje, 132 (9,02%) brisa rane, 122 (8,33%) brisa čmara, 108 (7,38%) briseva pazuha, 106 (7,24%) uzoraka urina, 94 (6,42%) endotrahealna aspirata, 63 (4,30%) brisa nosa, 56 (3,83%) aspirata, 43 (2,94%) sadržaja drenova, 15 (1,02%) sadržaja trbušne duplje, 15 (1,02%) vrhova centralnih venskih katetera, 11 (0,75%) vrhova drena, 10 (0,68%) aspirata iz bronhija i 112 (7,65%) ostalih uzoraka.

Distribucija bakterija prema vrsti bila je sledeća: *Klebsiella* sp. 20,15%, *Enterooccus faecalis* 19,26%, *Acinetobacter* sp. 14,41%, *Pseudomonas aeruginosa* 7,9%, *Escherichia coli* 7,17%, *Staphylococcus epidermidis* 4,99%, *Proteus mirabilis* 3,96%, *Enterococcus faecium* 2,94%, *Enetrobacter* sp. 2,94% i *Staphylococcus* sp. 2,19%. *Klebsiella* sp. je bila dominantno prisutna u brisu usne duplje (3,28%), *Enterococcus faecium* u brisu prepona (5,60%), *Acinetobacter* sp. u endotrahealnom aspiratu (1,91%), *Pseudomonas aeruginosa* u brisu rane (2,25%), a *Escherchia coli* u brisu čmara (1,84%).

Intrahospitalne infekcije izuzetno loše utiču na hospitalizovane pacijente. Dovode do dugotrajnog invaliditeta, produženog vremena boravka u bolnici i lečenja, povećanja smrtnosti, potrošnje medicinskih resursa i troškova lečenja. Uzročnici ovih infekcija zavise kako od bolničkih uslova, tako i od samih pacijenata i antibiotika koje prepisuju lekari. Trebalo bi da postoje smernice za davanje antibiotika u svakom regionu u skladu sa najčešćim uzročnicima i samom rezistencijom na antibiotike, budući da bi to pomoglo zdravstvenim radnicima pri odabiru najadekvatnije antimikrobne terapije za hospitalizovane pacijente.

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***Ključne reči**: intrahospitalne infekcije, nozokomijalne infekcije, bakterije, antibiotik*

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PERFORMANCE ASSESSMENT OF THREE PHENOTYPIC TESTS FOR CARBAPENEMASE DETECTION IN *ENTEROBACTERALES*

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Carbapenemase-producing *Enterobacterales* (CPE) pose a significant threat in hospital settings, especially in intensive care units, from both therapeutic and epidemiological perspectives. Rapid identification is crucial. This study aimed to evaluate the three phenotypic methods used in routine diagnostics. The study included 56 clinical isolates of carbapenem-resistant *Enterobacterales* collected from patients hospitalized at the University Clinical Center (UCC) in Niš. Among these isolates, 52 were confirmed as carbapenemase producers, while four lacked carbapenemase genes. Genotypic detection was performed using multiplex polymerase chain reaction targeting the *bla*KPC genes encoding *Klebsiella pneumoniae* carbapenemase, *blaVIM gene* encoding Verona integron metallo-beta-lactamase (VIM), *bla*NDM gene encoding New Delhi metallo-beta-lactamase, and *bla*OXA-48 genes encoding Oxacillinase-48 carbapenemase. The evaluated phenotypic methods included the NG-Test Carba 5, the RAPIDEC Carba NP test (RCNP), and a commercial combination disk test (CDT)—KPC, MBL, and OXA-48 Confirm Kit: Carbapenemases. Multiplex PCR revealed: 2 KPC producers; 24 NDM producers; 16 OXA-48-like producers; 10 isolates producing both NDM and OXA-48 enzymes. One isolate of *Enterobacter cloacae* was identified as a co-producer of NDM, KPC, and OXA-48 enzymes, and one isolate of *Klebsiella pneumoniae* was identified as a co-producer of NDM and KPC enzymes. The sensitivity and specificity of the NG-Test Carba 5 were 98.08% and 100.00%, respectively. In the Carba NP test, after 120 minutes, sensitivity and specificity were 90.38% and 100%, respectively. For the CDT method, the sensitivity and specificity for detecting metallo-β-lactamases using dipicolinic acid were 80.56% and 100%, respectively, while for detecting class D carbapenemases using temocillin, they were 95.65% and 100%, respectively. The best results in detecting specific classes of carbapenemases were achieved with the NG-Test Carba 5 and the CDT method. These methods could be employed for rapid and reliable detection of carbapenemases in routine diagnostics.

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Key words: *carbapenemase production, Enterobacterales, carbapenemase detection, phenotypic methods*

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Introduction

Carbapenemase-producing *Enterobacterales* (CPE) cause infections with limited treatment options and are associated with high morbidity and mortality, particularly in patients in intensive care units. The emergence of antibiotic resistance is often due to natural factors; however, the primary contributor to this issue is the misuse of antimicrobial agents. The presence of such strains in hospital settings, especially in intensive care units, poses a significant threat not only from a clinical perspective but also from an epidemiological standpoint, where as the spread of carbapenemases and potential outbreaks could lead to major health problems for hospitalized patients (1).

Carbapenem resistance in *Enterobacterales* involves two types of mechanisms. The first nec banisn, which is less significant, is based on the production of ESBL and AmpC enzymes combined with efflux mechanisms and alterations in porin channels. The other, much more significant, is the production of carbapenemases. This mechanism results not only in high levels of resistance but

also enables the rapid spread and colonization of these enzyme-producing strains in hospital settings. According to Ambler's classification, carbapenemases are divided into three major classes: Ambler class A (KPC), class B (metallo-β-lactamases—VIM, NDM, and IMP), and class D (OXA-48-like) (2). Considering the significance of carbapenemase production, the rapid detection of CPE is crucial for infection control, preventing hospital outbreaks, and optimizing antibiotic therapy for the infections they cause. Although PCR is regarded as the reference method for carbapenemase detection, phenotypic tests are much more suitable for routine use (3).

There are several phenotypic tests for detecting carbapenemase production, including biochemical (colorimetric) tests (e.g., Carba NP test), modified Hodge test (MHT), carbapenem inactivation method (mCIM) (4), rapid multiplex immunochromatographic assay (e.g. NG-Test Carba 5) (5), MIC MBL test (6), combined disc test (CDT) (7), synergistic tests—boronic acid, and ethylenediaminetetraacetic acid (EDTA) synergy tests (8). For detecting carbapenemase in *Enterobacterales*, especially for infection control purposes and public health purposes, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) proposed the use of combined disk test (CDT), Carba NP test, mCIM assays, Carbapenem Inactivation Method, detection of carbapenem hydrolysis with matrix-assisted laser desorption/ionization–time-of-flight (MALDI–TOF), and lateral flow assays (9). Our study compared the performance of three selected methods for detecting carbapenemase production in *Enterobacterales* with reduced susceptibility to carbapenems.

Materials and Methods

Bacterial Isolates

Identification and Selection

The study was conducted at the Microbiology Center of the Institute of Public Health in Niš. The study included 56 primary isolates of carbapenem-resistant *Enterobacterales* (CRE) obtained from samples of patients hospitalized at UCC Niš. Bacterial isolates were isolated and identified using standard bacteriological methods. Species-level identification and carbapenem susceptibility testing were performed using the automated VITEK®2 Compact system (BioMerieux, Marcyl'Etoile, France). Among the tested isolates, *Klebsiella pneumoniae* (*K. pneumoniae*) was the most prevalent (34 isolates), followed by *Enterobacter cloacae* (*E. cloacae*) (11 isolates), *Serratia marcescens (S. marcescens)* (3 isolates), *Enterobacter aerogenes* (*E. aerogenes*) (3 isolates), *Citrobacter freundii* (*C. freundii*) (2 isolates), and one isolate each of *Escherichia coli* (*E. coli*), *Morganella morganii* (*M. morganii*), and *Proteus mirabilis* (*P. mirabilis*).

Multiplex PCR was used to detect the *bla Klebsiella Pneumoniae carbapenemase* (KPC), *bla* Verona Integron-encoded Metallo-beta-lactamase (VIM), *bla* New Delhi metallo-beta-lactamase (NDM), and *bla*OXA-48 genes. This analysis revealed: 24 NDM producers (*10 K. pneumoniae, 8 E. cloacae, 2 C. freundii, 1 E. coli, 1 S. marcescens, 1 M. morganii,* and 1 *E. aerogenes*), 16 OXA-48-like producers (all *K. pneumoniae*), and 10 isolates producing both NDM and OXA-48 enzymes (7 *K. pneumoniae*, 2 *E. cloacae*, and 1 *E. aerogenes*). Additionally, one isolates of *E. cloacae* was identified as a co-producer of NDM, KPC, and OXA-48 enzymes, and one isolate of *K. pneumoniae* was identified as a co-producer of NDM and KPC enzymes. In the folloming isolates carbapenemase production was not confirmed: *S. marcescens* (2), *P. mirabilis* (1), and *E. aerogenes* (1). *K. pneumoniae* BAA 1705 (KPC-2), *K. pneumoniae* NCTC 13443 (NDM-1), *K. pneumoniae* NCTC 13440 (VIM), and *K. pneumoniae* NCTC 13442 (OXA-48) were used as positive controls, and *E. coli* ATCC 25922 was used as a negative control.

Phenotypic Methods for Detecting Carbapenemase Production

Combined Disk Test

The KPC, MBL, and OXA-48 Confirm Kit: Carbapenemases (Rosco Diagnostica, Denmark) was used as the combined disk test. A 10 µg meropenem disk was placed 30 mm apart from disks containing meropenem/dipicolinic acid (MBL inhibitor), meropenem/boronic acid (KPC inhibitor), meropenem/cloxacillin (AmpC inhibitor), and temocillin. After 24-hour incubation at 35 °C, the inhibition zones for each tested disk were measured. The CDT test interpretation was performed according to the manufacturer's instructions. For temocillin, isolates were considered positive if resistance to temocillin (≤ 11 mm) was detected, provided there was no difference greater than 3 mm in the inhibition zones between meropenem alone and its combination with dipicolinic acid (DPA), cloxacillin, and boronic acid. In the CDT test with EDTA, a ≥ 7 mm difference in the inhibition zone between imipenem and imipenem-EDTA (10 µg/750 µg) was considered a positive result.

Representation of the combined disk test for detecting carbapenemase production is shown in Figure 1.

Colorimetric Test—RAPIDEC Carba NP (RCNP)

The colorimetric test for carbapenemase detection is a classic acidimetric assay with a colorimetric endpoint, where the phenol red indicator turns yellow upon carbapenem hydrolysis. For this purpose, the commercial RAPIDEC® CARBA NP test (bioMérieux, France)

was employed. This test is designed for the rapid detection of carbapenemases in Gram-negative bacteria, including Enterobacterales, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii*. It is based on detecting the hydrolysis of the β-lactam ring in the imipenem molecule. The hydrolysis leads to acidification of the medium, which causes a visible color change in the pH indicator (phenol red). Results were interpreted after 30 minutes and again after 2 hours; the absence of a color change after 2 hours was considered a negative result. Figure 2 illustrates the colorimetric test for carbapenemase detection.

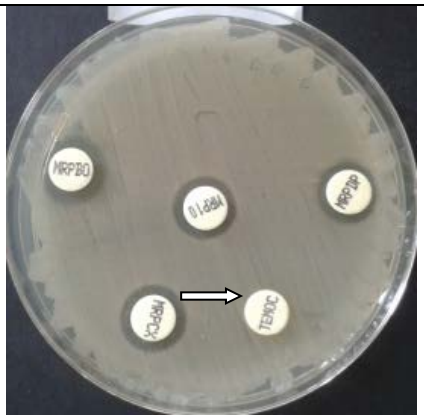
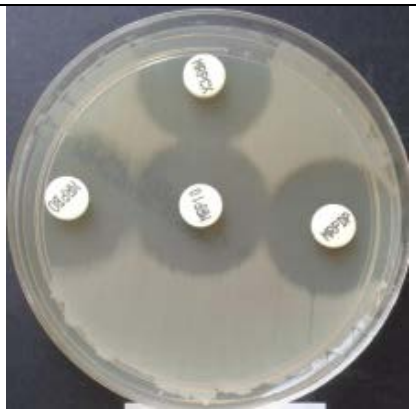
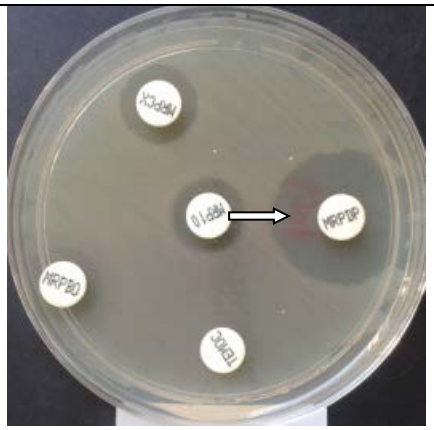
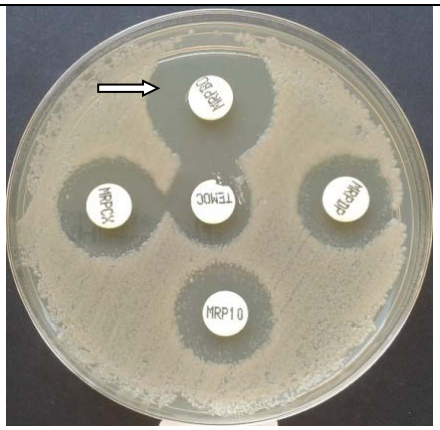
<table>
<tr><td>  </td><td>  </td></tr>
<tr><td>OXA-48 positive isolate—No inhibition zone around the temocillin disc ([5]TEMOC), and no difference in the inhibition zone between [1]MRP 10 and the combinations [2]MRP BO, [3]MRP DP, and [4]MRP CX</td><td>Carbapenemase-negative isolate—No differences in the inhibition zone between MRP 10 and the combinations [2]MRP BO, [3]MRP DP, and [4]MRP CX</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>NDM-positive isolate—Difference in the inhibition zone between [1]MRP10 and [3]MRP DP > 5 mm</td><td>KPC-positive isolate—Difference in the inhibition zone between [1]MRP10 and [2]MRP BO > 5 mm</td></tr>
</table>

Figure 1. Representation of the combined disk test for detecting carbapenemase production

[1]MRP 10—meropenem, [2]MRP BO—meropenem-boronic acid, [3]MRP DP—meropenem-dipicolinic acid, [4]MRP CX—meropenem-cloxacillin, [5]TEMOC—temocillin

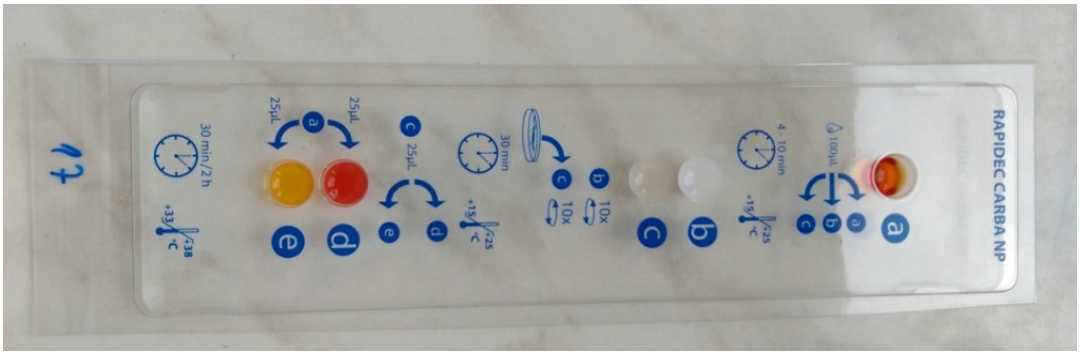


Figure 2. Positive result of the RAPIDEC Carba NP test

***In Vitro Multiplex Immunoassay*—NG-Test CARBA 5**

NG-Test CARBA 5 Protocol Description

A 1-µL loop was used to collect three bacterial colonies, which were then suspended in a 1.5 ml microcentrifuge tube prefilled with five drops of extraction buffer, as provided by the manufacturer. Following a brief vortexing step, 100 µL of the resulting suspension was transferred into the sample well (S) of the test cassette using the manufacturer-supplied disposable transfer pipette. After 15 minutes, the cassette was visually examined for the appearance of control and test lines. Figure 3 shows a positive test for different types of carbapenemases.

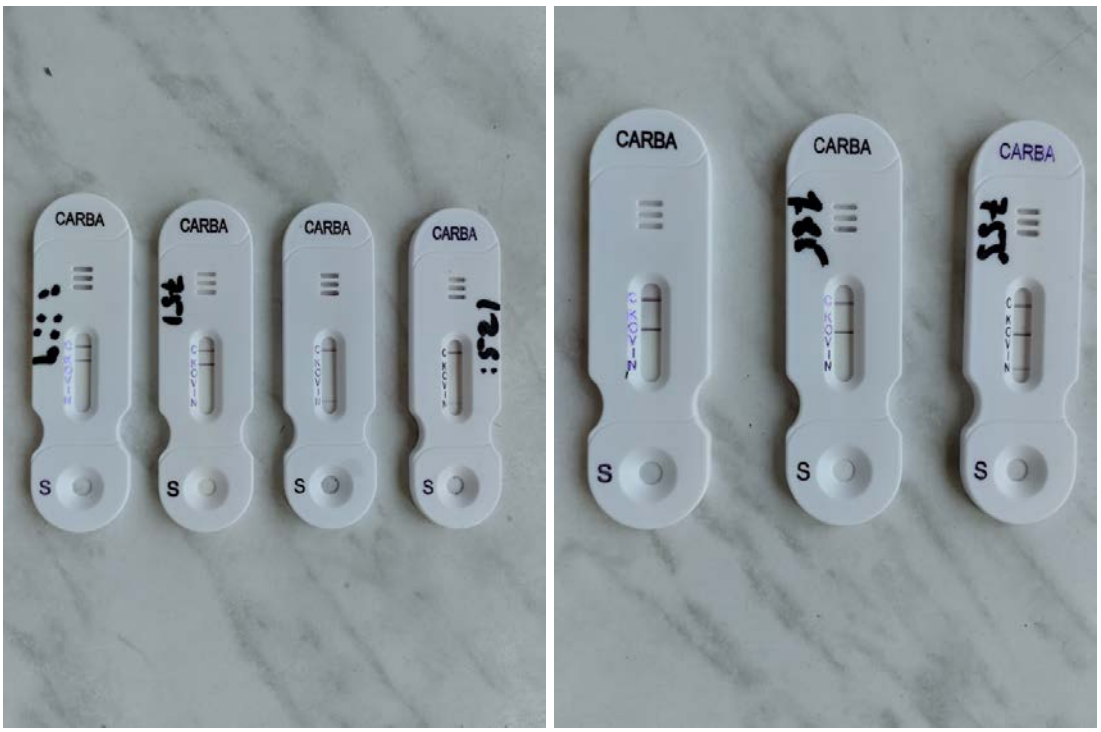


Figure 3. Positive NG-Test CARBA 5 test for different types of carbapenemases

*C-control line; K-KPC; O-OXA-48; V-VIM; I-IMP; N-NDM

Statistical Data Analysis

The validity of phenotypic tests was assessed and expressed in terms of sensitivity and specificity, using PCR-based detection of resistance genes as the gold standard. Sensitivity (the proportion of carbapenemase-producing isolates correctly identified) and specificity (the proportion of carbapenemase-negative isolates correctly distinguished) were determined for each method used, as well as positive predictive value (PPV) and negative predictive value (NPV) (10).

The data are presented according to the principles of descriptive statistics, and hypothesis testing was conducted using appropriate tests within the MedCalc statistical software at https://www.medcalc.org/calc/diagnostic_test.php.

Results

The susceptibility of carbapenemase-producing *Enterobacterales* isolates to antimicrobial agents was determined using the VITEK®2 method. The carbapenem susceptibility of carbapenemase-producing isolates, as well as positive and negative controls, is presented in Table 1. The isolates were grouped according to bacterial species and resistance genes. The susceptibility of carbapenemase-negative isolates is shown in Table 2.

Table 1. Carbapenem susceptibility of carbapenemase-producing isolates, positive and negative controls

Species name	Gene of resistance	Number of isolates	Ertapenem MIC (µg/ml)	Imipenem MIC (µg/ml)	Meropenem MIC (µg/ml)
K. pneumoniae	*bla*$_{NDM}$	10	4– ≥ 8	0.25– ≥ 16	1– ≥ 16
	bla$_{NDM}$/*bla*$_{OXA-48}$	7	4– ≥ 8	0.25– ≥ 16	1– ≥ 16
	bla$_{OXA-48}$	16	4– ≥8	0.25– ≥16	1– ≥ 16
	bla$_{KPC}$/*bla*$_{NDM}$	1	≥ 8	≥ 16	≥ 16
E. aerogenes	*bla*$_{NDM}$	1	4– ≥ 8	0.25– ≥ 16	1– ≥ 16
	bla$_{NDM}$/*bla*$_{OXA-48}$	1	4– ≥ 8	0.25– ≥ 16	1– ≥ 16
E. cloacae	*bla*$_{NDM}$	8	4– ≥ 8	0.25– ≥ 16	1– ≥ 16
	bla$_{NDM}$/*bla*$_{OXA-48}$	2	≥ 8	≥ 16	≥ 16
	bla$_{KPC}$/*bla*$_{NDM}$/*bla*$_{OXA-48}$	1	≥ 8	≥ 16	≥ 16
Citrobacter freundii	*bla*$_{NDM}$	2	≥ 8	≥ 16	≥ 16
S. marcescens		1	≥ 8	0.5	1.0
M. morganii	*bla*$_{NDM}$	1	1.0	1.0	4.0
E. coli	*bla*$_{NDM}$	1	≥ 8	≥ 16	≥ 16
Positive controls					
K. pneumoniae NCTC 13440	*bla*$_{VIM}$		≥ 8	≥ 16	≥ 16
K. pneumoniae NCTC 13443	*bla*$_{NDM}$		≥ 8	≥ 16	≥ 16
K. pneumoniae BAA 1705	*bla*$_{KPC}$		≥ 8	≥ 16	≥ 16
K. pneumoniae NCTC 13442	*bla*$_{OXA-48}$		≥ 8	≥ 16	≥ 16
Negative controls					
E. coli ATCC 25922	-		≤ 0.125	≤ 0.25	≤ 0.25
E. coli ATCC 35218	*bla*$_{TEM-1}$		≤ 0.125	≤ 0.25	≤ 0.25

Table 2. Carbapenem susceptibility of carbapenemase-negative isolates

Species name	Enzyme (phenotypic)	Number of isolates	Ertapenem MIC (μg/ml)	Imipenem MIC (μg/ml)	Meropenem MIC (μg/ml)
E. aerogenes	ESBL	1	0.5	2.0	0.5
S. marcescens	ESBL	1	≥ 8	0.5	1.0
S. marcescens	AmpC	1	0.5	1.0	0.25
P. mirabilis	ESBL	1	0.5	3.0	0.25

The RAPIDEC® CARBA NP test was used for colorimetric detection of carbapenemases. Out of 56 tested isolates, at the first reading (30 minutes), the test was positive for 32 carbapenemase-producing isolates, negative for 20 carbapenemase-producing isolates, and negative for all four carbapenemase-negative isolates. The sensitivity and specificity of the test were 61.54% and 100.00%, respectively. After the second reading (120 minutes), the test was positive for 47 carbapenemase-producing isolates, negative for all four carbapenemase-negative isolates (no false-positive results), and false-negative for five isolates. Among the 20 false-negative isolates from the first reading (30 minutes), 15 isolates tested positive after 120 minutes: three NDM-positive *K. pneumoniae* isolates, ten OXA-48/NDM-positive *K. pneumoniae* isolates (mucoid strains), one OXA-48/NDM-positive *K. pneumoniae* isolate, and one NDM-positive *K. pneumoniae* isolate. The last two isolates had MIC values of ertapenem/meropenem/imipenem at 4/0.25/1 μg/mL and NDM 8/0.25/1 μg/mL (respectively). False-negative results were most commonly observed in OXA-48/NDM-positive mucoid *K. pneumoniae* isolates. Thus, after 120 minutes, the test's sensitivity and specificity increased to 90.38% and 100.00%, respectively. The results for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Carba NP test are presented in Table 3.

The results of the false-negative tests observed in five isolates after 120 minutes are presented in Table 4.

Table 3. Carba NP test results

Procedure	Number of isolates after 30 min of incubation	Number of isolates after 120 min of incubation
True-positive	32	47
True-negative	4	4
False-positive	0	0
False-negative	20	5
Sensitivity	61.54%	90.38%
Specificity	100.00%	100.00%
PPV	100.00%	100.00%
NPV	16.67%	44.44%

Table 4. Composition of the five carbapenemase-positive isolates that tested negative after 120 minutes

No. of isolates	Species name	Carbapenemase (PCR)	Results
3	*K. pneumoniae*	OXA 48	-
1	*K. pneumoniae*	OXA 48/NDM	-
1	*S. marcescens*	NDM	-

Carbapenemase production was evaluated using the CDT method with the commercial KPC/Metallo-beta-lactamase and OXA-48 Confirm Kit from Rosco Diagnostica. For the detection of carbapenemases using this test, simultaneous testing was performed using a meropenem disk along with disks containing meropenem/dipicolinic acid, meropenem/boronic acid, meropenem/cloxacillin, and temocillin. The combined disk test is interpreted based on the difference in the inhibition zone around the carbapenem disk and the disk containing both the carbapenem and the enzyme inhibitor. An increase in the inhibition zone around the combined carbapenem/inhibitor disk compared to the carbapenem disk alone, exceeding the defined threshold values, indicates a positive result.

For carbapenemase-positive bacteria that produce only NDM enzymes, the combined disk test using EDTA was negative in only 2 of 24 isolates (one *M. morganii* isolate and one *K. pneumoniae* isolate), and positive in 22 of 24 isolates. The combined disk test using DPA yielded the same results, with 2 negatives of 24 isolates (one *E. cloacae* isolate and one *K. pneumoniae* isolate), and 22 of 24 isolates testing positive. The combined disk test with boronic acid was positive in eight OXA-48-positive isolates and two NDM-positive isolates. Among the OXA-48-positive isolates, none produced a positive result with either the CDT-BA or CDT DPA tests, and all were resistant to temocillin. Both the CDT cloxacillin and the boronic acid test were positive in only one NDM isolate, indicating co-production of AmpC.

The combined disk test using DPA was positive in 7 of 12 isolates, while the EDTA-based test was positive in 6 of 12 isolates. The combined disk test with boronic acid was negative in both KPC-positive isolates. According to the manufacturer's instructions, the temocillin test could only be interpreted as positive in three OXA-48/NDM-positive *K. pneumoniae* isolates; for the other isolates, interpretation was not possible due to the positive results in the DPA and boronic acid tests. The combined disk test with cloxacillin was negative for all tested isolates.

The combined disk tests using DPA/EDTA and the temocillin test were negative for all carbapenemase-negative isolates. The CDT test with boronic acid and cloxacillin, which serve as indicators of AmpC enzyme production, was positive in one *Serratia marcescens* isolate, this was also identified by VITEK®2 AES as a potential AmpC producer.

The NG-Test CARBA 5 was positive in all isolates of carbapenemase-producing bacteria that produced only NDM and OXA-80 enzymes (*K. pneumoniae* isolates).

For carbapenemase-positive bacteria that produce multiple enzyme types, NG-Test CARBA 5 was negative in only 1 of 12 isolates (two *K. pneumoniae* isolates) and positive in 11 of 12 isolates. In *K. pneumoniae* that produced all three enzymes, the test detected only the NDM enzyme.

The results for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are presented in Table 5.

The technical characteristics of the tests, including complexity and execution time, the number of steps required, time to result, and whether the test identifies specific carbapenemase classes or only detects their presence, are presented in Table 6.

Table 5. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of RAPIDEC Carba NP (RCNP), NG-Test CARBA 5, Rosco Combined Disk Test (RCDT) for Carbapenemase Production, and Combined Disk Test

Phenotypic test	Detection of carbapenemase	Sensitivity	Specificity	PPV	NPV
RCNP[1] after 120 min	Carbapenemase	90.38%	100.00%	100.00%	44.44%
NG-Test CARBA 5	KPC, NDM, OXA-48-like	98.08%	100.00%	100.00%	80.00%
RCDT[2] Temocilin	OXA-48	95.65%	100.00%	100.00%	83.33%
RCDT[2] Boronic acid	Class A	0.00%	77.78%	0.00%	95.45%
RCDT[2] DPA	Class B (MBL)	80.56%	100.00%	100.00%	74.07%
CDT[3] EDTA	Class B (MBL)	80.56%	100.00%	100.00%	74.07%

[1]RAPIDEC Carba NP [2]Rosco Combined Disk Test for Carbapenemase Production [3]Combined Disk Test

Table 6. The technical characteristics of the phenotypic tests

Characteristics	Phenotypic test		
	CDT	RCNP	NG-Test Carba 5
Validated isolate set	*Enterobacterales*		
	KPC, Class B (MBL), OXA-48-like	Carbapenemase	KPC, NDM, OXA-48-like
Maximum incubation time (hours)—Time to result readout	18–24	2	0.25
Total operator time (minutes)	5	5	3
Number of steps required	4	3	3
Operational simplicity	Easy, training required	Easy, little training required	Very easy, no training required

Discussion

Global dissemination of carbapenemase-producing *Enterobacterales* necessitates rapid and efficient detection methods in routine laboratory practice (11, 12). Monitoring and detecting these strains in hospital settings is essential not only for controlling hospital-acquired infections but also for tailoring individual therapeutic strategies for infections caused by carbapenemase-producing strains (13). The emergence of CRE requires that all *Enterobacterales* isolates exhibiting reduced susceptibility to one or more carbapenems be tested using efficient methods available in routine practice.

Rapid detection of carbapenemase production in *Enterobacterales* is crucial for preventing the spread of these strains, especially in hospital settings. Although PCR is regarded as the reference method for detecting carbapenemases, many authors report phenotypic methods as reliable and accessible means of detecting these enzymes.

A wide range of tests for detecting the production of carbapenemases is used in both routine diagnostics and epidemiological studies. In this research, isolates that are potentially carbapenemase producers—considered the most clinically and epidemiologically significant—were selected based on the threshold values provided by the European Committee on Antimicrobial Susceptibility Testing EUCAST (9). After selection, three phenotypic methods were used for the detection of carbapenemases and comparison with Multiplex PCR.

Detection of carbapenemases using the combined disk test (CDT) has been reported by many authors to provide satisfactory results, in terms of sensitivity and specificity (14, 15).

In the current study, the CDT demonstrated good performance in detecting MBL enzymes, with a sensitivity and specificity of 80.56% and 100%, and positive predictive value (PPV) and negative predictive value (NPV) of 100% and 74.07% when using DPA as the inhibitor. Identical results were obtained with the CDT using EDTA as the inhibitor. Solgi et al. reported sensitivity and specificity values of 82.61% and 96.22% for the CDT DPA test (16). These findings are consistent with previous reports (12).

Regarding the detection of KPC enzymes, the results showed low values. The combined disk test with boronic acid was positive in eight OXA-48-positive isolates and two NDM-positive isolates, but in both KPC producers, the test was negative. Sensitivity and specificity were 0% and 77.78%, and the PPV and NPV were 0% and 95.45%. It should be noted that the number of isolates evaluated was small (2), and both isolates were co-producers of NDM or OXA-48 enzymes. Certainly, the small number of KPC-producing organisms in our study limits our ability to draw robust conclusions from the data. Our findings do not align with those of Dijk et al. (7), who demonstrated that the PBA test effectively detects carbapenemase production in CRE isolates, with sensitivity and specificity rates of 95% and 99%, respectively.

Our sensitivity and specificity results indicate the good performance of temocillin in detecting OXA-48 enzymes (95.65% and 100%, respectively). It is crucial to note that the inhibition zone around the temocillin disk should only be considered valid when there is no difference in the inhibition zones between meropenem alone and meropenem combined with class A and MBL inhibitors. The sensitivity of this method has also been confirmed by other authors (17). The CDT, as currently designed, has been assessed in multiple studies, demonstrating high sensitivity ranging from 90% to 100%, depending on the carbapenemase type, and a specificity of 92% to 93% (7,18). Bartolini et al. reported 100% sensitivity and 100% specificity for CDT (19). However, this author, along with others (20, 21), highlights challenges in detecting isolates that produce multiple types of carbapenemases. In our study, the combined disk test using DPA was positive in 7 of 12

isolates, while the EDTA-based test was positive in 6 out of 12 isolates.

The sensitivity and specificity of the RAPIDEC® CARBA NP (RCNP) test in our study increased to 90.38% and 100.00%, PPV 100% and NPV 44.44% after 120 minutes, compared to the readings at 30 minutes. Noel et al. reported the sensitivity and specificity of 91.9% and 83.9% for the RCNP test (22). In Alizadeh et al. study, the sensitivity and specificity of the Carba NP test were 98% and 95%, respectively (23). In newer research, the RCNP test showed an overall sensitivity, specificity, PPV, NPV, and accuracy of 69.3%, 100%, 100%, 6.9%, respectively (24). Our research indicated that false-negative isolates were most frequently observed in OXA-48/NDM-positive mucoid isolates of *K. pneumoniae*. Similar findings, particularly regarding the detection of OXA-48 producers, have been reported in other studies (25, 26).

In the present study, the sensitivity and specificity of the NG-Test CARBA 5 were 98.08% and 100.00%, respectively, while the PPV and NPV were and 100.00% and 80.00% respectively. In a study by Hopkins et al., the overall sensitivity and specificity of the NG-Test CARBA 5 were 97.31% (95% CI 93.84–99.12%) and 99.75% (95% CI 99.12–99.97%), respectively (27). Saito et al. state that the NG-Test CARBA 5 demonstrated a sensitivity of 99.1% (106 of 107 strains of the five most common carbapenemase producers) and a specificity of 100% for *Enterobacterales* strains (28). The same study shows false-negative results for IMP producers, which has also been reported by other authors (29).

Regarding the strains with multiple carbapenemase genes, the NG-Test CARBA 5 successfully identified all these carbapenemases. However, the sensitivity and specificity of each method varied for different kinds of carbapenemases.

All three tests significantly reduced the turnaround time to under two hours, enabling direct identification of carbapenemases from clinical samples. For *Klebsiella* spp., the accuracy of the NG-Test CARBA 5 was 96.82% (5).

Study Limitations

The main limitation of this study is the relatively small number of isolates in which carbapenemases were detected using the reference PCR method. However, the Microbiology Center of the Institute of Public Health regularly monitors the occurrence of carbapenemases using phenotypic methods.

The types and distribution of specific carbapenemases correspond to the group of isolates defined by molecular methods. More extensive research, including a larger number of isolates and both genotypic and phenotypic methods for carbapenemase detection, is necessary.

Conclusion

Detection and differentiation of carbapenemases are no longer solely important for epidemiological surveillance and infection control but play a crucial role in selecting appropriate therapy and implementing antimicrobial stewardship strategies, especially considering the availability of novel antimicrobial agents targeting specific carbapenemases.

This study demonstrated that, based on their performance, available phenotypic tests can serve as useful methods for detecting carbapenemases in carbapenem-resistant *Enterobacterales* in routine practice. Although the RCNP test is simple to perform and provides results within two hours, it does not differentiate between different types of carbapenemases. Since susceptibility to newer antimicrobial agents is directly related to the enzyme type, it is essential for phenotypic methods to identify the specific carbapenemase present.

For this reason, the RCDT method represents a relatively inexpensive option that requires neither specialized training nor equipment, offers good performance, and can classify different carbapenemase classes. However, its main drawback is the longer turnaround time of 18–24 hours. This method is suitable for epidemiological surveillance and use during hospital outbreaks.

Finally, the NG-Test CARBA 5 demonstrated excellent accuracy in detecting carbapenemase-producing strains, with high sensitivity and specificity. The test is extremely simple, requires no special equipment or personnel training, and differentiates between five different carbapenemase types.

Phenotypic methods have limitations related to the types of carbapenemases they can detect, the challenge of identifying strains that produce multiple carbapenemases, and the varying distribution of specific enzymes across different geographical regions. Perhaps the most effective approach in selecting an appropriate test would be to conduct molecular screening in a given region, to determine the presence of specific carbapenemases, and then choose the most reliable test for those predominant enzymes.

Based on our molecular data indicating the predominance of NDM and OXA-48 enzymes, RCDT would be a suitable method for epidemiological studies, whereas the NG-Test CARBA 5 would be ideal for the rapid detection of carbapenemase producers, particularly in clinical samples requiring urgent processing (e.g., blood cultures, cerebrospinal fluid). An important future development would be refining this test to enable the direct detection of carbapenemases from patient samples (e.g., blood, urine).

Ultimately, continuous surveillance of the presence and spread of carbapenemases in hospital environments is essential for epidemiological monitoring, infection control, and the implementation of effective antimicrobial therapy.

References

1. Centers for Desease Control and Prevention. Carbapenem-resistant Enterobacterales (CRE): An urgent public health threat. Available from: URL:https://arpsp.cdc.gov/story/cre-urgent-public-health-threat
2. Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. Clin Microbiol Rev 2007; 20(3):440–458. [CrossRef] [PubMed]
3. Moloney E, Lee KW, Craig DA. Joy Allen AJ, Graziadio S, Power M et al. A PCR-based diagnostic testing strategy to identify carbapenemase-producing *Enterobacterales* carriers upon admission to UK hospitals: early economic modelling to assess costs and consequences. Diagn Progn 2019;3(8). [CrossRef] [PubMed]
4. Kumudunie WGM, Wijesooriya LI, Wijayasinghe YS. Comparison of four low-cost carbapenemase detection tests and a proposal of an algorithm for early detection of carbapenemase-producing *Enterobacterales* in resource-limited settings. PLoS One 2021;16(1):e0245290. [CrossRef] [PubMed]
5. Gu D, Yan Z, Cai C, Li J, Zhang Y, Wu Y et al. Comparison of the NG-Test Carba 5, Colloidal Gold Immunoassay (CGI) Test, and Xpert Carba-R for the Rapid Detection of Carbapenemases in Carbapenemase-Producing Organisms. Antibiotics 2023; 2;12(2):300. [CrossRef] [PubMed]
6. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H. Evaluation of different laboratory tests for the detection of metallo-β-lactamase production in *Enterobacterales*. J Antimicrob Chemoth 2008; 61(3):548–53. [CrossRef] [PubMed]
7. van Dijk K, Voets GM, Scharringa J, Voskuil S, Fluit AC, Rottier WC et al. A disc diffusion assay for detection of class A, B and OXA-48 carbapenemases in *Enterobacterales* using phenyl boronic acid, dipicolinic acid and temocillin. Clin Microbiol Infect 2014; 20(4):345-9. [CrossRef] [PubMed]
8. Josa MD, Leal R, Rojas J, Torres MI, Cortés-Muñoz F, Esparza G et al. Comparative Evaluation of Phenotypic Synergy Tests versus RESIST-4 O.K.N.V. and NG Test Carba 5 Lateral Flow Immunoassays for the Detection and Differentiation of Carbapenemases in *Enterobacterales* and *Pseudomonas aeruginosa*. Microbiol Spectr 2022;10(1):e0108021. [CrossRef] [PubMed]
9. The European Committee on Antimicrobial Susceptibility Testing - EUCAST. Resistance mechanisms. Available from: URL:https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/ Resistance_mechanisms)
10. Ilstrup DM. Statistical methods in microbiology. Clin Microbiol Rev 1990; 3(3):219–26. [CrossRef] [PubMed]
11. Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA. Carbapenemase-Producing Organisms: A Global Scourge. Clin Infect Dis 2018; 66(8):1290-7. [CrossRef] [PubMed]
12. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasevic AT, et al; European Survey of Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) Working Group. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE): a prospective, multinational study. Lancet Infect Dis 2017;17(2):153-63. [CrossRef] [PubMed]
13. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant *Enterobacteriaceae*: the impact and evolution of a global menace. J Infect Dis 2017; 215(1): S28–36. [CrossRef] [PubMed]
14. Giske CG, Gezelius L, Samuelsen Ø, Warner M, Sundsfjord A, Woodford N. A sensitive and specific phenotypic assay for detection of metallo-beta-lactamases and KPC in *Klebsiella pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. Clin Microbiol Infect 2011; 17(4):552–6. [CrossRef] [PubMed].
15. Tsakris A, Kristo I, Poulou A, Themeli-Digalaki K, Ikonomidis A, Petropoulou D, et al. Evaluation of boronic acid disk tests for differentiating KPC possessing *Klebsiella pneumoniae* isolates in the clinical laboratory. J Clin Microbiol 2009; 47(2): 362–7. [CrossRef] [PubMed]
16. Solgi H, Badamchi A, Shahcheraghi F, Badmasti F, Akbari M, Behzadfar M. A comparative evaluation of five phenotypic methods for identification of carbapenemase-producing *Enterobacteriaceae*: a modified carbapenemase detection test. Microbiol Spectr 2024; 12:e0038624. [CrossRef] [PubMed]
17. Sattler J, Brunke A, Hamprecht A. Systematic Comparison of Three Commercially Available Combination Disc Tests and the Zinc-Supplemented Carbapenem Inactivation Method (zCIM) for Carbapenemase Detection in *Enterobacterales* Isolates. J Clin Microbiol 2021; 59(9):e0314020. [CrossRef] [PubMed]
18. *Pantel A, Souzy D, Sotto A, Lavigne J-P. Evaluation of two phenotypic screening tests for carbapenemase-producing Enterobacteriaceae. J Clin Microbiol 2015; 53:3359–3362.* [CrossRef] [PubMed]
19. Bartolini A, Frasson I, Cavallaro A, Richter SN, Palù G. Comparison of phenotypic methods for the detection of carbapenem non-susceptible *Enterobacteriaceae.* Gut Pathog 2014; 6:13. [CrossRef] [PubMed]
20. *Hojabri Z, Arab M, Darabi N, Kia NS, Lopes BS, Pajand O. Evaluation of the commercial combined disk test and minimum inhibitory concentration (MIC) determination for detection of carbapenemase producers among Gram-negative bacilli isolated in a region with high prevalence of blaOXA-48 and blaNDM. Int Microbiol 2019; 22(1):81–9.* [CrossRef] [PubMed]
21. Miriagou V,Tzelepi E, Kotsakis SD, Bou Casals J,Tzouvelekis LS.Combined disc methods for the detection of KPC- and/or VIM-positive *Klebsiella pneumoniae*: improving reliability for the double

carbapenemase producers. Clin Microbiol Infect 2013; 19(9):E412-5. [CrossRef] [PubMed]
22. Noël A, Huang TD, Berhin C, Hoebeke M, Bouchahrouf W, Yunus S et al. Comparative Evaluation of Four Phenotypic Tests for Detection of Carbapenemase-Producing Gram-Negative Bacteria. J Clin Microbiol 2017; 55(2):510-8. [CrossRef] [PubMed].
23. Alizadeh N, Ahangarzadeh, Rezaee M, Samadi Kafil H, Hasani A, Soroush Barhaghi MH et al. Evaluation of Resistance Mechanisms in Carbapenem-Resistant *Enterobacteriaceae.* Infect Drug Resist 2020; 13:1377-85. [CrossRef] [PubMed]
24. Eltahlawi RA, Jiman-Fatani A, Gad NM, Ahmed SH, Al-Rabia MW, Zakai, S et al. Detection of Carbapenem-resistance in CRE by Comparative Assessment of RAPIDEC® CARBA NP and Xpert™Carba-R Assay. Infect Drug Resist 2023; 16:1123–31. [CrossRef] [PubMed]
25. Gallagher LC, Roundtree SS, Lancaster DP, Rudin SD, Bard JD, Roberts AL et al. Performance of the CLSI Carba NP and the Rosco Carb screen assays using North American carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates. J Clin Microbiol 2015; 53:3370–3. [CrossRef] [PubMed]
26. *Garg A, Garg J, Upadhyay GC, Agarwal A, Bhattacharjee A. Evaluation of the Rapidec Carba NP test kit for detection of carbapenemase-producing Gram-negative bacteria. Antimicrob Agents Chemother 2015; 59(12):7870–2.* [CrossRef] [PubMed]
27. Hopkins KL, Meunier D, Naas T, Volland H, Woodford N, Evaluation of the NG-Test CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases. J Antimicrob Chemother 2018; 73(12):3523–6. [CrossRef] [PubMed]
28. Saito K, Mizuno S, Nakano R, Tanouchi A, Mizuno T, Nakano A et al. Evaluation of NG-Test CARBA 5 for the detection of carbapenemase-producing Gram-negative bacilli. J Med Microbiol 2022; 71(6). [CrossRef] [PubMed]
29. Tarlton NJ, Wallace MA, Potter RF, Zhang K, Dantas G, Dubberke ER, et al. Evaluation of the NG-Test CARBA 5 Lateral Flow Assay with an IMP-27-Producing *Morganella morganii* and Other *Morganellaceae*. Microbiol Spectr 2023; 11(3):11:e00793-23. [CrossRef] [PubMed]

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PROCENA PERFORMANSI TRIJU FENOTIPSKIH TESTOVA ZA DETEKCIJU KARBAPENEMAZA U ENTEROBAKTERIJAMA

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Enterobakterije koje proizvode karbapenemaze (engl. *carbapemenase-producing enterobacterales* – CPE) predstavljaju značajan terapijski i epidemiološki problem u bolničkom okruženju, posebno na odeljenjima intenzivne nege. Brza identifikacija ovih mikroorganizama od ključnog je značaja. Cilj ove studije bila je procena triju fenotipskih metoda koje se koriste u rutinskoj dijagnostici. Studija je obuhvatila pedeset šest kliničkih izolata enterobakterija rezistentnih na karbapeneme, koji su izolovani kod pacijenata hospitalizovanih u Univerzitetskom kliničkom centru Niš. U pedeset dva izolata potvrđena je produkcija karbapenemaza, a četiri izolata nisu imala gene za karbapenemaze. Genotipska detekcija je sprovedena Multiplex PCR metodom, ispitujući blaKPC (engl. *beta-lactamase Klebsiella Pneumoniae carbapensemase*), blaVIM (engl. Verona Integron-encoded Metallo-beta-lactamase), blaNDM (engl. New Delhi metallo-beta-lactamase) i blaOXA (engl. *Beta lactamase* OXA)-48 gene obuhvatile. Fenotipske metode koje su analizirane uključivale su NG-Test Carba 5, RAPIDEC Carba NP test (RCNP) i komercijalni kombinovani disk test (engl. *combined disk test* – CDT) – KPC, MBL i OXA-48 *Confirm Kit: Carbapenemases*. Multiplex PCR je pokazao sledeću distribuciju karbapenemaza: dva izolata su bila produktori KPC-a, dvadeset četiri su produkovala NDM, šesnaest je produkovalo OXA-48 enzime, dok je kod deset izolata detektovana istovremena produkcija NDM-a i OXA-48 enzima. Takođe, jedan izolat *Enterobacter cloacae* identifikovan je kao koproduktor NDM-a, KPC-a i OXA-48 enzima, a jedan izolat *Klebsiella pneumoniae* istovremeno je produkovao NDM i KPC enzime. Osetljivost (senzitivnost – Se) i specifičnost (Sp) NG-Test Carba 5 iznosile su 98,08% i 100%. Kada je reč o Carba NP testu, Se i Sp su iznosile 90,38% i 100% posle sto dvadeset minuta. U CDT metodi Se i Sp za detekciju metalo-β-laktamaza (engl. *metallo-beta-lactamase*) pomoću dipikolinične kiseline (engl. *docosapentaenoic acid*) bile su 80,56% i 100%, dok su za detekciju klase D karbapenemaza pomoću temocilina iznosile 95,65% i 100%. Najbolje rezultate u detekciji specifičnih klasa karbapenemaza pokazali su NG-Test Carba 5 i CDT metoda. Ove metode bi se mogle koristiti za brzu i pouzdanu detekciju karbapenemaza u rutinskoj dijagnostici.

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BEHAVIORAL PATTERNS IN ONLINE GAMBLING IDENTIFIED THROUGH ARTIFICIAL INTELLIGENCE AND PSYCHIATRIC METHODS

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This study examined behavioral patterns in online gambling by using advanced technologies such as artificial intelligence (AI), machine learning, and the Internet of Behavior (IoB). The digital revolution has significantly increased access to online gambling, leading to the emergence of complex behavioral patterns among players. While many enjoy gambling as a form of recreation, the growing availability of online gambling poses a risks of addiction. The key role of AI and machine learning lies in the early detection of risky behavior among players. Algorithms can analyze gameplay data to identify patterns indicative of problematic behavior, including excessive spending and "chasing losses", which is the tendency to continue gambling or increase bets to recover losses. Appropriate interventions at the right time can mitigate the risk of developing gambling addiction. This research specifically focused on the use of machine learning and neural networks Multilayer Perceptron (MLP) to identify different player types, analyzing data from a group of online slot game players in the Republic of Srpska. Based on experience in clinical practice, models were trained on a sample of 200 players and tested on a broader group of 11,657 players to predict the risky behavior of players who played online slot games. Future research directions suggest the implementation of personalized tools for player control and support, with an emphasis on promoting responsible gambling and protecting public health. The results were evaluated using player data from the Republic of Srpska, a market governed by regulations designed to protect online gambling participants. This analysis underscored the role of regulatory frameworks and educational initiatives in mitigating the risk of gambling addiction.

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Key words: *behavioral patterns, Internet of Behavior, online gaming addiction, public health, artificial intelligence*

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Introduction

In the era of the digital revolution, online gambling is becoming increasingly widespread, creating complex behavioral patterns. These patterns, viewed through psychiatric methods and advanced artical intelligence (AI) technology, as well as through the concept of the Internet of Behavior (IoB), raise new questions and challenges in understanding human nature. Online gambling has been available since the mid-1990s. For some individuals, it serves as a form of entertainment and recreation, comparable to going to the cinema, whereas others perceive online gambling as a hobby. Unlike traditional casinos, online games are accessible from any location, without social stigma, and at any time of day, allowing players to spend more time gambling, thus providing an enjoyable experience.

These factors increase both the frequency and duration of play. As online gambling has an increase in popularity, the demand for systems that monitor player behavior has arisen, driven by the social responsibility to safeguard players. Global trends indicate that through the regulation of online gambling and the introduction of laws and measures to protect players, there is an effort to promote this as a recreational activity a fun and recreational industry, rather than an impulse control disorder.

In many countries around the world, online gambling is considered a socially acceptable and legal form of entertainment. However, the availability of online gambling raises public health concerns due to the potential for contributing to the development of addiction. Recently, gambling addiction has increasingly attracted the attention of clinicians and researchers. Therefore, it is crucial to identify players who are at high risk of developing problematic gambling behavior as early

as possible. Behavioral indicators and AI can predict the development of gambling addiction with high accuracy.

Addiction typically entails exposure to a particular stimulus, which is then followed by behavior directed at reliving that experience. After a certain number of repetitions, addiction is established. The nature and frequency of addiction can fluctuate over time and may occasionally be disrupted by the individual's efforts to regain control. Problematic gambling manifests as repetitive behavior, in which individuals are unable to control their betting activities. Regulators worldwide aim to make online gambling an entertainment industry, rather than a public health issue.

Pathological gambling (1), as a form of addiction, is the most widespread and severe type of non-substance addiction. This disorder is characterized by an overwhelming urge to gamble, which exceeds the boundaries of social or recreational activity, and it is accompanied by a willingness to risk money to achieve greater gain. Addictions are often associated with impulsive behavior. Historically, pathological gambling was considered an impulse control disorder, but it has recently been reclassified as a behavioral addiction within the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (2) and International Classification of Diseases, 11th Revision (ICD-11) (3). This type of gambling is recognized as a specific addiction disorder of global proportions, manifesting through an uncontrollable need to gamble, leading to serious consequences for a person's mental and physical health, as well as negative effects on other aspects of their life. Artical intelligence (AI) algorithms analyze player behavior to recognize patterns indicative of problematic behavior, such as excessive spending or extended periods of gameplay. Identifying these issues at on early stages enables timely interventions and support for players whose behavior may escalate into more severe gambling problems.

The COVID-19 pandemic had a significant impact on gambling in many jurisdictions worldwide. Literature review on online gambling during the pandemic (4) systematically identified and described survey findings regarding the effects on gambling behavior and gambling disorders during the pandemic.

This paper examines behavioral patterns in online gambling, using psychiatric methods in combination with machine learning, AI, and the IoB concept. Internet of Behavior (IoB) refers to the collection of data that provides key insights into user behavior, interests, and preferences. From the perspective of behavioral psychology, IoB seeks to understand data generated by user activities on the internet. This study analyzed individual player activity data from the Republic of Srpska to determine whether a player belonged to the category of recreational slot game players, displaying no signs of addiction, or showing indications of risky behavior or clear signs of addiction. The application of AI to these analyses at an early stage enabled the reliable prediction of addiction risk, allowing for timely interventions to prevent its progression.

One of the goals of this study was to identify playing patterns by tracking the behavior of active players in the Republic of Srpska over 12 weeks of playing online slot games. The study was conducted to identify behavioral markers that can predict the occurrence of problematic gambling. The dataset was obtained from an online slot game provider, encompassing over two hundred different games available through multiple operators in the Republic of Srpska. The data was provided in accordance with a Non-Disclosure Agreement, preventing the sharing of details or the identification of individual players.

Previous research has encountered difficulties in identifying at-risk players, primarily due to the absence of clinical verification, relying instead on self-reported cases of problematic gambling behavior. In this study, Multilayer Perceptron (MLP) 200 players were analyzed and categorized by experts in addiction psychology and psychiatry. The players were categorized into recreational players, those showing signs of risky behavior, and those showing clear signs of addiction. Machine learning was applied to this sample using a neural network, specifically an MLP, which was then tested on a control group of 11,657 players to identify these player types in the Republic of Srpska.

The study presents an overview of previous research, challenges in developing machine learning models, and detecting problematic player behavior, as well as directions for future research. The proposed solution includes selecting relevant behavioral pattern markers and training machine learning models. The implementation of the Multilayer Perceptron (MLP) algorithm for supervised machine learning and the detection of risky player behavior was carried out using clinical experience in selecting harmful behavioral markers.

Previous Research

In recent years, the number of projects utilizing AI, and IoB has increased significantly. However, only a small portion of these projects focuses on improving public health and preventive measures for online slot players. Most research primarily concentrates on player behavior monitoring, with only a few examining the influence of various factors identified through the analysis of behavioral patterns, as defined by psychiatric methods. Since online slot players face different levels of risk for developing gambling disorders, there is a need to develop more tools and resources to mitigate the harmful consequences of gambling. Previous research has emphasized the importance of accuracy in machine learning algorithms, highlighting the need to balance the

prevention of the negative effects of gambling with the preservation of privacy while minimizing the use of sensitive data. The approaches analyzed have also considered the ethical application of AI in the gambling industry, particularly regarding voluntary self-exclusion programs and responsible gambling initiatives that allow players to voluntarily limit their access to games. However, no new methods have been proposed. The data analyzed generally consisted of a limited set of indicators, and comprehensive statistics on the proportions of recreational, at-risk, and problematic players were often lacking. Additionally, players from multiple operators within the same country were not included in these analyses.

Behavioral addictions, particularly online gambling, internet addiction, and gaming disorder, are increasingly recognized as significant public health challenges, as noted in reference (5). While not all behavioral addictions are yet formally classified in systems like ICD-11 or DSM-5, online gambling and gaming disorder have gained official recognition. Online gambling is classified as a mental disorder in ICD-11, and more recently, gaming disorder has been added as a new category. Various countries, especially in Europe and parts of Asia, have begun developing national strategies and opening treatment centers to address these issues. Research highlights the importance of early intervention, education, and prevention campaigns, particularly targeting youth.

A study conducted in 2012 examined the potential for early identification of players at high risk of developing gambling problems by analyzing their behavior during the first month of play (6). The study hypothesized that behavioral markers such as gambling frequency, intensity, and the tendency to increase or decrease bets during the first month of live play could segment players who would later close their accounts due to problematic gambling. A sample of over 48,000 sports bettors was segmented using K-means cluster analysis into groups with similar gambling patterns. Out of 48,000 players, 1,758 closed their accounts after one month of play. Of these, 530 (33%) confirmed they had closed their accounts due to gambling problems, 19% were dissatisfied with the gambling provider's services, and 48% stated they were no longer interested in gambling. Participants were residents of Germany, Turkey, Poland, and Spain. The findings revealed that only a small percentage (3%) of those who closed their accounts due to gambling-related issues belonged to the high-risk group, highlighting the complexity of early detection and the need to improve player segmentation algorithms.

An earlier study (7) analyzed data from 22,500 players in England to explore the role of gender in predicting problematic gambling behavior. It emphasized the importance of recognizing gender-specific behavioral patterns and their potential link to risk-taking tendencies. It was suggested that testosterone levels could be linked to risk-taking behavior and pathological gambling, and gender-based behavioral patterns in problematic gambling were identified. Using the Random Forest algorithm (8), researchers developed a model in which gender served as the primary classification variable. To address concerns about using gender data directly, they proposed training separate models for each gender and merging them into a final model that does not require gender as an input, while still preserving predictive accuracy.

BetBuddy (9) used risk curve analysis to identify online players whose behavior resembled that of those who had self-excluded from gambling. Focusing on UK players of bingo and slot games, the model evaluated behavioral indicators such as nighttime play and declined deposits. The dataset used for training the model included variables describing playing behavior, deposits, withdrawals, and player-set limits. Findings showed that certain behaviors, like moderate night time gambling or frequent deposit refusals, were not inherently problematic unless combined with other risk factors. Using a Random Forest algorithm, the model effectively predicted self-exclusion risk and demonstrated potential for harm reduction by enabling timely interventions and supporting the development of safer gambling guidelines.

A study using data from a Canadian gambling operator tested the performance of machine learning, specifically the Random Forest algorithm, in classifying online gambling players with a history of self-exclusion (10). The model was trained on behavioral data such as frequency, intensity, and variability of play, with bet variability per session emerging as the most significant predictor. Of all the input variables, bet variability per session was the most significant for prediction, contributing 32% to the predictive signal. While psychological factors like “chasing losses”, were not included, the model demonstrated solid predictive accuracy, confirming the potential of behavioral data for identifying self-excluding players.

A review from 2011 highlighted key challenges in using machine learning for predicting online gambling disorders. It emphasized that commonly used indicators, such as self-exclusion or account closure, were insufficient on their own and should be complemented by behavioral and contextual variables, including demographics, deposit patterns, night-time play, and customer support interactions (11). The review also stressed the importance of tracking bet escalation to detect behaviors like "chasing losses." A central challenge remains the integration of data science insights with psychological theories to better understand cognitive processes, such as impulsivity, and their role in problem gambling.

Seo et al. explored the use of machine learning to detect problematic gambling among adolescents in South Korea, aiming to support early prevention (12). Based on survey data from over 5,000 students, key predictors included recent online gambling activity, peer betting, and

gambling-related behaviors and demographics. Four models were trained: Random Forest, Support Vector Machine (13), Extra Trees (14), and Ridge Regression (15), with Random Forest showing the highest reliability in predictions. Algorithms trained on a sample of 5,045 students provided moderate accuracy in predicting problematic gambling. Of the 5,045 participants, 51.1% were male, 48.9% female, with an average age of approximaty 15 years. Among the tested models, Random Forest demonstrated the highest predictive reliability. Although mental stress was not included, results highlighted the influence of psychological, social, and environmental factors. The study recommended gambling education for both adolescents and parents, and proposed implementing the model in mobile apps to enable self-assessment of gambling risk.

In 2020, Auer M et al. analyzed data from 70,789 players of a Norwegian operator to predict changes in self-imposed monetary limits using machine learning (16). Only 6.7% of players adjusted their monthly loss limits, with most increases occurring after receiving feedback that 80% of the limit had been reached, which often resulted in greater losses. Among players who received feedback that they had reached 80% of their spending limit, 18% adjusted their limit, most often increasing it, while only 0.7% lowered it. Ten percent of players selected the maximum monthly spending limit, and 5% were classified as problematic gamblers. Key predictors included prior limit increases, total wagers, theoretical loss, and feedback messages. Machine learning algorithms used included Logistic Regression (17), Linear Discriminant Analysis (18), Random Forest, Gradient Boost Machine Learning (19), and Naive Bayes (20). Among the tested algorithms, Gradient Boost outperformed others in predicting limit changes. The study highlighted the potential of predictive analytics to support timely and personalized responsible gambling interventions.

It is important to note that most previous studies were based exclusively on data from a single operator in a single country. Such data provide only a partial view of behavioral patterns during playing the online slots, excluding other operators. Previous studies did not involve clinical professionals, nor did they consider the mentality and habits of a particular population when selecting harmful markers for machine learning model training. Several hypotheses have been proposed regarding which types of online gambling are more strongly associated with harmful behavioral markers and which have a higher potential to encourage risky behavior as player engagement increases. Behavioral markers leading to future self-exclusion were analyzed, as well as whether the monetary characteristics of players' gambling further improve prediction. Methodologically, supervised learning was used relatively more frequently than unsupervised learning in the included addiction studies. In earlier studies, models such as Random Forest, Logistic Regression, Linear Discriminant, Naive Bayes, and Gradient Boosting Machine were used. The Random Forest model achieved the highest training accuracy, exceeding 90%, while testing accuracy was below 80%. Other models demonstrated significantly lower accuracy during training and testing, i.e., below 80%.

By proposing multiple hypotheses in this study, several goals were achieved, which will be described in the following sections. Harmful behavioral markers were selected for slot players. Using IoB and machine learning algorithms, data were interpreted with the aim of reducing the negative impact on mental health and creating a positive impact on overall well-being by preventing risky players from escalating. The model was trained and evaluated using data obtained from an online slot game provider, with a portfolio of 279 different slot games played across various online gambling operators in the Republic of Srpska, and a database of 11,857 players.

Dataset and Methodology

Using markers derived from psychiatric methods and clinical practice, the objective was to train a machine learning model with data from recreational players, those demonstrating risky behavior, and players showing problematic gambling behavior.

The dataset was obtained from an online slot game provider, featuring a portfolio of over two hundred different slot games. Slot games have shorter event frequencies, minimal pauses between bets, enable continuous betting opportunities, facilitate impulsive decision-making, and are highly accessible, playable at any time of day or night. These slot games are played across various online operators within the Republic of Srpska. When considering both the training and testing datasets, 279 games and 11,857 players were processed. The model was trained on a sample of 200 players and tested on 11,657 players. The data was provided under a Non-Disclosure Agreement, which prevents sharing or reporting individual player data. The player's behavior information was anonymized, ensuring that no individual could be identified or traced in any way. The raw data included every game played and every bet placed between January and April 2024. For the model training and subsequent predictions, randomly selected players who played during that period and participated in at least one session per week were included. Millions of individual game rounds were aggregated through sessions, days, and weeks for each player to extract relevant behavioral markers. A session is defined as a continuous period of play in which the player places successive bets within a specific time frame.

The dataset, consisting of a total of 15,413.076 individual rounds (bets) played in slot games, was grouped by weeks for each individual player. The raw data was aggregated such that for

each data point in the dataset, 10 input variables, i.e., behavioral markers, were generated for model training, with one output variable as the result. The information about the dataset used for training the neural network to detect player behavioral patterns is as follows:

- Country: Republic of Srpska
- Legally regulated online gambling: Yes
- Number of analyzed games: 279
- Number of players: 200
- Number of individual rounds/spins: 15,413,076
- Number of training samples: 2,400.

Behavioral Markers

Harmful behavioral indicators play a critical role in implementing responsible gambling strategies, enabling personalized interventions for players. It is expected that such tailored interventions will more effectively raise awareness, reduce risky behavior, and prevent harmful outcomes. Certain characteristics can be grouped to monitor specific activities, trends, or fluctuations in player behavior over time. For example, the number of minutes a player spends gambling daily, the increase in average playing time over a certain period, and variations in playtine throughout the day can indicate risk. Nighttime gambling is especially risky, as it often indicates that players may be making irrational decisions, which can adversely impact their work and personal life. Frequent gambling is associated with impulse control disorders, while stable betting amounts may indicate better control, whereas dramatic shifts in bets signal potential problems. Additionally, tracking the amount of money players spend, their wins and losses lover a one-week period, helps in analyzing the frequency, intensity, session duration, and variability. Frequency refers to the number of sessions within a given period, intensity to the length of the sessions and behavior during them, while variability indicates changes in player behavior, such as "chasing losses". Changes in these indicators over time enable the identification of shifts in gambling behavior.

Multiple variables are included in the model to achieve optimal prediction. Behavioral indicators can be divided into those tracking activity levels (such as the number of minutes spent gambling daily) or those analyzing trends over time (e.g., an increase in average daily playing time over the past month) and variability (e.g., changes in playing time throughout the day or shifts in betting amounts). To maximize model performance, a diverse range of characteristics and variables is necessary.

After extracting the markers, the challenge was to identify the most relevant ones for grouping players and classifying their risk-pronebehavior. Precise selection of behavioral indicators is crucial for the rapid and accurate training of the model to provide reliable predictions.

During the marker selection process, with clinical practice as a foundation, 10 key input variables were chosen for training the machine learning model. All variables were numerical, and the output classified players as follows: 0—recreational players, 1—at-risk players, and 2—problematic players. According to psychiatric methods, players were divided into three groups. The first group consists of those who play infrequently, with low bets and minimal bet variability. The second group includes players who play more frequently and intensively, but with stable bets. The third group involves players who gamble more intensively, with significant fluctuations in their betting amounts.

Recreational players, categorized in the first group, typically do not play more than two games simultaneously, place small bets, and their sessions last less than two hours. The time between their gombling sessions and rounds is consistent, and their bets remain stable. At-risk and problematic players engage in shorter sessions with higher bets that often increase, while the time between sessions is very short. Their bets and volatility fluctuate significantly compared to average values. The key difference between the second and third groups lies in larger bets, greater session variability, night-time gambling, and shorter intervals between sessions.

Sampling and Statistical Analysis

As previously mentioned, player behavior was monitored for training and validation the machine learning model, with players selected randomly. The dataset used for training and validating the model tracked 200 players over 12 weeks, resulting in 2.400 samples, each containing 10 input markers. The samples were labeled based on psychiatric methods and clinical practice, and grouped into non-risky, at-risk, and problematic gombling behavior categories. The dataset was split into a training set (80% of the samples) and a validation set (20% of the samples).

Demographic and statistical Data for the Republic of Srpska:

- Total population (millions): 1.2 (21)
- Average monthly net salary in 2024 (EUR): 710.2 (22)
- Unemployment rate in 2024: 11.2% (23)
- Literacy rate in 2024: 96.8% (24)
- Internet penetration rate: 83% (25)
- Percentage of the population engaged in gambling activities: 15% (26).

According to available data (27), the religious composition in the Republic of Srpska is as follows:

- Eastern Orthodox Christianity: 82%
- Islam: 12.76%
- Catholicism: 2.20%
- Protestantism: 0.02%

●Other Christian denominations: 0.21%
●Other religions: 0.49%
●Agnosticism: 0.10% and
●Atheism: 0.50%.

Online gambling is regulated by law in the Republic of Srpska. As online gambling is legally governed, players engage with licensed gambling operators (28).
However, there are no mandatory measures to enforce responsible gambling practices.

Dataset for Training the Model for Players in the Republic of Srpska

In the dataset comprising 200 players, with 12 weeks of data per player, a total of 15,413,076 individual bets were aggregated for training the model for the territory of the Republic of Srpska.

For those classified as extreme cases, the average nightly playtime was approximately 3 hours, while the 24-hour average was around 5.4 hours per day. Additionally, the following were obtained:

●The average number of different games played per week was approximately 4.

●The average number of rounds per day was 2,868.

●The average daily loss was around 229 BAM.

●The average daily bet amount was approximately 2,368 BAM.

For players considered at risk, the average nightly playtime was around 40 minutes, and the 24-hour average was approximately 1.4 hours per day. Additionally, the following were obtained:

●The average number of different games played per week was around 3.

●The average number of rounds per day was approximately 534.

●The average daily loss was around 6 BAM.

●The average daily wage was close to 65 BAM.

Considering the average net salary in the Republic of Srpska, cultural norms, customs, and the population's mentality, as well as insights from psychiatric practice, it is concluded that more than five hours of gambling per day constitutes problematic gambling. Additionally, any daily wager exceeding 60 BAM goes beyond recreational play and enters the at-risk category. A continuous weekly loss exceeding 120 BAM, combined with "chasing losses" and high bets, is an indicator of problematic gambling. Playing four or more different games daily is considered extreme. An average of more than two hours of nighttime gambling indicates a high risk of impulse control disorders. If all observed parameters increase or fluctuate significantly over the 12-week observation period, the player's behavior transitions into the category of players at-risk players with behavior. Any extreme variations in intensity, frequency, variability, or session length indicate a loss of control by the player.

The dataset used to train the model for the Republic of Srpska was imbalanced, consisting of 31% recreational players, 41% players at-risk, and 28% problematic players, as determined after the samples were labeled by experts with clinical experience in the fields of psychology and addiction psychiatry.

Proposed Solution

For a long time, AI has played an important role in player protection systems against the negative consequences of gambling, with its application significantly increasing over the past year. Platforms now frequently use machine learning models for player prediction and segmentation, offering standardized approaches. Generative artificial intelligence enables the creation of personalized content for each player. In the future, machine learning is expected to advance with a deeper understanding of player behavior while considering legal regulations related to the use of AI in areas like gambling.

In previous research, a key obstacle to identifying players with risky behavior was the lack of external verification, as there was no clinical identification of the samples used for model training. Instead, researchers relied on players who self-identified their behavior as problematic and stopped gambling, and their behavioral indicators were used to train machine learning models. In this study, players from the Republic of Srpska were analyzed, with their behavior classified by experts in psychology and addiction psychiatry. The behavior of each player in each week was labeled as non-risky, at-risk, or problematic, and this data was then used to train the machine learning model. In classifying behavior, factors such as average net salary, culture, customs, and the local population's mentality, along with insights from psychiatric practice, were considered.

The most prominent characteristics of at-risk players included frequent and intense gambling, as well as significant variations in bets. A noticeable pattern was the players' need to increase bets to achieve the excitement they once gained with smaller bets, while attempts to recoup losses were identified as an indicator of pathological gambling. Changes in bets serve as a significant indicator for distinguishing at-risk from non-risky players, as most recreational players show consistent playing patterns. Another important indicator is playtime, how often a player gambles at night, and whether they are more active on weekdays or weekends. The logic behind this is that players with less control over their behavior tend to gamble more frequently at night, attempting to hide these activities from others.

Machine learning models vary in complexity. Innovations in machine learning research often focus on new techniques for processing large datasets. The aim of this study was not to

introduce new machine learning techniques for improved prediction but rather to apply an existing predictive model to the topic of player behavior prediction and risk assessment, specifically trained for the Republic of Srpska, to promote responsible gambling. Unlike most previous studies that used Random Forest, this study employed a neural network approach, specifically MLP classification. The MLP model was trained on an imbalanced dataset of randomly selected data samples.

To develop and train the model in the most appropriate way, it was necessary to bring together expertise from multiple fields. In this study, the training data were prepared, and the machine learning model was trained and tested with the help of experts in software engineering and addiction psychiatry.

For each individual player, every bet placed was stored in the database. By aggregating such raw data, every round played by the player over a specific week, a set of input variables was created to describe the player's behavior, frequency, intensity of play, and time spent gambling during that week. Repeating this process for each player over a 12 weeks period resulted in a set of input variables for training, validating, and testing the MLP model.

The set of input variables resulting from the aggregation and processing of individual rounds included: player ID, number of the week of the year, number of different games played, total loss, average daily playtime, average number of daily rounds, total wager, total morning playtime, total daytime playtime, and total night-time playtime. Morning, daytime, and night-time play were categorized within the time frames of 6:00 AM to 12:00 PM, 12:00 PM to 7:00 PM, and 7:00 PM to 6:00 AM, respectively.

For model training and validation, a total of 2,400 data samples were aggregated, representing 200 randomly selected players with behavioral data over a 12 weeks period. Each week, for each individual player, was labeled by an expert with domain knowledge in clinical practice and psychiatric methods for identifying addiction, classifying player behavior with output variable values of 0, 1, or 2 for recreational play, risky play, and problematic play, respectively. The test set for the model, i.e., the control group of players, consisted of a total of 139,884 previously unseen samples of behavioral data, representing the behavior of 11,657 players per week over 12 weeks.

Model Architecture

The artificial neural network (ANN), specifically the MLP model used in this study, was designed with an input layer, two hidden layers, and an output layer. Experimental methods were employed to determine the most suitable model architecture that would provide the highest accuracy. The best results were obtained with the number and arrangement of neurons per layer, as shown in Figure 5. The input layer consists of 13 neurons, the first hidden layer has 22 neurons, and the second hidden layer has 11 neurons. The output is a single neuron with three possible values (recreational behavior, at-risk behavior, and problematic behavior).

The trained MLP model achieved an accuracy of 99.446% during training and 95.928% during testing. Between 50 and 200 epochs, the test loss and test accuracy remained relatively stable at approximately 96.83% accuracy. From 250 to 400 epochs, there was a slight improvement in testing accuracy, reaching 97.29%, indicating performance improvement. After that, training between 500 and 700 epochs showed an increase in test loss and a decrease in testing accuracy, indicating potential overfitting. Between 800 and 1,000 epochs, test losses continued to rise, and testing accuracy fluctuated between 95.48% and 95.93%, indicating that overfitting likely began between 600 and 700 epochs.

Model's Confusion Matrix

In this case, since the model has three output values (classes), the confusion matrix is 3x3 and displays the performance of the classification model. The rows represent the true classes, i.e., the labels, while the columns represent the predicted classes. Each cell (c[i,j]) in the matrix shows the number of instances of class iiii that were predicted as class jjj.

●Class 0. A total of 125 instances were correctly classified. Only 1 instance was incorrectly classified as class 1, while 0 instances were incorrectly classified as class 2.

●Class 1. A total of 20 instances were correctly classified. A total of 5 instances were incorrectly classified as class 0, while 2 instances were incorrectly classified as class 2.

●Class 2. A total of 67 instances were correctly classified. A total of 0 instances were incorrectly classified as class 0, while 1 instance was incorrectly classified as class 1. Figure 1 illustrates the confusion matrix for the classification algorithm's performance evaluation.

It is concluded that the model performs very well for classes 0 and 2, while it makes slight errors with class 1, having difficulty distinguishing class 1 from classes 0 and 2. The model more easily identifies recreational and problematic players with clear signs of addiction, while at-risk players are the most difficult to recognize

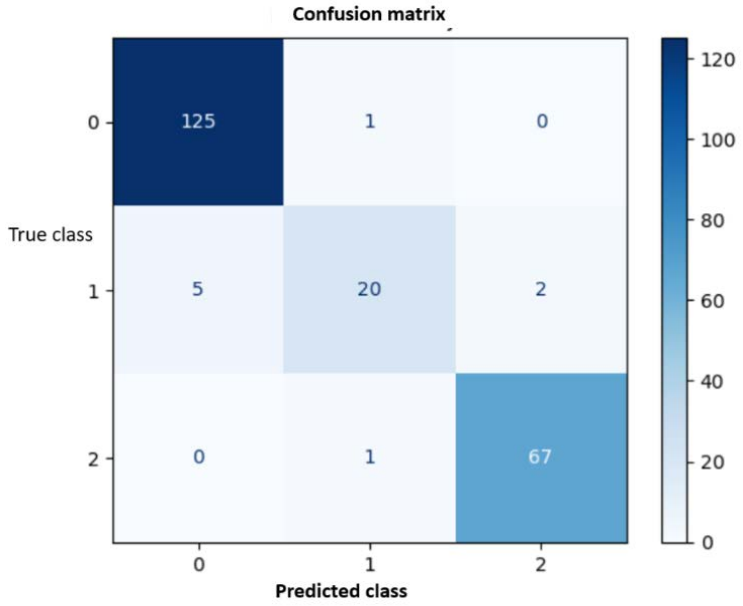

Figure 1. Confusion Matrix from model training on players in the Republic of Srpska

Table 1. Overview of Confusion Matrix Metrics for the trained model for players from the Republic of Srpska

Metrics	Class 0	Class 1	Class 2
Recall	99.21%	80%	98.53%
Accuracy	96.15%	95.24%	97.10%
Specificity	94.74%	98.97%	98.69%
Prevalence	57.27%	11.36%	31.36%
F score	97.65%	85.11%	97.10%

ROC Curve

Receiver Operating Characteristic (ROC) Curves show metrics like accuracy and precision, which are good indicators when the data is balanced. For an imbalanced dataset, high accuracy does not necessarily mean the classifier is effective. For example, if 90 out of 100 players are recreational, even if the algorithm classifies all 100 as recreational, its accuracy would be 90%, which could give a misleading impression of the model's performance. In cases of imbalanced datasets, metrics like ROC are more effective for evaluation. The ROC curve visually represents the performance of a binary classification model at various classification thresholds. It is a plot of the true positive rate (TPR) versus the false positive rate (FPR), calculated as specificity at different threshold values. The point closest to the 45-degree line on the graph is considered the most accurate threshold. If the threshold is too high, there will be fewer false positives, but more false negatives, and vice versa.

●Class 0: A TPR (precision) of 99.21% and an FPR of 5.26% indicate that the model classifies class 0 instances very well.

●Class 1: A TPR of 80.00% and an FPR of 1.03% suggest that the model identifies class 1 with sufficient precision, but there is room for improvement.

●Class 2: A TPR of 97.10% and an FPR of 1.31% show that the model performs excellently for class 2.

Classes 0 and 2 have AUC (Area Under the ROC Curve) values close to 1, indicating excellent performance. Class 1 has a slightly lower AUC, reflecting a trade-off between detection probability and specificity.

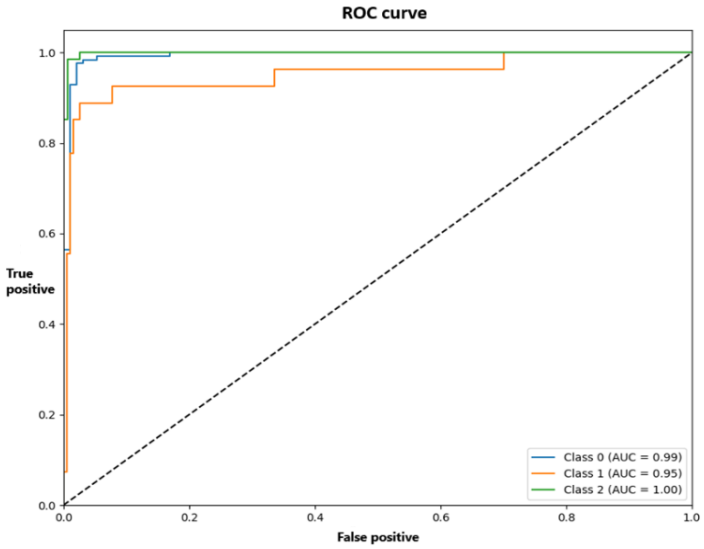

Figure 2. ROC Curve for the model trained for players from the Republic of Srpska

Evaluation and Discussion

The main objective of this study was to discover behavioral patterns related to online gambling, identified through psychiatric methods in combination with artificial intelligence and IoB. To achieve this goal, it was necessary to implement and evaluate an algorithm for detecting the harmful effects of online gambling.

The dataset, comprising a total of 268,062,490 individual bets placed in slot games, was grouped by weeks for each individual player. The test set for the model, i.e., the control group of players, consisted of a total of 138,804 previously unseen behavioral data samples, representing the weekly behavior of 11,567 players over a 12 week period. The results obtained were as follows:

●Number of games: 279

●Number of players: 11,657

●Control group sample collected from 8 different gambling operators in the Republic of Srpska

●Number of individual rounds/bets: 268,062,490

●Number of samples analyzed: 139,884

●Detected percentage of recreational players: 86.38%

●Detected percentage of players at-risk: 9.02%

●Detected percentage of problematic players: 4.61%.

Out of the 11,657 players, the model classified 4.61% as problematic gamblers, 9.02% as at-risk players, while the majority of players, 86.38%, were identified as recreational gamblers.

Online gambling datasets offer a valuable and rich resource for understanding problematic gambling and proposing interventions to mitigate the harmful impact of gambling in an online environment. Unlike most behavioral gambling studies, this research focuses on players who use their own funds to play games of their choice. It is evident that problematic gambling among such players is expressed through multiple behavioral indicators, including both monetary and non-monetary behavioral markers.

The challenge in this study was how to integrate data science results with existing clinical knowledge about the detailed characterization of behavior and cognitive processes in problematic gambling.

One of the advantages of this study is that it does not rely on players who resort to self-exclusion, as this may be less effective in identifying the harmful impact of gambling among players, some of whom may exhibit signs of problematic gambling or gambling addiction without even considering self-exclusion.

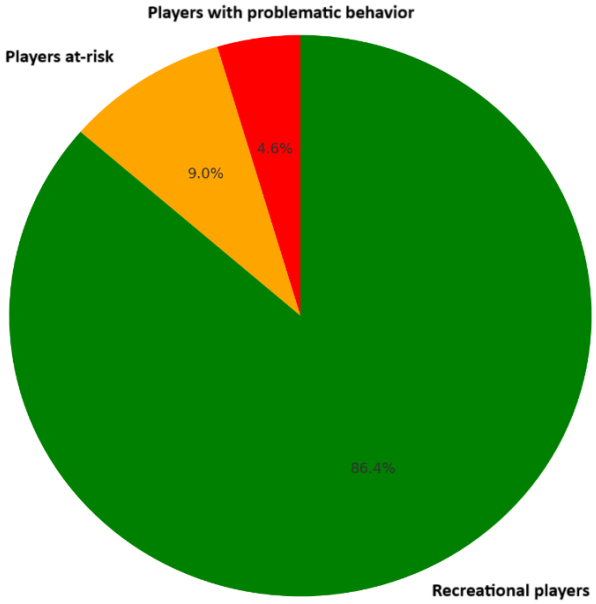

Figure 3. Classification of the control group of players using the trained model

Conclusion

There is an increasing need to develop effective strategies for preventing serious conditons caused by unhealthy lifestyle habits. Given the scope of this issue, personalized healthcare systems that apply innovative preventive strategies are becoming crucial. Previous research suggests that advances in machine learning enable the application of analytical methods in studying addictive behaviors. These innovations have the potential to revolutionize medical practice, particularly in terms of preventing and mitigating certain diseases. The quality of preventive care can be greatly enhanced as average users can better understand their health status and potential risks, while working closely with healthcare professionals.

When it comes to applying AI in healthcare, historical data on player behavior during gambling is essential for machine learning models that identify potentially problematic gambling. In this study, many harmful indicators were derived from behavioral variables that are independent of the financial weight of gambling. Although this study included a large sample and incorporated behavioral data from players who gambled with eight different operators in the Republic of Srpska, it still has certain limitations. If gambling operators were to share more data on actual player behavior, this would enable further research into responsible gambling and enhance algorithms for detecting problematic behavior. Factors such as player deposits, age, and frequency of contact with customer support could then be considered. Additionally, this study did not include sports betting, which is a very popular form of gambling in the Republic of Srpska.

The results of this study indicate that the application of machine learning algorithms, combined with psychiatric methods, can successfully identify behavioral patterns associated with recreational, at-risk, and problematic gambling. Based on the analysis of 11,657 players, 4.61% were classified as problematic gamblers, and 9.02% showed behaviors indicative of risk. These findings confirm that a significant portion of players fall into a risk zone even before developing full-blown gambling addiction, creating an opportunity for early intervention.

One of the key conclusions is that harmful behavioral patterns can be detected even when the financial aspect of gambling is not dominant, emphasizing the importance of including non-monetary behavioral indicators in predictive models. Furthermore, the study demonstrates that models can be trained on actual player behavior without relying on self-reporting mechanisms, such as self-exclusion, thereby increasing their practical applicability.

Based on these findings, several key recommendations are proposed to support the early detection of risky online gambling. One important approach involves incorporating a broad set of behavioral indicators, including bet variability, frequency of play, nightatime gambling activity, and shifts in gambling intensity. Another important recommendation is the use of machine learning algorithms to enable continuous monitoring and automatic classification of players according to their risk level. Personalized

interventions should also be implemented, particularly for individuals identified as "at-risk", to prevent the development of addictive behaviors. Furthermore, closer collaboration between gambling operators and researchers is essential to improve access to larger datasets and to integrate additional variables such as demographic characteristics, interactions with customer support, and deposit histories. Lastly, educating players and introducing regulatory measures that require operators to apply responsible gambling tools based on behavioral analytics can further enhance efforts to mitigate gambling-related harm.

Future research should also include other forms of online gambling, such as sports betting, and compare behavioral patterns across jurisdictions and socioeconomic contexts. This would allow for further model optimization and improvement of global practices in gambling addiction prevention.

Machine learning is particularly well-suited for identifying behavioral patterns in problematic gamblers compared to recreational players. Gambling operators can provide "responsible gambling" tools to all users or target such interventions at specific player groups.

In future research, one of the objectives will be the specialized training of models for players from specific countries and comparing their performance. The reasoning is that previous studies have not provided statistics on the percentage of recreational, at-risk, and problematic players when comparing data from game providers whose online slots are played across multiple operators, spanning both regulated and unregulated markets, as well as countries with varying average net incomes and those located on different continents.

References

1. Čomić M, Knezevic V, Dickov A, Ratković D, Abazović M. Pathological gambling: Addiction or impulse control disorder? Timocki medicinski glasnik 2022; 47: 157-62. [CrossRef]
2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Washington, DC: American Psychiatric Publishing; 2013. [CrossRef]
3. World Health Organization. International classification of diseases for mortality and morbidity statistics. 11th ed. [Internet]. 2019 [cited 2025 Jan 9]. Available from: https://icd.who.int/
4. Hodgins DC, Stevens RMG. The impact of COVID-19 on gambling and gambling disorder: emerging data. Curr Opin Psychiatry 2021;34(4):332-43. [CrossRef] [PubMed]
5. Das S, Pandey MK. Behavioral Addictions: An Emerging Public Mental Health Crisis? Indian J Soc Psychiatry 2023;39(3):230-5. [CrossRef]
6. Braverman J, Shaffer HJ. How do gamblers start gambling: identifying behavioural markers for high-risk internet gambling. Eur J Public Health 2012;22(2):273-8. [CrossRef] [PubMed]
7. Percy C, Garcez Ad, Dragicevic S, Sarkar S. Lessons Learned from Problem Gambling Classification: Indirect Discrimination and Algorithmic Fairness. In: Proceedings of the CEUR Workshop; 2020; Virtual Symposium. Vol. 2884.
8. Breiman L. Random forests. Mach Learn 2001;45(1):5-32. [CrossRef]
9. Dragicevic SA, Garcez Cd, Percy C, Sarkar S. Understanding the Risk Profile of Gambling Behaviour through Machine Learning Predictive Modelling and Explanation. In: Proceedings of the 33rd Conference on Neural Information Processing Systems (NeurIPS 2019); 2019; Vancouver, Canada.
10. Finkenwirth S, MacDonald K, Deng X, Lesch T, Clark L. Using machine learning to predict self-exclusion status in online gamblers on the PlayNow.com platform in British Columbia. Int Gambl Stud 2020;21:1-18. [CrossRef]
11. Deng X, Lesch T, Clark L. Applying Data Science to Behavioral Analysis of Online Gambling. Curr Addict Rep 2019;6. [CrossRef]
12. Seo W, Kim N, Lee SK, Park SM. Machine learning-based analysis of adolescent gambling factors. J Behav Addict 2020;9:734-43. [CrossRef] [PubMed]
13. Cortes C, Vapnik V. Support-vector networks. Mach Learn 1995;20(3):273-97. [CrossRef]
14. Geurts P, Ernst D, Wehenkel L. Extremely randomized trees. Mach Learn 2006;63(1):3-42. [CrossRef]
15. Hoerl AE, Kennard RW. Ridge regression: Biased estimation for nonorthogonal problems. Technometrics 1970;12(1):55-67. [CrossRef]
16. Auer M, Griffiths M. Predicting Limit-Setting Behavior of Gamblers Using Machine Learning Algorithms: A Real-World Study of Norwegian Gamblers Using Account Data. International Journal of Mental Health and Addiction 2022;20:1-18. [CrossRef]
17. Cox DR. The regression analysis of binary sequences. J R Stat Soc Ser B 1958;20(2):215-32. [CrossRef]
18. Fisher RA. The use of multiple measurements in taxonomic problems. Ann Eugen 1936;7(2):179-88. [CrossRef]
19. Friedman JH. Greedy function approximation: A gradient boosting machine. Ann Stat. 2001;29(5):1189-232. [CrossRef]
20. Maron ME, Kuhns JL. On relevance, probabilistic indexing and information retrieval. J ACM. 1960;7(3):216-44. [CrossRef]
21. United Nations, Department of Economic and Social Affairs, Population Division. World Population Prospects: The 2022 Revision. [Internet]. 2024 [cited 2025 Jan 9]. Available from: https://population.un.org.
22. Salary Explorer. Salary Survey in Republika Srpska. [Internet]. 2024 [cited 2025 Jan 9]. Available from: https://www.salaryexplorer.com/salary-survey.php?loc=42&loctype=1.
23. IRBRS. Database of Economic indicators of RS. [Internet]. 2024 [cited 2025 Jan 9]. Available from: https://www.irbrs.net/statistika/UporedniPrikaz.aspx?tab=3&lang=eng.
24. World Population Review. Republika Srpska Population. [Internet]. 2024 [cited 2025 Jan 9]. Available from: https://worldpopulationreview.com/countries/republic-of-srpska-population.
25. DataReportal. Digital in Bosnia and Herzegovina. [Internet]. 2024 [cited 2025 Jan 9]. Available from: https://datareportal.com/digital-in-bosnia-and-herzegovina.
26. World Population Review. Republika Srpska Population. [Internet]. 2024 [cited 2025 Jan 9]. Available from: https://worldpopulationreview.com/countries/republic-of-srpska-population.
27. Slotegrator. Gambling in Bosnia and Herzegovina in 2022. [Internet]. 2022 [cited 2025 Jan 9]. Available from: https://slotegrator.pro/analytical_articles/gambling-in-bosnia-and-herzegovina-in-2022/.
28. Republika Srpska Online. Gambling Statistics. [Internet]. 2024 [cited 2025 Jan 9]. Available from: https://www.republikasrpskaonline.com.

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BIHEVIORALNI OBRASCI U KOCKANJU PREKO INTERNETA IDENTIFIKOVANI POMOĆU VEŠTAČKE INTELIGENCIJE U KOMBINACIJI SA PSIHIJATRIJSKIM METODAMA

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Ovaj rad istražuje bihevioralne obrasce u kockanju preko interneta koristeći napredne tehnologije poput veštačke inteligencije, mašinskog učenja i koncepta Interneta ponašanja (engl. *Internet of Behaviour* – IoB). Digitalna revolucija je značajno olakšala pristup igrama na sreću dostupnim na internetu, što je dovelo do nastanka složenih obrazaca ponašanja igrača. Dok mnogi uživaju u kockanju kao obliku rekreacije, sve veća dostupnost igara na internetu može stvoriti rizike od razvijanja zavisnosti. Ključna je uloga veštačke inteligencije i mašinskog učenja u ranom prepoznavanju rizičnog ponašanja kod igrača. Algoritmi mogu analizirati podatke o igranju kako bi se identifikovali obrasci koji ukazuju na problematično ponašanje, uključujući preterano trošenje i „jurenje gubitaka", tj. tendenciju da se nastavi kockanje ili da se poveća opklada u nastojanju da se gubici vrate. Pravovremene intervencije mogu pomoći u sprečavanju razvoja bolesti zavisnosti. Rad se posebno fokusira na upotrebu mašinskog učenja i neuronskih mreža (engl. *multilayer perceptron* – MLP) za identifikaciju različitih tipova igrača, analizirajući podatke o grupi igrača slot-igara dostupnih na internetu sa teritorije Republike Srpske. Pomoću iskustva iz kliničke prakse, modeli su trenirani na uzorku od 200 igrača i testirani na široj grupi sačinjenoj od 11.657 igrača kako bi predvideli rizično ponašanje u slot-igrama dostupnim na internetu. Pravci daljeg istraživanja predlažu implementaciju personalizovanih alata za kontrolu i podršku igračima. Pritom, akcenat se stavlja na promociju odgovornog kockanja i zaštitu javnog zdravlja. Rezultati su evaluirani na osnovu podataka igrača iz Republike Srpske, tržišta regulisanog zakonima koji štite igrače igara na sreću, i pokazuju kako regulisanje i edukacija mogu pomoći u smanjenju problema zavisnosti od kockanja.

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***Ključne reči**: bihevioralni obrasci, Internet ponašanja, zavisnost od onlajn-igara, javno zdravlje, veštačka inteligencija*

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PROGNOSTIC FACTORS FOR LOCOREGIONAL RECURRENCES AFTER SUPRACRICOID LARYNGECTOMY

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Supracricoid laryngectomy, also named open partial horizontal laryngectomy type II (OPH II), involves resection of the ventricular folds, vocal folds, paraglottic space, thyroid cartilage, optionally up to one arytenoid cartilage, and, depending on whether it is an OPH II A or B, of the epiglottis. Locoregional control of the disease is a complex phenomenon that includes the characteristics of the tumor, the patient and genetic factors. This study aimed to identify factors indicating an increased possibility of locoregional recurrences following supracricoid laryngectomy.

Retrospective study included 104 patients who underwent supracricoid laryngectomy at the Otorhinolaryngology and Head and Neck Surgery Clinic of the University Clinical Center of Vojvodina in the period 2002–2020. Pathohistologically verified TN stage, thyroid cartilage invasion, positive margins, vascular invasion, positive Delphi lymph node and extranodal extension of metastatic disease were estimated as predictive factors for locoregional recurrences.

The median time for a local recurrence was 22 months (9.5–59.5), and the median time for regional recurrence was 21 months (10–38.5). Statistical significance in local recurrences of the disease was recorded in the presence of pathohistologically confirmed positive margins ($p = 0.033$) and vascular invasion ($p = 0.029$). The statistical significance of vascular invasion was also observed in regional ($p = 0.041$) and locoregional recurrences ($p = 0.004$). Pathohistologically confirmed higher T stage showed statistical significance in the occurrence of regional metastases ($p = 0.035$).

Pathohistologically confirmed vascular invasion stands out as an independent factor that should be paid attention to in further postoperative therapeutic care in order to achieve high-quality locoregional control of the disease, while in local control, along with vascular invasion, the importance of positive resection margins is also emphasized.

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Key words: *laryngeal carcinoma, organ preservative protocol, open partial horizontal laryngectomy type II, supracricoid laryngectomy*

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Introduction

Laryngeal carcinoma is one of the most common head and neck carcinomas accounting for about 1% of all cancer cases and cancer-related deaths (1). The incidence and mortality of laryngeal carcinoma in 2020 were estimated at 184,615 newly diagnosed cases and 99,840 deaths (2). With the reduction in tobacco use, the incidence of laryngeal carcinoma has decreased by 2.4% each year over the last 10 years (3). The 5-year survival rate of patients with laryngeal carcinomas is estimated at 80% to 90% for early-stage, T1 and T2 tumors (1, 3). In contrast, survival decreases to 40% in patients diagnosed with stage IV disease (3).

Open partial horizontal laryngectomy type II (OPH II), in literature also named supracricoid partial laryngectomy with cricohyoidopexy, was first introduced as an alternative to total laryngectomy for the treatment of selected cases of glottic and supraglottic carcinomas by Mayer in 1959, while in 1974, Piquet described cricohyoidepiglottopexy as a modification of the previously mentioned procedure in the case of glottic carcinoma. This type of partial laryngectomy involves resection of the ventricular

folds, vocal folds, paraglottic space, thyroid cartilage, optionally up to one arytenoid cartilage, and, depending on whether it is a subgroup of OPH II A or B of the epiglottis (resection of the epiglottis present in the case of OPH II B) (4). Preservation of at least one cricoarytenoid unit is crucial for functional recovery after surgery (5). Retrospectively analyzed results of this method showed excellent oncological and functional success in the treatment of glottic and supraglottic carcinomas (6). The main advantage of such a surgical procedure compared to total laryngectomy is reflected in the preserved ability of speech created by bringing air from the lungs, avoiding transient or permanent swallowing disorders as well as permanent tracheostomy, with the presence of very good local control of the disease (4). It is believed that the excellent results of the organ preservation protocol, including OPH II, will be maintained if the indications for its application are strictly followed (7). Traditional guidelines for performing OPH type II in glottic carcinomas include T1a and T1b carcinoma with radiologically suspicious invasion of the l cartilage, T2 carcinoma, T3 carcinoma of the glottis without cancerous invasion of the cricoarytenoid joint but with possible invasion of the anterior commissure and preepiglottic space, as well as carefully selected patients with stage T4 tumors with limited cartilage involvement and extralaryngeal spread in the anterior compartment. OPH II is indicated for T2 supraglottic carcinomas in which supraglottic laryngectomy is contraindicated due to invasion of the floor of the ventricle, extension to the glottis and/or reduced vocal cord mobility, as well as for T3 supraglottic carcinomas involving the preepiglottic space (6, 8, 9). Determining the stage of laryngeal cancer has long been considered a criterion that correlates well with locoregional control of the disease and overall survival, however prognosis is a complex phenomenon that also includes characteristics of the tumor, the patient and genetic factors. Prognosis is further complicated by the fact that negative prognostic factors have not been universally determined, although defining them would greatly facilitate the choice of therapeutic modality (10).

This study aimed to identify factors that might indicate an increased possibility of both local and regional recurrences of laryngeal carcinoma in patients treated with OPH II.

Materials and Methods

This retrospective study included 104 patients who underwent open partial horizontal laryngectomy type II as part of laryngeal cancer treatment at the Otorhinolaryngology and Head and Neck Surgery Clinic of the University Clinical Center of Vojvodina between 2002 and 2020. Of 104 patients, 86 (83%) patients were male, while 18 (17%) were female. The average age of patients at the time of operative treatment was 60 years (ranging from 56 to 67 years). Patients who, in addition to laryngeal carcinoma, had one or more primary localizations of cancer in the head and neck region, as well as patients who had previously been surgically treated for laryngeal carcinoma, were excluded from the study.

After the pathohistological verification of laryngeal carcinoma, all patients were presented at the Oncology Council for Malignancies of the Head and Neck Region of the University Clinical Center of Vojvodina where a decision was made regarding the patient`s surgical management. As part of preoperative assessment, patients underwent contrast-enhanced computer tomography (CT scan) of the head, neck and chest as well as abdominal ultrasound. Before the procedure, each patient was counseled about the planned course of the procedure, potential complications, and the expected postoperative recovery. After the induction of general endotracheal anesthesia, a direct laryngomicroscopy was performed on each patient to gain a better insight into the size and extent of the laryngeal involvement by the tumor. OPH II A was performed in 93 cases, which involved cricohyoidepiglottopexy as part of the neolarynx reconstruction, while OPH II B was performed in 29 patients, which meant cricohyoidopexy as part of reconstruction. Neck dissections were done as diagnostic procedure in 87 patients, and as a therapeutic procedure in 17 patients, in the same acts as OPH II. After the operative treatment, and upon the arrival of the definitive postoperative pathohistological findings, the patients were diagnosed with pathological tumor-node-metastasis (pTNM), and then they were again presented at the Oncology Council for Malignancies of the Head and Neck Region of the University Clinical Center of Vojvodina, where a decision was made to administer adjuvant therapy, which was applied to 20 patients (radiotherapy to 18 patients (17%), chemotherapy to 1 patient (1.0%), and chemoradiotherapy to 1 patient (1.0%)). In the following period, the patients were regularly controlled and monitored by the operator according to the protocol for monitoring patients after surgical treatment of malignancy in the head and neck region. Patients in whom the existence of local or regional recurrence was suspected by clinical examination underwent a CT scan of the head and neck region, on which the existence of recurrence was radiologically verified. After radiological verification of local recurrence, the patients were again presented to the Oncology Council for Malignancies of the Head and Neck Region of the University Clinical Center of Vojvodina, where a decision was made to treat the recurrence of laryngeal malignancy in the form of a conservative approach (radiotherapy, chemoradiotherapy, chemotherapy, symptomatic therapy) or surgical approach (total laryngectomy in case of local recurrence, or modified radical, radical neck dissection or extirpation of a metastatic lymph node).

In order to determine predictive factors for local and regional recurrences after OPH II, the pathohistologically verified TN stage of each patient was taken into account. In addition, the pathohistological parameters obtained after surgical treatment (invasion of the thyroid cartilage, positive resection margins, vascular invasion, positive Delphian lymph node, and extranodal spread of metastatic disease) were considered. Recurrence, both local and regional, was defined as any occurrence of carcinoma at the same localization in the case of local recurrence, or regional occurrence of metastatic disease within 5 years of surgical treatment.

Statistical analyzes were performed using RStudio 2024.04.2 + 764 "Chocolate Cosmos" version. The normality of continuous variables was assessed using the Kolmogorov–Smirnov test. Continuous variables were compared between independent groups using the Wilcoxon rank sum test. Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. Cox proportional hazards modeling was used to examine associations between the outcome and predictor variables. Hazard ratios (HRs) and their 95% confidence intervals (CIs) were used to qualify the magnitude and direction of associations. Kaplan–Maier curves were plotted to visualize survival probabilities, stratified by relevant variables. All statistical tests were two-tailed, and an alpha level of 0.005 was set as the significance threshold. No imputation was used for missing data.

This study was approved by the Ethics Committee of the Clinical Center of Vojvodina, approval number 00-365, approval date: October 15, 2024.

Results

A total of 104 patients participated in this retrospective study (86 men (83%) and 18 women (17%)), with previously histologically verified laryngeal carcinoma, who underwent OPH II as part of the treatment. The average age of the patients was 60 years (range 56 to 67 years). OPH II A was performed in 78 cases (75%), while OPH II B was performed in 26 patients (25%).

In 92 patients (88%), the primary site of the tumor was the glottis, while in the remaining 12 patients (12%), supraglottic carcinoma was surgically treated as the primary origin. Over half of the patients at the time of treatment had T2 stage (54 patients; 52%), while the following were slightly less represented: T3 (30 patients; 29%), T1b (17 patients; 16%), T4a (2 patients; 1.9%) and finally T1a (1 patient; 1.0%). The largest number of patients at the time of operative treatment was in the N0 stage (87 patients; 84%), then N1 (5 patients; 4.8%), while the other groups were equally represented (N2b, N2c, N3b stages—3 patients; 2.9% in each of four groups) (Table 1). All patients were in M0 stage at the time of surgery (104 patients; 100%).

After obtaining definitive pathohistological findings, it was observed that 24 patients (23%) had verified cartilage invasion. Vascular invasion was experienced by 9 patients (8.7%), while perineural invasion was experienced by 10 patients (9.6%). Positive margins were present in 5 patients (4.8%). The Delphian node was affected by metastatic disease in 6 patients (5.8%), while extranodal spread of metastatic disease (extranodal extension (ENE) at the time of surgical treatment) was present in 3 patients (2.9%) (Table 2).

After OPH II, 84 patients (81%) did not receive adjuvant therapy, while 20 patients (19%) received adjuvant therapy, namely, 18 patients (17%) received radiotherapy, while polychemotherapy and chemoradiotherapy were administered to 1 patient each (1.0%).

The average patient follow-up time, the period of time during which the patients regularly came for scheduled check-ups, was 59 months (range from 29 to 100 months).

Of the 104 patients who underwent surgery and were retrospectively reviewed, 86 patients (83%) experienced no local or regional recurrences. The number of patients with local recurrences was 9 (8.7%), while the number of patients with regional recurrences was 15 (14.42%). Only 3 patients (2.5%) had isolated local recurrence of laryngeal cancer, isolated regional recurrence was observed in 9 patients (8.7%), while local and regional recurrences occurred simultaneously in 6 patients (5.8%) (Table 3).

Table 1. Pathohistologically confirmed TN stage of patients at the time of surgery

T stage	***N (%)***
T1a	1 (1.0%)
T1b	17 (16%)
T2	54 (52%)
T3	30 (29%)
T4a	2 (1.9%)
N stage	***N (%)***
N0	87 (84%)
N1	5 (4.8%)
N2a	3 (2.9%)
N2b	3 (2.9%)
N2c	3 (2.9%)
N3b	3 (2.9%)

Table 2. Findings of pathohistological analyses

Cartilage invasion	***N (%)***
Yes	24 (23%)
No	80 (77%)
Vascular invasion	***N (%)***
Yes	9 (8.7%)
No	95 (91.3%)
Perineural invasion	***N (%)***
Yes	10 (9.6%)
No	94 (90.4%)
Positive margins	***N (%)***
Yes	5 (4.8%)
No	99 (95.2%)
Metastasis in Delphian lymph node	***N (%)***
Yes	6 (5.8%)
No	98 (94.2%)
ENE	***N (%)***
Yes	3 (2.9%)
No	101 (97.1%)

Table 3. Local and regional recurrences after OPH II

Cancer recurrence	***N (%)***
No	86 (83%)
Local	3 (2.5%)
Regional	9 (8.7%)
Local + Regional	6 (5.8%)

The median time for local recurrence was 22 months (9.5–59.5) (Figure 1), while the median for regional recurrence was 21 months (10–38.5) (Figure 2).

Regarding the pathohistologically confirmed T stage of laryngeal carcinoma at the time of surgical treatment, statistical significance was observed in predicting regional recurrences (p = 0.035), but no statistical significance was observed in local recurrences (p = 0.18). Although without statistical significance in terms of local recurrences, a higher frequency of recurrences was observed in laryngeal carcinomas treated with OPH II, which at the time of treatment had pathohistologically confirmed T4a stage disease (50%) (Table 4).

When it comes to pathohistologically confirmed N stages of metastatic disease of laryngeal carcinoma at the time of surgical treatment, no statistical significance was observed in both local recurrences (p = 0.71) and regional recurrences (p = 0.36). Although without statistical significance, a higher percentage of regional recurrences can still be observed in patients who, at the time of OPH II, had pathohistologically confirmed N3b stage (67%) (Table 5).

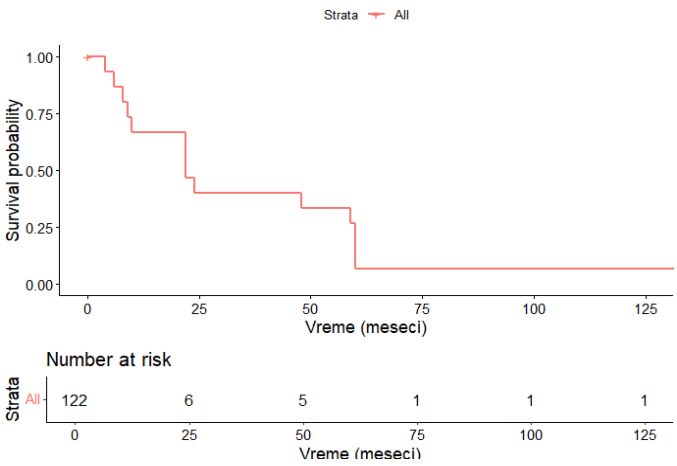

Figure 1. Kaplain–Maier risk curve of local recurrence

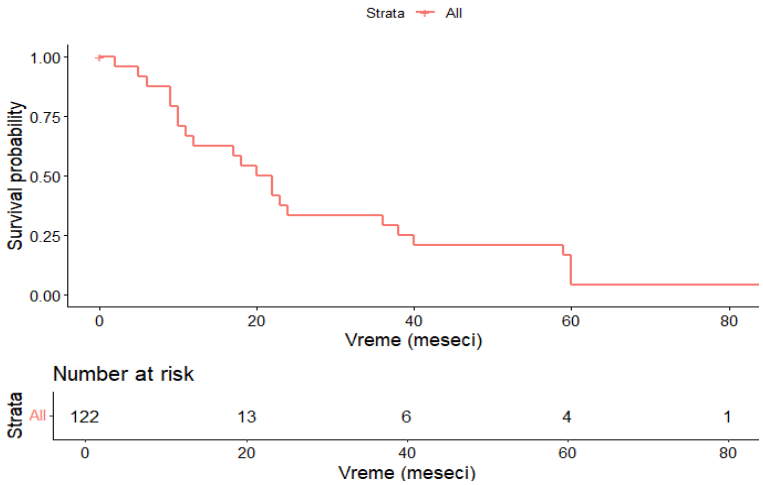

Figure 2. Kaplain–Maier risk curve of regional recurrence

Table 4. Local and regional recurrences in relation to pathohistologically confirmed T stage of laryngeal carcinoma at the time of surgical treatment

	T1a	***T1b***	***T2***	***T3***	***T4a***	**p**
	N = 1	N = 18	N = 60	N = 39	N = 4	
Local recurrence N (%)						0.18
No	1 (100%)	17 (100%)	48 (89%)	28 (93.3%)	1 (50%)	
Yes	0 (0%)	0 (0%)	6 (11%)	2 (6.7%)	1 (50%)	
Regional recurrence N (%)						***0.035***
No	1 (100%)	17 (100%)	42 (78%)	28 (93.3%)	1 (50%)	
Yes	0 (0%)	0 (0%)	12 (22%)	2 (6.7%)	1 (50%)	

Table 5. Local and regional recurrences in relation to pathohistologically confirmed N stage of metastatic disease of laryngeal carcinoma at the time of surgical treatment

	N0	***N1***	***N2a***	***N2b***	***N2c***	***N3b***	**p**
	N = 102	N = 5	N = 3	N = 4	N = 4	N = 4	
Local recurrence N (%)							0.71
No	79 (90.8%)	5 (100%)	3 (100%)	3 (100%)	2 (67%)	3 (100%)	
Yes	8 (9.2%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)	0 (0%)	
Regional recurrence N (%)							0.36
No	75 (86%)	5 (100%)	2 (67%)	3 (100%)	2 (67%)	2 (67%)	
Yes	12 (14%)	0 (0%)	1 (33%)	0 (0%)	1 (33%)	1 (33%)	

Statistical significance in local recurrences of the disease was recorded in the presence of pathohistologically confirmed positive resection margins (p = 0.033), as well as vascular invasion (p = 0.029) (Figure 3).

Statistical significance in regional recurrences was observed in vascular invasion (p = 0.041). Although without proven statistical significance in the Forest plot for the COX proportional hazard model with a confidence interval of 95%, the presence of ENE can indicate an overall greater possibility of regional recurrences of laryngeal cancer (Figure 4).

Locoregional recurrences were associated with a significantly higher frequency of

pathohistologically confirmed vascular invasion (p = 0.004) (Figure 5).

Surgical management of local recurrences included total laryngectomy (6 patients; 67%) and pharyngolaryngectomy (1 patient; 16.5%) (Table 6).

In most cases of regional recurrences (40%), conservative therapy (chemotherapy, radiotherapy, chemoradiotherapy or symptomatic therapy) was applied. Surgical management of regional recurrences in the same number of cases included radical dissection (3 patients; 20%) and selective dissection (3 patients; 20%), then modified radical dissection (2 patients; 13.3%) and finally node extirpation (1 patient; 6.7 %) (Table 7).

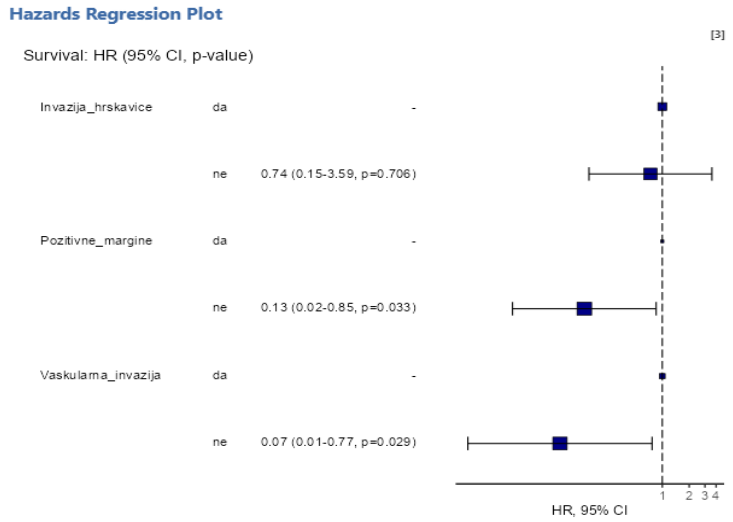

Figure 3. Forest plot of multivariate COX analysis of risk of local recurrence

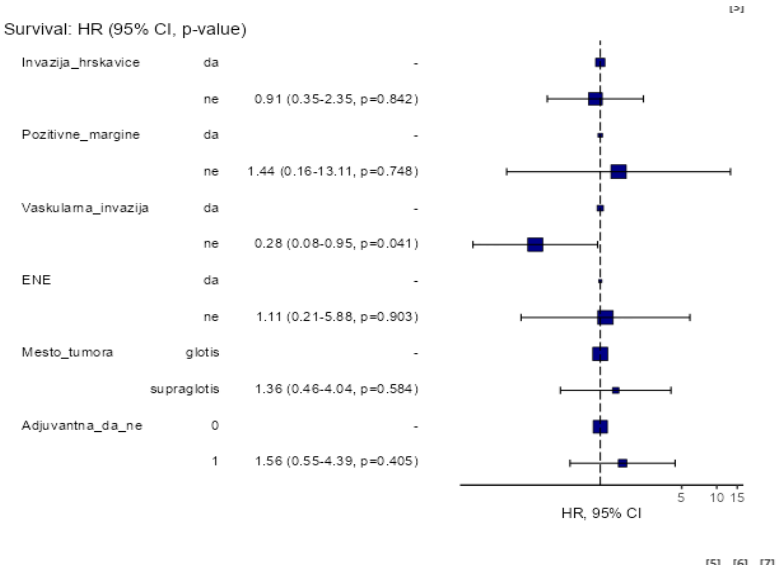

Figure 4. Forest plot of multivariate COX analysis of risk of regional recurrence

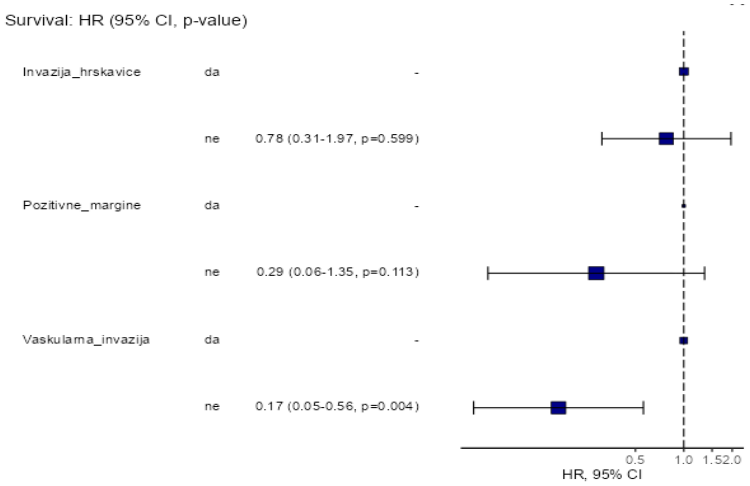

Figure 5. Forest plot of the multivariate COX analysis of the risk of simultaneous local and regional recurrence

Table 6. Surgical management of local recurrences of laryngeal carcinomas

Surgical management	***N (%)***
Without surgery	1 (16.5%)
Total laryngectomy	6 (67%)
Pharyngolaryngectomy	1 (16.5%)

Table 7. Surgical treatment of regional recurrences of laryngeal carcinomas

Surgical management	***N (%)***
Without surgery	6 (40%)
Extirpation of the metastatic lymph node	1 (6.7%)
Selective neck dissection	3 (20%)
Radical neck dissection	3 (20%)
Modified radical neck dissection	2 (13.3%)

Discussion

In 70% of cases, laryngeal carcinomas originate in the glottis, then the supraglottis and finally the subglottis, and squamous cell carcinoma is the main histological type of laryngeal malignancies. The National Comprehensive Cancer Network (NCCN) has developed clinical practice guidelines for the screening, prevention, diagnosis, treatment, and follow-up of patients with various types and locations of cancer, including cancers of the head and neck region (2). Historically, the main therapeutic modality for patients with advanced laryngeal carcinoma has been total laryngectomy, which is associated with functional deficits and was often followed by poor survival if not by adjuvant therapy. The treatment strategy for T1/T2 cancer of the larynx involves an organ preservation protocol, either surgical or conservative, emphasizing the importance of adequate patient selection. The spread of the disease, adequate visualization and comorbidities must be taken into account when choosing the treatment modality (11). As a possible source of confusion, the large heterogeneity within the T3 and T4a groups of tumors can be mentioned (T3 minimal vs. massive involvement of the paraglottic space, with or without cartilage invasion, with or without involvement of the preepiglottic space, different levels of reduced mobility of the vocal cords; T4a anterior extralaryngeal expansion vs. postero-inferior extension through the lateral part of the cricothyroid membrane and the cricothyroid space). The level of diagnostic confusion has been reduced by endoscopic and radiological visualization of laryngeal carcinoma, but none of these methods has yet established a definitive way to determine the cause of vocal cord fixation (7). Currently, multiple surgical and nonsurgical organ-preserving options are available for the treatment of T3 and T4a cancers that are competitive in terms of locoregional control, overall survival, and survival without the need for total laryngectomy. Multiple studies with a large number of patients as one of the options of an organ preservation protocol with a high rate of locoregional control, overall survival, and better functional results compared to total laryngectomy have highlighted OPH (7). In a multicentric study conducted in Italy, it was observed that the overall five-year survival after OPH II was 87.92% and the five-year survival without signs of the disease was 81.87%, which in itself indicates its success (5). OPH II has been highlighted as a type of

laryngectomy with relatively low morbidity, good overall survival, better oncological control than OPH III and long survival without the need for total laryngectomy (7, 12, 13). A systematic review of several studies conducted in the USA indicated that the percentage of local recurrences after a supracricoid laryngectomy in a five-year period was 1.85% (9). Low percentages of local recurrences in the same period of 2.5% were also observed in our study. In our study, locoregional control was achieved in 83% of patients, and 5.77% of all treated patients required total laryngectomy. Only 1 patient (0.96%) underwent pharyngolaryngectomy as part of the surgical management of local recurrences of laryngeal carcinomas, confirming previous claims. If only patients with present local disease were considered, 67% were candidates for total laryngectomy, 16.5% for pharyngolaryngectomy, thus leaving 83.5% of these patients manageable at the time of local recurrence of the disease. The same frequency in the surgical management of regional recurrences was equally distributed between radical and selective neck dissection (20% each). However, the majority of patients with regional recurrences (40%) were not candidates for surgical treatment.

In a multicentric study conducted across several centers in Italy, it was noted that, in case of relapse, the highest percentage involved local relapses, followed by regional and then locoregional with rates of 40%, 25% and 5.7% of patients experiencing relapses, respectively (7). A somewhat different trend was observed in the present study, where the highest percentage was represented by regional recurrences (8.7%), then locoregional (5.8%), and finally local (2.5%). Such a discrepancy between the results can be interpreted as a discrepancy between the pTN patients who participated in the study. While the previously mentioned study included only patients with pT3 and pT4a laryngeal carcinomas, in the current study, only 30.9% of all surgically treated patients were in these two stages. This can actually lead to a conclusion about the importance of the pT stage in the development of local recurrences.

In a multicentric study conducted in two centers in Italy, the results indicate that only pathohistologically confirmed vascular invasion ($p = 0.001$) affects overall survival, while disease-free period was influenced by pT ($p = 0.0001$), pN stage ($p = 0.083$) and vascular invasion ($p = 0.006$). Based on this, perivascular invasion was defined as an independent prognostic factor associated with lower overall survival and disease-free period (5). In our study, vascular invasion proved to be a statistically significant factor in local recurrences ($p = 0.029$), regional recurrences ($p = 0.041$), as well as locoregional recurrences ($p = 0.004$). Pathohistologically confirmed vascular invasion, the only factor that appeared in all three groups of recurrence, is a very strong predictive factor for recurrence. By correlating these results with the results of the previously mentioned study, we underline the importance of this factor in the predictive model of recurrence and in taking it into account when deciding on the application of adjuvant therapy. In addition to vascular invasion, concerning local recurrences, the statistically significant factor in the current study was positive resection margins ($p = 0.033$). Positive margins stood out as an important factor for predicting locoregional recurrences in a study conducted at the University Hospital of Santa Lucia, where it was observed that disease recurrence was present in 22.5% of patients with positive margins, as opposed to 6.1% of patients who did not have positive margins (14). In this study, statistical significance was observed in pT stage and regional recurrences ($p = 0.035$). Statistical significance in local recurrences was not recorded, however, it can be noted that in the pT4a stage, recurrences were present in 50% of cases, which highlights the importance of this factor in the case of local disease control. The absence of statistical significance regarding the pN stage in the prediction of both regional and locoregional recurrences can be explained by the great heterogeneity between the groups, which is the origin of the indications when performing surgery. It is noted that the majority of patients with OPH II were treated in the pN0 stage (84%), while only 2.9% of patients were treated in the pN3b stage. Similar results regarding the importance of vascular invasion and positive margins ($p < 0.001$) were published in a study that subsumed the results from two centers in Italy—Rome and Milan. Also, like the previously mentioned study, this one highlights the importance of pN status in predicting recurrence (15).

In a multicentric study conducted at two centers in Italy, loco-regional recurrences occurred 23.5 ± 14.8 SD months after OPH II (5). In the current study, the median time to local recurrence was 22 months (9.5–59.5), while the median time to regional recurrence was 21 months (10–38.5). These results particularly emphasize the importance of regular monitoring of the patient, especially in the first two to three postoperative years, considering the highest likelihood of recurrence during that period.

Conclusion

With proper consideration of factors related to the patient and tumor, adequate locoregional control of patients treated with an organ preservation protocol is achieved. Patho-histologically confirmed vascular invasion emerges as an independent factor that warrants close attention in postoperative management to achieve optimal locoregional disease control, while in local control, along with vascular invasion, the importance of positive resection margins is also emphasized.

References

1. Huang J, Chan CS, Ko S, Lok V, Zhang L, Lin X, et al. Update disease distributions, risk factors, and trends of laryngeal cancer: a global analysis of cancer registers. International journal of surgeru 2024;110:810-9. [CrossRef] [PubMed]
2. Arboleda LPA, Neves AB,Kohler HF, Vartanian JG, Candelaria LM, Borges MF, et al. Overview of glottic laryngeal cancer treatment recommendation changes in the NCCN guidelines from 2011 to 2022. Cancer reports 2023;6:e1837. [CrossRef] [PubMed]
3. Obid R, Redlich M, Tomeh C. The treatment of laryngeal cancer. Oral Maxillofacial surg. 2019;31:1-11. [CrossRef] [PubMed]
4. Mesolella M, Iorio B, Buono S, Cimmino M, Motta G. Supracricoid partial laryngectomy: Oncological and functional outcomes. Int arch otorhinolaryngol 2022;26(1):75-84. [CrossRef] [PubMed]
5. Allegra E, Bianco MR, Modica DM, Azzolina A, Privitera E, Latella D, et al. Multicentric study on oncological outcomes and prognostic factors of open partial horizontal laryngectomies. Ear, nose and troath journal 2024; 0(0):1-10. [CrossRef] [PubMed]
6. Bron L, Brossard E. Supracricoid partial laryngectomy with cricoidoepiglottopexy and cricohyoidopexy for glottic and supraglotic carcinoma. Laryngoscope 2000;110:627-34. [CrossRef] [PubMed]
7. Succo G, Crosetti E, Bertolin A, Piazza C, Molteni G, Cirillo S, et al. Treatment for T3 to T4a laryngeal cancer by open partial horizontal laryngectomies: Prognostic impact of differen.t pathologic tumor subcategories. Head & Neck 2018;40(9):1897-908. [CrossRef] [PubMed]
8. Brasnu DF. Supracricoid partial laryngectomy with cricohyoidopexy in the menagment of laryngeal carcinoma. World J Surg 2003;27(7):817-23. [CrossRef] [PubMed]
9. Saturno M, Shaari AL, Yun J, Wein LE, Shaari D, Kappauf C, et al. Outcomes of supracricoid partial laryngectomy performed in the United States: A systematic review. The Laryngoscope 2024;134:3003-11. [CrossRef] [PubMed]
10. Crosetti E, Bertolin A, Molteni G, Bertotto I, Balmativola D, Carraro M, et al. Patterns of recurrence after open partial horizontal laryngectomy types II and III: univariate and logistic regression analysis of risk factors. Acta otorhinolaryngologica italica 2019;39:235-43. [CrossRef] [PubMed]
11. Baird BJ, Sung CK, Beadle BM, Divi V. Treatment of early-stage laryngeal cancer: A compariosn of treatment options. Oral Oncology. 2018;87:8-16. [CrossRef] [PubMed]
12. Succo G, Crosseti E, Bertolin A, Lucioni M, Arrigoni G, Panetta V, et al. Intermediate and selected advanced stage laryngeal carcinoma. Head & Neck 2016;38:649-57. [CrossRef] [PubMed]
13. Gallo A, Manciocco V, Simonelli M, Pagliuca G, D'Arcangelo E, De Vincentiis M. Supracricoid partial laryngectomy in the treatmen of laryngeal cancer. Univariate and Multivariate analysis of prognostic factors. Arch otolaryngol head and neck surg. 2005;131:620-5. [CrossRef] [PubMed]
14. De Vincentiis M, Greco A, Campo F, Candelori F, Ralli M, Di Traglia M, et al. Open partial horizontal laryngectomy for T2/T3/T4a laryngeal cancer: oncological outcomes and prognostic factors of two Italian hospitals. European Archives of Oto-Rhino-Laryngology 2022;279:2997-3004. [CrossRef] [PubMed]

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PROGNOSTIČKI FAKTORI LOKOREGIONALNIH RECIDIVA NAKON SUPRAKRIKOIDNE LARINGEKTOMIJE

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Suprakrikoidna laringektomija, koja se imenuje i kao otvorena parcijalna horizontalna laringektomija tip II (OPH II), podrazumeva resekciju ventrikularnih nabora, glasnica, paraglotisnog prostora, tireoidne hrskavice (opciono jedne aritenoidne hrskavice) i, u zavisnosti od toga da li je reč o podgrupi OPH II A ili B, epiglotisa. Lokoregionalna kontrola bolesti predstavlja kompleksan fenomen koji obuhvata karakteristike tumora, pacijenta i genetske faktore. Cilj ove studije bila je identifikacija faktora koji mogu ukazati na povećanu mogućnost pojave lokoregionalnih recidiva posle suprakrikoidne laringektomije.

Ova retrospektivna studija obuhvatila je sto četiri pacijenta lečena na Klinici za otorinolaringologiju i hirurgiju glave i vrata Univerzitetskog kliničkog centra Vojvodine između 2002. i 2020. godine koja su bila podvrgnuta suprakrikoidnoj laringektomiji. Kao prediktivni faktori za pojavu lokoregionalnih recidiva tumora u obzir su uzeti patohistološki verifikovan TN stadijum, invazija tireoidne hrskavice, pozitivne margine, vaskularna invazija, pozitivan delfski limfni čvor i ekstranodalna propagacija metastatske bolesti.

Medijana vremena pojave lokalnog recidiva iznosila je dvadeset dva meseca (9,5–59,5), a medijana pojave regionalnog recidiva dvadeset i jedan mesec (10–38,5). Statistička značajnost u pojavi lokalnih recidiva bolesti zabeležena je u vezi sa prisustvom patohistološki potvrđenih pozitivnih margina ($p = 0,033$) i vaskularnom invazijom karcinoma ($p = 0,029$). Statistička značajnost vaskularne invazije zapažena je i u pojavi regionalnih ($p = 0,041$) i lokoregionalnih recidiva ($p = 0,004$). Patohistološki potvrđen T stadijum pokazao je statističku značajnost u pojavi regionalnih metastaza ($p = 0,035$).

Patohistološki potvrđena vaskularna invazija izdvaja se kao nezavisan faktor na koji treba obratiti pažnju u terapijskom zbrinjavanju posle operacije radi postizanja kvalitetne lokoregionalne kontrole bolesti. U lokalnoj kontroli se uz vaskularnu invaziju ističe i važnost pozitivnih margina resekata.

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***Ključne reči**: laringealni karcinomi, protokol usmeren na očuvanje organa, otvorena parcijalna horizontalna laringektomija tip II, suprakrikoidna laringektomija*

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HORMONE IMBALANCE IN A PATIENT WITH HYPOGONADISM AND CRANIAL BASE TOXOPLASMOSIS

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Toxoplasmosis is a globally prevalent infection caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii*) and represents a widespread and highly adaptable parasitic disease. This case report describes alterations in endocrine function in a patient with *T. gondii* infection. A 45-year-old man was examined by a neurosurgeon due to visual impairment and intermittent headaches. Magnetic resonance imaging of the brain revealed an extracranial presentation characterized by symmetrical subcentimeter cystic lesions at the skull base. There were no signs of intracranial involvement or abnormalities of the *sella turcica*. The patient had secondary hypogonadism with decreased levels of both luteinizing hormone and follicle-stimulating hormone. However, *T. gondii* infection did not result in an increase in testosterone levels. This rare case of a patient with *T. gondii* infection and hypogonadism highlights the requirement for further research to better understand the pathophysiological mechanisms underlying endocrine alterations associated with this parasite.

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Key words: *toxoplasmosis, hypogonadism, skull base*

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Introduction

Toxoplasmosis is a globally prevalent infection caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii*) and represents a widespread and highly adaptable parasitic disease (1). This parasite can infect humans, but it is more commonly found in various animal species, with prevalence rates above 50% in dogs, rabbits, and sea otters, exceeding 60% in rodents and wild birds, and up to 70% in felines, bears, and deer (2, 3). Based on epidemiological assessments, it is highly likely that more than one-third of the global human population has been exposed to this parasite (4). Male hypogonadism is characterized by reduced or absent secretion of sex hormones and impaired reproductive function of the testicles. It is classified as primary hypogonadism due to dysfunction of the gonadal tissue, or central (secondary) hypogonadism, due to dysfunction of the hypothalamic-pituitary axis. Patients present with decreased serum testosterone levels, with variable levels of gonadotropin concentrations. Clinically, hypogonadism may manifest as infertility, reproductive dysfunction, decreased bone mineral density, reduced libido, emotional instability, and fatigue (5).

Recent evidence suggests that *T. gondii* infection may influence human behavior by affecting hormonal regulation, including alterations in testosterone levels. This case report examines endocrine alterations in a patient with *T. gondii* infection.

Case Presentation

A 45-year-old man was examined by a neurosurgeon due to visual impairment and intermittent headaches. Magnetic resonance imaging (MRI) of the pituitary gland revealed a microadenoma located in the left lateral aspect of the gland (Figure 1).

Brain MRI demonstrated an extracranial pattern characterized by symmetrical subcentimeter cystic lesions in both sphenoid bones, the retromaxillary region, the right medial and superior rectus muscles, as well as in both infratemporal fossae adjacent to the parotid glands (Figures 2 and 3). There were no signs of intracranial involvement or involvement of the *sella turcica*.

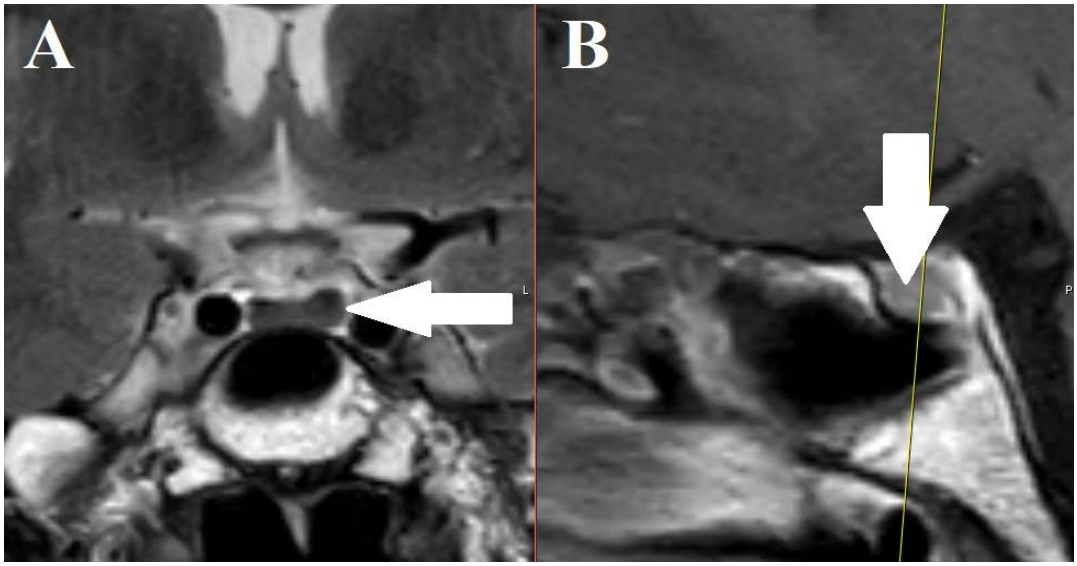

Figure 1. In the left lateral aspect of the adenohypophysis (A, B), an irregular T2-weighted hypointense lesion (arrow), measuring 3 x 5 mm, is observed. The lesion demonstrates delayed post-contrast enhancement and is consistent with a pituitary microadenoma

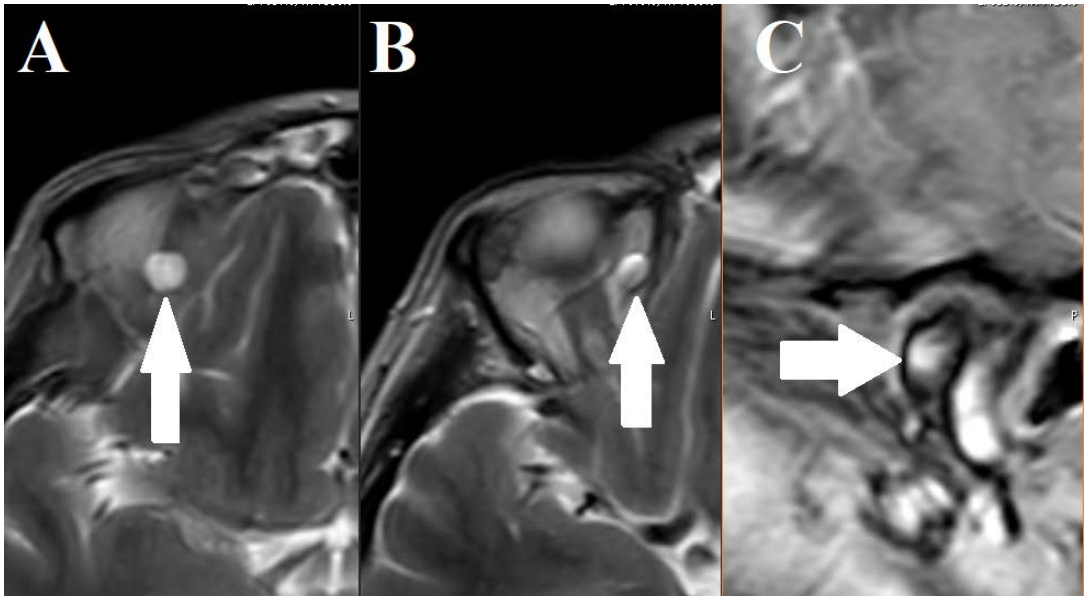

Figure 2. Multiple cystic lesions are present along the superior (A) and medial rectus muscles (B) of the right orbit, as well as within the deep lobe of the left parotid gland (C), as indicated by the white arrows

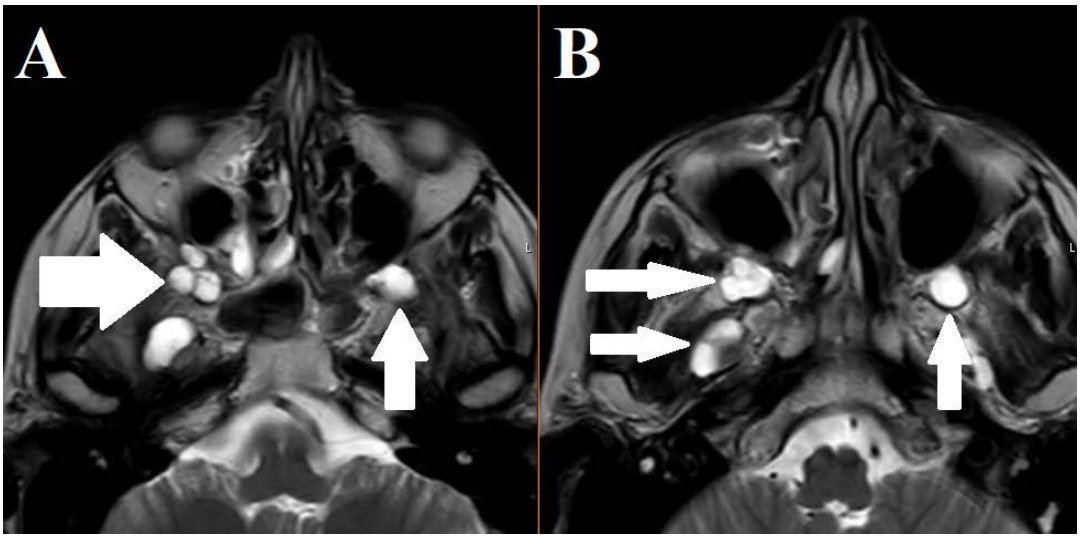

Figure 3. Multiple cystic lesions are present in the bilateral retromaxillary regions (A, B), as indicated by the white arrows

The physical appearance of the patient was specific. He exhibited tall stature, a slender physique, pale skin, and the absence of facial hair. Additionally, the patient had no children and reported no history of marriage or long-term relationships. For the past 20 years, he had lived with several cats. A complete neurological examination exhibited no abnormalities.

As the brain MRI showed no findings of surgical significance, the patient was referred to an infectious disease specialist. Western blot testing for *Toxoplasma gondii* immunoglobulin G and immunoglobulin M antibodies was positive, so conservative treatment was initiated with albendazole (400 mg twice daily for seven days) and prednisone (20 mg once daily for four weeks). In addition, gastroprotective agents, probiotics, and analgesic therapy were prescribed. Follow-up examination after one year showed no progression of disease on the control brain MRI.

Hormonal assessment was performed using an enzyme-linked immunosorbent assay (ELISA) test, with the following results: growth hormone 3.40 ng/ml (reference range < 2.47), serum oxytocin was 248.88 pg/ml (reference range 15.0–242), luteinizing hormone (LH) 0.2 IU/L (reference range 1.2–8.6), follicle-stimulating hormone (FSH) 0.4 IU/L (reference range 1.3–19.3), and testosterone 2.9 nmol/l (reference range 9.9–27.8). Levels of adrenocorticotropic hormone (ACTH), free thyroxine (FT4), thyroid-stimulating hormone (TSH), and prolactin were within the normal limits. The patient was referred to an endocrinologist for further assessment and management of secondary hypogonadism.

At the 12-month follow-up, control brain MRI showed no significant changes in the number or size of the cystic lesions, nor in the size of the microadenoma. Hormonal evaluation was repeated using the ELISA, with the following results: growth hormone 2.97 ng/ml (reference range < 2.47), serum oxytocin was 165.1 pg/ml (reference range 15.0–242), LH 0.1 IU/L (reference values 1.2–8.6), FSH levels were 0.3 IU/L (reference range 1.3–19.3), and testosterone 1.22 nmol/l (reference range 9.9–27.8). The values of ACTH, FT4, TSH, and prolactin remained within the normal limits. Conservative management was continued with analgesic therapy as needed for occasional headaches.

Discussion

It has been estimated that approximately 20–60% of the population in Eastern Europe is infected with *T. gondii* (6). The parasite is most commonly transmitted through the consumption of undercooked food containing tissue cysts, contact with infected feline feces, or vertical transmission during pregnancy. In symptomatic individuals, the most common clinical manifestations are flu-like symptoms, headache, lymphadenopathy, fever, chills, malaise, and fatigue (7). In more severe cases, particularly in immunocompromised patients and neonates, encephalitis, chorioretinitis, and neurodevelopmental impairment may occur (8). Miscarriages and congenital anomalies are also frequently reported. However, in most cases, *T. gondii* infection is asymptomatic, whereas latent toxoplasmosis has been suggested to contribute to sex hormone imbalance and hypogonadism (9).

Furthermore, *Toxoplasma gondii* infection has been associated with various behavioral and psychological alterations in infected individuals (10). The sexual reproduction of this parasite occurs in the feline definitive host, while selective evolutionary pressures have favored mechanisms that enhance transmission from intermediate hosts (e.g., rodents) to cats, thus completing its life cycle. The neurotropic affinity of *T. gondii* for the central nervous system of intermediate hosts

is thought to contribute to host manipulation (11). Additional experimental studies have elaborated on these hypotheses, examining whether *T. gondii* infection alters rodent perception of the predators, particularly cat odor (12, 13). An experimental study analyzed how rodents react to cat odors, as cat odor induces a strong innate aversion, which persists even after multiple generations of laboratory breeding. The results showed that uninfected rats clearly avoided areas with cat odor, while infected rats showed a pathological, potentially suicidal, attraction to areas marked with cat-associated odors (13). Some studies have indicated changes in social and reproductive behavior, including increased interaction between uninfected females and infected males, resulting in a higher number of matings between them, which is one of the confirmed modes of transmission of this parasite (14).

Other studies have investigated the pathophysiology of behavioral changes in rats and have demonstrated a positive correlation between behavioral alterations and the expression of genes involved in testosterone synthesis in experimentally infected male rats (15).

Behavioral alterations in infected patients may be preceded by encephalitis, with different predilection sites depending on the *T. gondii* strains (16). Some authors have analyzed the effects of *T. gondii* infection on libido and perceived attractiveness in men and women, reporting increased libido and attractiveness among infected individuals (15). Some of the results in infected participants had lower fluctuating facial asymmetry, while women with *T. gondii* infection had lower body mass index, a higher number of sexual partners, and higher levels of self-rated attractiveness compared with uninfected controls.

On the other hand, one study reported that both *T. gondii*-infected men and women were rated as attractive and healthier by uninfected individuals (15). A potential route of interhuman transmission of the infection may include unprotected sexual intercourse and oral sex (17). These hypotheses are supported by studies involving female sex workers (18) and individuals with a history of multiple sexual partners (19) in Latin America. On the other hand, seropositive men infected with *T. gondii* are significantly more promiscuous than uninfected men (19). Furthermore, *T. gondii* infection has been proposed as a potential contributing factor in the development of schizophrenia (10), depression, anxiety disorders, obsessive-compulsive disorder (19), and autism spectrum disorder (10, 20–22).

Numerous studies have shown a positive correlation between *T. gondii* infection and elevated testosterone levels (9). Most human studies have demonstrated elevated testosterone levels after latent toxoplasmosis in men with latent toxoplasmosis, while three studies have noted decreased levels, and two studies observed no significant changes. These discrepancies in results could be explained by the incompletely understood pathophysiological mechanisms of *T. gondii* infection in humans, which selectively stimulate libido and sexual behavior in ways that may facilitate transmission. Whether *T. Gondii* infection influences secondary hypogonadism and reduced libido remains unclear. In the present case report, no such association was observed. Although our patient had secondary (central) hypogonadism and pituitary microadenoma, with low levels of both LH and FSH, *T. gondii* infection did not lead to an increase in testosterone levels. During a two-year follow-up, the patient's clinical appearance and sex hormones remained unchanged. His serological findings showed the persistence of infection despite therapy, without any increase in testosterone levels.

Additionally, lower testosterone levels observed in infected humans and animals may contribute to infertility and lower libido. A potential explanation is that different *T. gondii* strains and varying intensities of infection—detected by serological tests, polymerase chain reaction, and microscopic methods— may play an important role in the observed variation in testosterone levels (23).

Conclusion

This rare case of a *Toxoplasma gondii* infection associated with hypogonadism highlights the need for further research to better understand the underlying pathophysiological mechanisms.

References

1. Boothroyd JC, Grigg ME. Population biology of Toxoplasma gondii and its relevance to human infection: do different strains cause different diseases? Curr Opin Microbiol 2002;5(4):438-42. [PubMed]
2. Lafferty KD. Can the common brain parasite, Toxoplasma gondii, influence human culture? Proc R Soc B 2006;273(1602):2749-55. [CrossRef] [PubMed]
3. Webster JP. Prevalence and transmission of Toxoplasma gondii in wild brown rats, Rattus norvegicus. Parasitology 1994;108(Pt 4):407-11. [CrossRef] [PubMed]
4. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. Int J Parasitol 2000;30(12):1217-58. [CrossRef] [PubMed]
5. Barber TM, Kyrou I, Kaltsas G, Grossman AB, Randeva HS, Weickert MO. Mechanisms of Central Hypogonadism. Int J Mol Sci 2021;22(15):8217. [CrossRef] [PubMed]
6. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol 2009;39(12):1385-94. [CrossRef] [PubMed]
7. Dubey JP, Jones JL. *Toxoplasma gondii* infection in humans and animals in the United States. Int J Parasitol 2008;38(11):1257-78. [CrossRef] [PubMed]
8. Flegr Jaroslav. Effects of *Toxoplasma* on human behavior. Schizophr Bull 2007;33(3): 757-60. [CrossRef] [PubMed]
9. Abdoli A, Ghaffarifar F, Sharifi Z, Taghipour A. Toxoplasma gondii infection and testosterone alteration: A systematic review and meta-analyses. PLoS One 2024;19(4):e0297362. [CrossRef] [PubMed]
10. Webster JP. The effect of Toxoplasma gondii on animal behavior: playing cat and mouse. Schizophr Bull 2007;33(3):752-6. [CrossRef] [PubMed]
11. Werner H, Masihi KN, Senk U. Latent Toxoplasma infection as a possible risk factor for CNS disorders. Zentralbl Bakteriol Mikrobiol Hyg A 1981;250(3):368-75. [CrossRef] [PubMed]
12. Berdoy M, Webster JP, Macdonald DW. Fatal attraction in Toxoplasma-infected rats: a case of parasite manipulation of its mammalian host. Proc R Soc B 2000;267(1452):1591–4. [CrossRef] [PubMed]
13. Webster JP, Lamberton PHL, Donnelly CA, Torrey EF. Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer, and anti-protozoan medication on T. gondii's ability to alter host behaviour. Proc Biol Sci 2006;273(1589):1023-30. [CrossRef] [PubMed]
14. Dass SAH, Vasudevan A, Dutta D, Soh LJT, Sapolsky RM, Vyas A. Protozoan parasite *Toxoplasma gondii* manipulates mate choice in rats by enhancing attractiveness of males. PloS One 2011;6(11):e27229. [CrossRef] [PubMed]
15. Lim A, Kumar V, Hari Dass SA, Vyas A. Toxoplasma gondii infection enhances testicular steroidogenesis in rats. Mol Ecol 2013;22(1):102-10. [CrossRef] [PubMed]
16. Borráz-León JI, Rantala MJ, Krams IA, Cerda-Molina AL, Contreras-Garduño J. Are *Toxoplasma*-infected subjects more attractive, symmetrical, or healthier than non-infected ones? Evidence from subjective and objective measurements. PeerJ 2022;10:e13122. [CrossRef] [PubMed]
17. Flegr J, Klapilová K, Kaňková Š. Toxoplasmosis can be a sexually transmitted infection with serious clinical consequences. Not all routes of infection are created equal. Med Hypotheses 2014;83(3):286-9. [CrossRef] [PubMed]
18. Alvarado-Esquivel C, Sánchez-Anguiano LF, Hernández-Tinoco J, Arreola-Cháidez E, López J, Salcido-Meraz KI, et al. High seroprevalence of *Toxoplasma gondii* infection in female sex workers: a case-control study. Eur J Microbiol Immunol 2015;5(4):285-92. [CrossRef] [PubMed]
19. Alvarado-Esquivel C, Estrada-Martínez S, Ramos-Nevárez A, Pérez-Álamos AR, Beristain-Garcia I, Alvarado-Félix ÁO, et al. Is *Toxoplasma gondii* Infection Associated with Sexual Promiscuity? A Cross-Sectional Study. Pathogens 2021;10(11):[1393 p]. [CrossRef] [PubMed]
20. Groër MW, Yolken RH, Xiao JC, Beckstead JW, Fuchs D, Mohapatra SS, et al. Prenatal depression and anxiety in *Toxoplasma gondii*–positive women. Am J Obstet Gynecol 2011;204(5):433. [CrossRef] [PubMed]
21. Nayeri Chegeni T, Sarvi S, Amouei A, Moosazadeh M, Hosseininejad Z, A. Aghayan S, et al. Relationship between toxoplasmosis and obsessive compulsive disorder: A systematic review and meta-analysis. PLoS Negl Trop Dis 2019;13(4):e0007306. [CrossRef] [PubMed]
22. Al Malki JS, Hussien NA, Al Malki F. Maternal toxoplasmosis and the risk of childhood autism: serological and molecular small-scale studies. BMC Pediatr 2021;21(1):133. [CrossRef] [PubMed]
23. Abdoli A. *Toxoplasma*, testosterone, and behavior manipulation: the role of parasite strain, host variations, and intensity of infection. *Front Biol* 2014;9(2):151–60. [CrossRef]

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HORMONSKI DISBALANS KOD PACIJENTA SA HIPOGONADIZMOM I TOKSOPLAZMOZOM BAZE LOBANJE

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Toksoplazmoza predstavlja globalno rasprostranjenu infekciju koju izaziva parazit Toxoplasma gondii (*T. gondii*), prilagodljiv i široko rasprostranjen organizam. Ovaj prikaz slučaja zasnovan je na ispitivanju promena u hormonskom disbalansu kod pacijenta sa infekcijom parazitom *T. gondii*. Kod četrdesetpetogodišnjeg muškarca, kojeg je neurohirurg pregledao zbog problema sa vidom i povremenih glavobolja, magnetnom rezonancom mozga utvrđeno je prisustvo ekstrakranijalnih simetričnih cističnih lezija manjih od jednog centimetra u bazi lobanje. Nije bilo znakova intrakranijalnog zahvatanja, kao ni zahvatanja u selarnoj regiji. Iako je ovaj pacijent imao sekundarni hipogonadizam, sa niskim vrednostima i luteinizirajućeg hormona (LH) i folikostimulirajućeg hormona (FSH), parazit *T. gondii* nije doveo do povećanja nivoa testosterona. Ovaj redak slučaj pacijenta sa infekcijom parazitom *T. gondii* i hipogonadizmom naglašava potrebu za daljim istraživanjem, koje je neophodno sprovesti da bi se bolje razumeli patofiziološki mehanizmi koji leže u osnovi infekcije ovim parazitom.

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***Ključne reči**: toksoplazmoza, hipogonadizam, baza lobanje*

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THE INFLUENCE OF NIGHT WORK, DIET, ALCOHOL CONSUMPTION, AND CIGARETTE SMOKING ON THE STRESS LEVEL OF SECURITY WORKERS

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Night work, characteristic of occupations such as security guard work, presents unique challenges to workers' health on biological, psychological, and social levels. Circadian rhythms are disrupted due to night shifts, leading to sleep disorders, increased stress, and metabolic imbalances. This study analyzes how age group, night work, smoking, alcohol consumption, diet, and body mass index (BMI) affect stress levels among security workers. A sample of 154 workers was assessed through surveys and standardized stress measurement tools. Key findings indicate significant effects of night work, age, smoking, alcohol consumption, and BMI on stress levels, with a balanced diet being associated with reduced stress. Practical suggestions include promoting healthy habits to reduce stress and improve workers' overall health.

Night work, typical jobs such as security guard work, poses distinct challenges to worker health on biological, psychological, and social levels. Circadian rhythms, which regulate biological processes in the body, are disrupted due to shift work, especially night shifts, potentially leading to sleep disorders, increased stress, metabolic disturbances, and poor eating habits. Lack of sleep and improper diet contribute to reduced work performance and increase the risk of chronic illnesses such as diabetes and cardiovascular diseases. Additionally, alcohol and cigarette use are often coping mechanisms for stress and insomnia, further exacerbating health issues.

The research aimed to analyze how different age groups, night work, smoking, alcohol consumption, diet, and BMI influence stress levels in security workers, with a special focus on how night shifts shape health habits and overall well-being. The study was conducted on a sample of 154 workers from various age groups and work environments, using a survey to assess work-related habits, health, diet, smoking, alcohol use, and BMI measurement.

The results show that age group and night work significantly affect stress levels; older workers and those on night shifts report higher levels of stress. Smoking and alcohol use further increase stress, while BMI shows a significant impact on stress, particularly during night shifts. A balanced diet is associated with reduced stress. The conclusion is that night work, age, smoking, alcohol consumption, BMI, and diet significantly influence stress levels among security workers, and changes in unhealthy habits can help reduce stress.

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Key words: *stress, diet, night work, alcohol*

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Introduction

Since ancient times, people have sought to protect themselves and their property. The concept of security is as old as civilization itself, with the mechanisms of protection evolving over time (1). In the 21st century, with significant technological advancements, the role of security has become a sophisticated and professional occupation. Modern security guards are equipped with advanced devices, artificial intelligence, drones, and robotics, and they undergo rigorous training to be prepared for the challenges of contemporary society.

To perform their duties successfully, they must possess physical fitness, observational skills, decision-making and communication abilities, conflict resolution skills, emergency response capabilities, first aid knowledge, and technological literacy (2). Considering all these factors, working in security services entails a high level of psychophysiological strain, stemming from several

key factors that contribute to both physical and emotional stress. Security guards are often exposed to verbal and physical aggression, violence, and even the use of force, which further intensifies stress, especially in situations where they must carry or use firearms. This exposure can lead to the development of post-traumatic stress and burnout syndrome, significantly affecting their health and well-being (3–6).

Workplace stress is generally on the rise. In major global economies, daily stress levels among employees have reached record highs (7). The professional services sector is among the industries with the highest stress levels, with a large percentage of employees reporting work-related stress (8). As a result, approximately 17 million workdays are lost annually worldwide due to mental health disorders caused by stress, depression, or anxiety, accounting for more than half of all work-related illness cases (8, 9).

The work environment is also another source of stress for security personnel (10). These workers are often exposed to unfavorable micro- and macro-climatic working conditions, working in noisy or confined spaces, with fixed body positions and static exertion, along with irregular and extended working hours, shift work, and night shifts.

In recent years, many sectors have organized their work in shifts. In Europe, a smaller percentage of workers regularly work night shifts, while more than 15 million Americans work night shifts (11, 12). In the Republic of Serbia, night work is defined by the Labor Law, Article 62: "Work performed between 10:00 p.m. and 6:00 a.m. the following day is considered night work" (13).

Shift work, particularly night shifts, is one of the leading sources of stress, as it involves working and sleeping against normal chronobiological rhythms. This unnatural routine, forcing people to work at night when the body promotes sleep and to sleep during the day when the body is most active, can lead to changes in diet, misalignment of meal schedules with biological rhythms of hunger, satiety, and metabolism (14), and an increased risk of developing many chronic diseases. All these factors trigger a range of health problems among security personnel, affecting their work capacity (15, 16).

Methodology

The study was conducted on a sample of 154 workers across different age groups and work environments to assess stress levels in relation to factors such as age group, night work, smoking, alcohol consumption, diet, and BMI. Data were collected using a survey questionnaire that covered work habits, health habits, diet, and stress perception. Stress levels were measured using the standardized Perceived Stress Scale (PSS) (17).

BMI was calculated using the following formula:

BMI = Weight (kg)/Height (m)²

The study was conducted over a six-month period. For data analysis, Analysis of Covariance (ANCOVA) was used to assess the impact of independent variables (age group, night work, smoking, alcohol consumption, BMI) on stress levels, while controlling for the effects of diet. The study was conducted with participants' informed consent in accordance with privacy and ethical standards for data protection.

Results

The connection between age, night work, and stress levels is shown in Table 1.

Statistical Analysis

ANCOVA analysis of the influence of age group and night work on stress level

·Age group:
Sum of squares (SS): 120.5
·Mean square (MS): 40.17
·F-value: 5.23
·p-value: 0.002
Interpretation: The effect of age group on stress level was statistically significant ($p < 0.05$), meaning that different age groups had different levels of stress, even after accounting for covariates such as diet.

2. Night work
·Sum of squares (SS): 95.3
·Mean square (MS): 95.3
·F-value: 12.41
·p-value: 0.0005
Interpretation: The effect of night work on the stress level was highly statistically significant ($p < 0.01$), showing that night shift work had a significant impact on stress levels.

3. Interaction (age group x night work)

·Sum of squares (SS): 45.7
·Mean square (MS): 15.23
·F-value: 1.98
·p-value: 0.119
Interpretation: The interaction between age group and night work was not statistically significant ($p > 0.05$), indicating that the effect of age group on stress level did not significantly depend on whether a person worked at night or not.

4. Covariate (diet)

·Sum of squares (SS): 70.2
·Mean square (MS): 70.2
·F-value: 9.14
·p-value: 0.003

Interpretation: The covariate, in this case diet, had a significant effect on the stress level ($p < 0.01$). Differences in diet were associated with differences in stress levels.

5. Error:

·Sum of squares (SS): 985.0

·Mean square (MS): 6.74

6. Total:

·Sum of squares (SS): 1316.7

The results shown in Table 1 indicate that the age of the respondents and night work had a significant impact on the stress level, while the interaction between age group and night work was not significant. Diet, as a covariate, had a significant impact on stress levels.

Table 2 shows the connection between the age of the respondents and the level of stress.

The results showed that the level of stress was higher in workers who worked at night across all age groups. Older workers (51+) experienced the highest levels of stress, while younger workers (20–30 years old) reported the lowest levels of stress, regardless of their working hours. The effect of night work was constant across all age groups, indicating that night work generally affects stress levels, regardless of age. It can be concluded that night work was the main factor that increased stress levels, while the age group also had a significant impact, but not in interaction with night work. Diet, as a covariate, also contributed to differences in stress levels. Older workers generally showed higher levels of stress compared to younger workers, especially when working at night.

Statistical Analysis

The results of the ANCOVA analysis are shown in Tables 1 and 2. Key findings include:

1. **Age group** had a significant effect on stress level ($F = 5.23$, $p = 0.002$). Older workers (51+) reported a higher level of stress compared to younger groups (20–30 years old), likely due to physiological changes and greater responsibilities that come with age.

2. **Night work** significantly increased the level of stress ($F = 12.41$, $p = 0.0005$). Night shift workers reported higher levels of stress compared to day shift workers, probably due to disruption of circadian rhythms and lack of sleep.

3. **Smoking** was also a significant factor that increased the level of stress ($F = 6.59$, $p = 0.011$). Smokers reported higher levels of stress compared to nonsmokers.

4. **Alcohol consumption** had a significant effect on the stress level ($F = 5.23$, $p = 0.024$). People who regularly consumed alcohol showed higher levels of stress.

5. **Body mass index** showed a significant effect on stress level ($F = 8.13$, $p = 0.004$). People with a higher BMI reported higher levels of stress, potentially related to physical burdens and self-perception of health.

6. **Diet**, regarded as a covariate, showed a significant effect on the level of stress ($F = 9.14$, $p = 0.003$). Workers maintaining a balanced diet reported lower levels of stress compared to those with an irregular or unhealthy diet.

7. **Interactions between factors** did not show significant results, suggesting that the effects of age group, night work, smoking, alcohol, and BMI on stress level were not mutually dependent.

The relationship between the age of the respondents, night work, cigarette smoking, and alcohol consumption with new stress is shown in Table 3.

Table 1. ANCOVA analysis of the effect of age group and night work on stress level

Factor	Sum of squares (SS)	df	Mean square (MS)	F-value	p-value
Age group	120.5	3	40.17	5.23	0.002
Night work	95.3	1	95.3	12.41	0.0005
Interaction (age group x night work)	45.7	3	15.23	1.98	0.119
Covariate (diet)	70.2	1	70.2	9.14	0.003
Error	985.0	146	6.74	-	-
Total	1316.7	154	-	-	-

Table 1 shows the key statistical parameters of the ANCOVA analysis, including the F-value and p-value, which indicate the significance of the effects

Table 2. Mean values of stress levels by age groups and night work (with covariate control)

Age group	Night work	Stress level (median value)
20–30	Yes	25.6
20–30	No	22.4
31–40	Yes	27.9
31–40	No	24.1
41–50	Yes	30.5
41–50	No	26.7
51+	Yes	32.2
51+	No	28.9

Table 3. ANCOVA analysis of the effect of age group, night work, smoking, and alcohol on stress level

Factor	Sum of squares (SS)	df	Mean square (MS)	F-value	p-value
Age group	120.5	3	40.17	5.23	0.002
Night work	95.3	1	95.3	12.41	0.0005
Smoking	50.4	1	50.4	6.59	0.011
Alcohol	40.1	1	40.1	5.23	0.024
Interaction (age group x night work)	45.7	3	15.23	1.98	0.119
Interaction (age group x smoking)	20.5	3	6.83	0.88	0.455
Interaction (age group x alcohol)	18.3	3	6.10	0.78	0.508
Interaction (night work x smoking)	15.2	1	15.2	1.94	0.167
Interaction (night work x alcohol)	12.7	1	12.7	1.62	0.210
Covariate (diet)	70.2	1	70.2	9.14	0.003
Error	900.0	142	6.34	-	-
Total	1316.7	153	-	-	-

Table 3 shows the key statistical parameters of the ANCOVA analysis, including the F-value and p-value for the effects of age group, night work, smoking, alcohol, and their interactions

Detailed mean stress levels by age group, night work, smoking, and alcohol consumption are presented in Table 4.

The connection between age, night work, cigarette smoking, and alcohol consumption with the level of stress is shown in Table 4.

The combination of smoking and alcohol consumption further increased the level of stress, especially in older age groups and night workers. The analysis showed that age group, night work, smoking, alcohol consumption, and diet were all significant factors affecting stress levels:

- **Age group and night work** had a significant effect on stress levels, with older workers and night shift workers reporting higher levels of stress.
- **Smoking and alcohol** additionally increased the level of stress, indicating the need for interventions focused on changing lifestyle habits.
- **Diet** had a significant impact on stress levels; workers with a balanced diet experienced lower levels of stress.
- **Interactions between these factors** indicated that the effects of smoking, alcohol, and diet did not change significantly depending on age group or night work status, but all these factors together contributed to the overall stress level.

The results of ANCOVA analysis, including body mass index (BMI) as an additional factor, are shown in Table 5, confirming BMI as a significant predictor of stress levels.

Statistical analysis

1. BMI as a factor:

• BMI was a significant factor ($p = 0.002$) affecting the level of stress. People with an increased BMI (overweight or obese) had higher levels of stress compared to people with a normal BMI.

2. Interactions of BMI with other factors:

• BMI x night work: There was a marginal interaction between BMI and night work ($p = 0.056$), suggesting that the effect of night work on stress levels may be enhanced in individuals with a higher BMI.

• BMI x smoking and alcohol: No significant interactions were found between BMI and smoking or alcohol, meaning that the presence of these factors did not further modify the effects of BMI on stress levels.

3. Median values of stress level:

• The level of stress increased with increasing BMI in all age groups and for both night work statuses.

• The combination of increased BMI, smoking, and alcohol consumption further increased stress levels, especially in older workers and those working night shifts.

Table 6 presents the adjusted mean stress values across combinations of all key variables—age, night work, BMI, smoking, and alcohol—while controlling for the effect of diet.

By introducing BMI as an additional factor in the ANCOVA analysis, it was observed that an increased BMI significantly contributed to a higher level of stress, independent of age group, night work, smoking, and alcohol consumption. This suggests the need for interventions aimed at controlling body mass, taking into account other factors such as night work and unhealthy habits, in order to reduce stress levels among workers.

Table 4. Median values of stress levels by age groups, night work, smoking, and alcohol consumption (with covariate control)

Age group	Night work	Smoking	Alcohol	Stress level (median value)
20–30	Yes	Yes	Yes	27.0
20–30	Yes	Yes	No	26.1
20–30	Yes	No	Yes	26.5
20–30	Yes	No	No	24.5
20−30	No	Yes	Yes	23.0
20−30	No	Yes	No	22.0
20–30	Doesn’t work	No	Yes	22.4
20–30	Doesn’t work	Ne	Ne	20.5
31–40	Works	Yes	Yes	30.0
31–40	Works	Yes	No	28.5
31–40	Works	No	Yes	29.0
31–40	Works	No	No	26.5
31–40	Doesn’t work	Yes	Yes	25.0
31–40	Doesn’t work	Yes	No	24.0
31–40	Doesn’t work	No	Yes	24.5
31–40	Doesn’t work	No	No	22.0
41–50	Works	Yes	Yes	33.0
41–50	Works	Yes	No	31.5
41–50	Works	No	Yes	32.0
41–50	Works	No	No	29.5
41–50	Doesn’t work	Yes	Yes	28.0
41–50	Doesn’t work	Yes	No	26.5
41–50	Doesn’t work	No	Yes	27.0
41–50	Doesn’t work	No	No	24.0
51+	Works	Yes	Yes	35.0
51+	Works	Yes	No	33.5
51+	Works	No	Yes	34.0
51+	Works	No	No	31.5
51+	Doesn’t work	Yes	Yes	30.0
51+	Doesn’t work	Yes	No	28.5
51+	Doesn’t work	No	Yes	29.0
51+	Doesn’t work	No	No	26.5

The results show that the stress level increases with the number of covariates (smoking and alcohol) and with age, which indicates the cumulative effect of several unhealthy habits and age on the stress level

Night shift work consistently contributes to higher levels of stress, regardless of smoking or alcohol consumption

Table 5. ANCOVA analysis of the effect of age group, night work, BMI, smoking, and alcohol on stress level

Factor	Sum of squares (SS)	df	Mean square (MS)	F-value	p-value
Age group	115.0	3	38.33	4.75	0.003
Night work	92.0	1	92.0	11.42	0.001
BMI	85.0	1	85.0	10.55	0.002
Smoking	50.4	1	50.4	6.25	0.013
Alcohol	40.1	1	40.1	5.00	0.027
Interaction (age group x night work)	42.0	3	14.0	1.74	0.159
Interaction (BMI x night work)	30.0	1	30.0	3.72	0.056
Interaction (BMI x smoking)	20.5	1	20.5	2.54	0.113
Interaction (BMI x alcohol)	18.3	1	18.3	2.27	0.135
Covariant (diet)	70.2	1	70.2	8.68	0.004
Error	880.0	140	6.29	-	-
Total	1315.0	153	-	-	-

Table 5 shows the key statistical parameters of the ANCOVA analysis, including the F-value and p-value for the effects of age group, night work, BMI, smoking, alcohol, and their interactions

Table 6. Median values of stress levels by age groups, night work, BMI, smoking, and alcohol consumption (with covariate control)

Age Group	Night work	BMI	Smoking	Alcohol	Stress level (median value)
20–30	Works	Normal	Yes	Yes	26.5
20–30	Works	Increased	Yes	No	28.0
20–30	Doesn't work	Normal	No	Yes	22.4
20–30	Doesn't work	Increased	No	No	24.5
31–40	Works	Normal	Yes	Yes	29.5
31–40	Works	Increased	Yes	No	31.0
31–40	Doesn't work	Normal	No	Yes	25.5
31–40	Doesn't work	Increased	No	No	27.0
41–50	Works	Normal	Yes	Yes	31.5
41–50	Works	Increased	Yes	No	33.5
41–50	Doesn't work	Normal	No	Yes	28.0
41–50	Doesn't work	Increased	No	No	30.0
51+	Works	Normal	Yes	Yes	33.5
51+	Works	Increased	Yes	No	35.5
51+	Doesn't work	Normal	No	Yes	30.0
51+	Doesn't work	Increased	No	No	32.0

Table 6 shows the median values of stress levels taking into account age group, night work, BMI, smoking, and alcohol consumption, while controlling for the effect of diet

Discussion

Alongside changes in the world of work, processes of privatization, transition, rationalization, and job loss, a large number of people in our country have secured their livelihood by working in the physical-technical security. On the other hand, the rise in insecurity, aggression, and violence—both in everyday life and the workplace—necessitates the employment of workers in this field. From the perspective of occupational health measures, preserving their health within the framework of occupational medicine is essential. According to the literature, shift work, particularly night work, poses a significant health challenge for workers, affecting their circadian rhythm, sleep quality, and long-term risk of various health problems (18).

By 2040, a significant increase in the number of older workers is expected, which will impact workforce structure, productivity, and economic growth. According to OECD data, if current patterns of work and retirement do not change, the number of inactive elderly individuals that each worker will have to support is projected to increase by approximately 40% between 2018 and 2050. This presents a challenge to the sustainability of labor markets and economic systems (19). Stress, defined as an adverse reaction to excessive pressure, as well as burnout syndrome, has been recognized as a major health risk for all workers, particularly those working night shifts.

However, it is clear that older workers likely face different stressors, not only workplace pressures but also because of additional responsibilities outside work, such as caring for their families. Moreover, although laws prohibit discrimination, evidence suggests that older workers remain vulnerable to age discrimination (20, 21).

One of the causes of stress among night workers is the disruption of sleep duration and quality, which, to some extent, depends on genetics, physical and mental health, and external factors. Poor sleep reduces work performance, causes chronic fatigue, and increases the risk of accidents, malignancies, and autoimmune diseases.

In this regard, according to the results of this analysis, workers over the age of 50 exhibit the highest stress levels, while younger workers experience the lowest stress levels, regardless of whether they work night shifts or not. The effect of night work remains consistent across all age groups, indicating that night shifts may have a universal impact on stress levels. In general, older workers show higher levels of stress compared to younger ones, especially when working night shifts.

Night work is the primary factor that increases stress levels, while age group also has a significant impact, though not in interaction with night work, according to our results. Diet, as a covariate, also contributes to differences in stress levels.

The high levels of stress that security workers are exposed to may contribute to weight gain and obesity due to increased cortisol secretion (22, 23), a hormone that stimulates appetite for high-calorie foods. Additionally, stress can disrupt the secretion of hunger and satiety hormones (leptin and ghrelin), which are regulated by the circadian rhythm, thereby promoting nighttime eating and affecting sleep patterns (24, 25).

On the other hand, obesity can contribute to increased stress due to physical health issues, reduced energy, low self-esteem, and social pressure. This creates a vicious cycle in which stress leads to obesity, and obesity further increases stress, ultimately affecting physical health and harming employees' family and social lives (26). Additionally, it may lead to compensatory maladaptive behaviors such as excessive alcohol consumption and smoking.

From a chronobiological perspective, humans are diurnal beings, meaning they are naturally active during the day. This explains why shift work, particularly night shifts, can be challenging. Due to circadian rhythm disruption, the health of night shift workers often suffers more than that of those who work only during the day. Night workers are at higher risk of various metabolic disorders and diseases, resulting from a disrupted biological clock, lack of sleep, increased psychosocial stress, physical inactivity, fatigue syndrome due to insufficient rest and recovery, and attempts to mitigate exhaustion through excessive consumption of high-calorie foods, smoking, or alcohol intake.

Shift workers, especially those working night shifts, often experience problems such as reduced sleep quality and duration, as well as symptoms of insomnia. Their daytime sleep periods typically last between 4 and 6 hours, after which they frequently wake up and struggle to fall back asleep (27–30). These workers find it difficult to maintain good sleep quality, as their sleep is often disturbed by external factors such as street noise, family obligations, and daylight exposure. All of these factors contribute to the development of stress (31, 32).

Melatonin, the hormone that regulates the sleep-wake cycle, is secreted at night and reaches its highest levels during childhood. As people age, melatonin secretion gradually decreases, particularly after the age of 45, leading to circadian rhythm disturbances and poorer sleep quality in older adults (33, 34).

Lack of sleep significantly increases stress levels by stimulating the production of hormones such as cortisol. This can lead to elevated blood pressure, an increased heart rate, and heightened anxiety. Chronic sleep deprivation weakens the body's ability to cope with stress, increasing the risk of mental disorders such as depression and anxiety (35, 36).

Additionally, sleep deprivation impairs decision-making abilities and emotional control, further intensifying feelings of stress and frustration. Disrupted sleep can seriously endanger mental health by increasing the risk of mood disorders, anxiety, and substance abuse, while also making recovery from these conditions more difficult.

The results of this study indicate that night work, smoking, alcohol consumption, age group, and diet significantly impact stress levels. These findings are consistent with previous research, which has shown that working night shifts can severely disrupt circadian rhythms, increase the risk of insomnia and metabolic disorders, and contribute to chronic stress due to endocrine dysfunction and sleep deprivation (36–38).

Specifically, a study by Ferri et al. highlights the negative psychological consequences of shift work, including increased stress, anxiety, and mood disorders, while Bonnell et al. point out that dietary changes among shift workers, such as irregular meals and consumption of nutritionally poor foods, may further contribute to elevated stress levels (39, 40).

Although the results largely align with existing literature, no significant interaction was found between age group and smoking. This contrasts with some previous studies that have demonstrated a relationship between work-related stress, shift work, and smoking frequency (41–44). This discrepancy may be due to the specific characteristics of the sample or the methodology used in this study.

The obtained results confirm the importance of a proper diet as a protective factor. Workers with a balanced diet exhibited lower stress levels,

which is consistent with studies highlighting the role of dietary habits in stress regulation (45). On the other hand, smoking and alcohol consumption emerged as significant stressors, reaffirming previous findings on their negative impact on mental health (46, 47).

The results of this study indicate the universal impact of night work and unhealthy habits on stress. This applies not only to security workers but also to other professional groups engaged in night shifts. The generalizability of these findings is further supported by similarities with global data on the effects of shift work on health (48, 49).

One unexpected finding is the relatively weak influence of interactions between the examined factors, which may suggest the need for more complex analytical models or larger sample sizes. For example, the impact of BMI on stress could be examined in greater detail through longitudinal studies (50–52).

This study contributes to the understanding of the impact of night work on health and highlights the importance of integrating dietary and lifestyle habits into health protection programs. Theoretically, it confirms the independent effects of various factors on stress, while its practical contribution includes recommendations for promoting healthy lifestyles among workers.

Practical recommendations include implementing stress management programs, educating workers on healthy nutrition, and encouraging smoking cessation and reduced alcohol consumption. Employers should provide better working conditions for night shifts, including adequate breaks and opportunities for physical activity during shifts (52, 53).

Based on the aforementioned findings, night shift workers are advised to have their meals in a relaxed, pleasant environment, which may help reduce stress. Additionally, during meal breaks, they are encouraged to engage in activities such as stretching exercises, brisk walking, or relaxing conversations with colleagues. These practices can improve mood, increase energy levels for the remainder of the shift, and contribute to better sleep after work (54).

To maintain their health, night shift workers must also adopt a healthy lifestyle outside of work. First and foremost, ensuring sufficient sleep and quality rest is essential for the body's recovery. A healthy and balanced diet plays a crucial role in maintaining energy levels and physical resilience. Regular physical activity helps reduce stress and maintain a healthy body weight, which is especially important for those working in rotating shifts.

Avoiding harmful habits such as smoking and excessive alcohol consumption contributes to long-term health. Preserving mental well-being is equally important, so workers should practice relaxation techniques and seek support if they encounter difficulties. All these measures collectively contribute to a better quality of life and reduce the risk of health problems associated with night work (55).

Conclusion

Night shift workers face unique health challenges at the biological, psychological, and social levels.

The results of this study indicate that night work, age, smoking, alcohol consumption, and dietary habits significantly influence stress levels among security workers.

Night work has a major impact on the health of security workers, increasing stress levels, leading to poor dietary habits, and raising the risk of obesity. The findings of this study highlight the need for preventive and promotional workplace activities and better worker education to improve employee health, reduce healthcare costs, decrease absenteeism, and enhance work productivity.

References

1. Xpressguards. Historical Evolution of the Security Guard Profession [Internet]. Available at: https://xpressguards-com.translate.goog/historical-evolution-of-the-security-guard-profession/?_x_tr_sl=en&_x_tr_tl=sr&_x_tr_hl=sr&_x_tr_pto=sc
2. The Impact of Artificial Intelligence on the Security Industry [Internet]. Security Magazine; 2020 [cited 2025 Feb 10]. Available from: https://www.securitymagazine.com/articles/92332-the-impact-of-artificial-intelligence-on-the-security-industry
3. Talas R, Button M, Doyle M, Das J. Violence, abuse and the implications for mental health and wellbeing of security operatives in the United Kingdom: the invisible problem. Policing Soc 2020;1–16. [CrossRef]
4. Money U, Ehimewenma EI. The Nigeria police stress: its organization and operations. Indian J Commerce Manag Stud. 2016;7(1):67–74.
5. Tutenges S, Sogaard TF, Kroll LT, Bloomfield K, Hesse M. Violent work environments. Int J Workplace Health Manag 2015;8:129-141. [CrossRef]
6. Veljković D. Burnout Syndrome at Work. *Southeast Eur J Emerg Disaster Med.* 2021;7(1):8–14. [CrossRef]
7. Gallup. State of the global workplace 2022 report [Internet]. Dostupno na: https://www.gallup.com/workplace/349484/state-of-the-global-workplace-2022-report.aspx [Accessed: 10.02.2025].
8. Press Office HSE. HSE publishes annual work-related ill health and injury statistics for 2021-22 [Internet]. Dostupno na: https://press.hse.gov.uk/2022/11/23/hse-publishes-annual-work-related-ill-health-and-injury-statistics-for-2021-22/ [Accessed: 10.02.2025].
9. HR Magazine. UK loses 17 million working days to stress, depression, and anxiety [Internet]. Dostupno na: https://www.hrmagazine.co.uk/content/news/uk-loses-17-million-working-days-to-stress-depression-and-anxiety [Accessed: 10.02.2025].
10. Jovanović J, Šarac I, Jovanović S, Sokolović D, Govedarović N, Jovanović J. The relationship between occupational stress, health status, and temporary and permanent work disability among security guards in Serbia. Int J Occup Saf Ergon 2019;1–17. [CrossRef] [PubMed]
11. Härmä M, Ropponen A, Hakola T, Koskinen A, Vanttola P, Puttonen S, et al. Developing register-based measures for assessment of working time patterns for epidemiologic studies. Scand J Work Environ Health 2015;41(3):268-79. [CrossRef] [PubMed]
12. Eurostat. Working at night – Statistics on working time [Internet]. Dostupno na: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Working_at_night [Accessed: 10.02.2025].
13. *Labor Law* ("Official Gazette of the RS", No. 24/2005, 61/2005, 54/2009, 32/2013, 75/2014, 13/2017 - Constitutional Court Decision, 113/2017, and 95/2018 – Authentic Interpretation). Available at: https://www.paragraf.rs/propisi/zakon_o_radu.html.
14. Kecklund G, Axelsson J. Health consequences of shift work and insufficient sleep. BMJ 2016;355:i5210. [CrossRef] [PubMed]
15. Zamanian Z, Dehghani M, Mohammady H, Rezaeiani M, Daneshmandi H. Investigation of shift work disorders among security personnel. Int J Occup Hyg 2012;4:39–42.
16. Lazaridis K, Jovanović J, Jovanović J, Šarac I, Jovanović S. The impact of occupational stress factors on temporary work disability related to arterial hypertension and its complications. Int J Occup Saf Ergon 2017;23:259–66. [CrossRef] [PubMed]
17. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav 1983;24(4):385–96. [CrossRef] [PubMed]
18. Costa G. Shift work and health: Current problems and preventive actions. Saf Health Work 2010;1(2):112–23. [CrossRef] [PubMed]
19. OECD. Working Better with Age: Policies to Support Older Workers. OECD Publishing; 2019. [CrossRef]
20. Black C, Frost D. Health at work—An independent review of sickness absence [Internet]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/181060/health-at-work.pdf [Accessed 10 Feb 2025].
21. Jones M, Latreille P, Sloane P, Staneva A. Work-related health risks in Europe: are older workers more vulnerable? Soc Sci Med 2013;88:18–29. [CrossRef] [PubMed]
22. Balkin TJ, Rupp T, Picchioni D, Wesensten NJ. Sleep loss and sleepiness: current issues. Chest. 2008;134(3):653-60. [CrossRef] [PubMed]
23. Chrousos GP. Stress and obesity: The neuroendocrinological basis of a complex relationship. Metabolism. 2009;58(10):20-28.
24. Zisapel N. New perspectives on the role of melatonin in human sleep, circadian rhythms, and their regulation. Br J Pharmacol 2018; 175(16):3190-9. [CrossRef] [PubMed]
25. Klok MD, Jakobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obes Rev 2007;8(1):21–34. [CrossRef] [PubMed]
26. Tomiyama AJ. Stress and obesity. Annu Rev Psychol 2019;70:703–18. [CrossRef] [PubMed]
27. Gan Y, Yang C, Tong X, Sun H, Cong Y, Yin X, et al. Shift work and diabetes mellitus: A meta-analysis of observational studies. Occup Environ Med 2015;72(1):72–8. [CrossRef] [PubMed]
28. Depner CM, Stothard ER, Wright KP. Metabolic consequences of sleep and circadian disorders. Curr Diab Rep 2014;14(7):507. [CrossRef] [PubMed]

29. Kivimäki M, Jokela M, Nyberg ST, Singh-Manoux A, Fransson EI, Alfredsson L, et al. Long working hours and risk of coronary heart disease and stroke: A systematic review and meta-analysis. Lancet 2015;386(10005):1739–46. [CrossRef] [PubMed]
30. Kecklund G, Axelsson J. Health consequences of shift work and insufficient sleep. BMJ 2016;355:i5210. [CrossRef] [PubMed]
31. Åkerstedt T, Wright KP. Sleep loss and fatigue in shift work and shift work disorder. Sleep Med Clin 2009;4(2):257–71. [CrossRef] [PubMed]
32. Dijk DJ, Cajochen C, Kräuchi K, et al. Circadian and sleep-dependent influences on cognitive performance and mood during the night. J Sleep Res 2003;12(1):22-7. [CrossRef] [PubMed]
33. Ferracioli-Oda E, Qawasmi A, Bloch MH. Meta-analysis: melatonin for the treatment of primary sleep disorders. PLoS One 2013;8(5):e63773. [CrossRef] [PubMed]
34. Ohashi N, Kohyama J. The significant role of melatonin in antioxidative stress—The association with aging. Antioxidants (Basel) 2021;10(10):1528. [CrossRef] [PubMed]
35. Walker MP. The role of sleep in cognition and emotion. Ann N Y Acad Sci 2009;1156:168-97. [CrossRef] [PubMed]
36. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. Lancet 1999;354(9188):1435-9. [CrossRef] [PubMed]
37. Viklund A, Andersson T, Selander J, Kader M, Albin M, Bodin T, et al. Night and shiftwork patterns and incidence of type 2 diabetes and hypertension in a prospective cohortstudy of healthcare employees. Scand J Work Environ Health. 2023;49(6):439-448.doi:10.5271/sjweh.4103.
38. Vgontzas AN, Liao D, Pejovic S, Calhoun S, Karataraki M, Basta M, et al. *Insomnia with objective short sleep duration is associated with type 2 diabetes: A population-based study.* Diabetes Care 2009;32(11):1980-85. [CrossRef] [PubMed]
39. Ferri P, Guadi M, Marcheselli L, Balduzzi S, Magnani D, Di Lorenzo R. The impact of shift work on the psychological and physical health of nurses in a general hospital: a comparison between rotating night shifts and day shifts. Risk Manag Health Policy. 2016 Sep 14;9:203-211. [CrossRef] [PubMed]
40. Bonnell EK, Huggins CE, Huggins CT, McCaffrey TA, Palermo C, Bonham MP. Influences on dietary choices during shift work. Nutrients 2017;9(3):193. [CrossRef] [PubMed]
41. Xiao C, Zhou CY, Jiang JH, Yin C. Neural circuits and nicotinic acetylcholine receptors mediate the cholinergic regulation of midbrain dopaminergic neurons and nicotine dependence. Acta Pharmacol Sin 2020;41(1):1–9. [CrossRef] [PubMed]
42. Khorrami Z, Zolala F, Haghdoost AA, Sadatmoosavi A, Ben Taleb Z, Kondracki A, et al. Job-related stress and tobacco smoking: A systematic review. J Workplace Behav Health. 2021;36(4):333-354. [CrossRef]
43. Bae MJ, Song YM, Shin JY, Choi BY, Keum JH, Lee EA. The Association Between Shift Work and Health Behavior: Findings from the Korean National Health and Nutrition Examination Survey. Korean J Fam Med 2017;38(2):86-92. [CrossRef] [PubMed]
44. Hamieh N, Airagnes G, Descatha A, Goldberg M, Limosin F, Roquelaure Y, et al. Atypical working hours are associated with tobacco, cannabis and alcohol use: longitudinal analyses from the CONSTANCES cohort. BMC Public Health 2022;22:1834. [CrossRef] [PubMed]
45. Anđelković S, Babić M. Dietary habits of night shift workers. Srp Arh Celok Lek. 2024;5(1):75-88.
46. Wyse CA, Celis Morales CA, Graham N, Fan Y, Ward J, Anderson J, et al. Adverse metabolic and mental health outcomes associated with shift work. Ann Med 2017;49(5):411-20. [CrossRef] [PubMed]
47. Schnall PL, Dobson M, Landsbergis P. Work, stress and cardiovascular disease. Int J Environ Res Public Health. 2016;13(2):208. [CrossRef]
48. 48.Wang Y, Chen HJ. The effect of body mass index on stress levels: A systematic review. Obes Rev 2015;16(11):903-911. [CrossRef] [PubMed]
49. López-González A, Sánchez-Muñoz LA, Martínez-López E, Aparicio VA. Body mass index and stress in workers: A review of the evidence. Occup Med (Lond) 2016;66(5):378-383. [CrossRef] [PubMed]
50. Kosmadopoulos A, Kervezee L, Boudreau P, Gonzales-Aste F, Vujovic N, Scheer FAJL, et al. Effects of shift work on eating behavior. Nutrients 2020;12(4):999. [CrossRef] [PubMed]
51. Bannai A, Tamakoshi A. The association between shift work and mental health: a systematic review. Sleep Med Rev 2014;17(6):435-47. [CrossRef] [PubMed]
52. Richter K, Hovenic M, Baumgärtner J, Peter L, Niklewski G, Spijkerman J, et al. Shift work and alcohol consumption: A systematic review. Eur Addict Res 2021;27(1):9-15. [CrossRef] [PubMed]
53. Easton DF, Gupta CC, Vincent GE, Dorrian J, Ferguson SA. Move the night way: how can physical activity facilitate adaptation to shift work? Commun Biol. 2024;7:259. [CrossRef] [PubMed]
54. Brajović Z, Bogdanović M, Šuštran B, Milanović Čabarkapa M, Bogdanović V. The impact of shift and night work on health. Zdravstvena Zaštita 2007;36(3):43-6. [CrossRef]
55. Caruso CC. Negative impacts of shift work and long work hours. Rehabil Nurs 2014;39(1):16-25. [CrossRef] [PubMed]

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UTICAJ NOĆNOG RADA, NAČINA ISHRANE, KONZUMIRANJA ALKOHOLA I PUŠENJA CIGARETA NA NIVO STRESA KOD RADNIKA OBEZBEĐENJA

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Noćni rad, koji je karakterističan za zanimanja poput radnika obezbeđenja, donosi jedinstvene izazove kada je posredi zdravlje radnika na biološkom, psihološkom i socijalnom nivou. Cirkadijalni ritmovi su poremećeni zbog noćnih smena, što dovodi do poremećaja sna, povećanog stresa i metaboličkih poremećaja. Ova studija analizira kako starosna grupa, noćni rad, pušenje, konzumacija alkohola, ishrana i indeks telesne mase (engl. *body mass index* – BMI) utiču na nivo stresa kod radnika obezbeđenja, s posebnim osvrtom na to kako noćne smene utiču na zdravstvene navike i opšte zdravstveno stanje. Za procenu uzorka sačinjenog od sto pedeset četiri radnika korišćene su ankete i standardizovane mere stresa. Ključni rezultati ukazuju na značajne efekte koji noćni rad, starost, pušenje, konzumacija alkohola i BMI imaju na nivo stresa; pritom, uravnotežena ishrana je povezana sa smanjenjem stresa.

Nedostatak sna i nepravilna ishrana doprinose smanjenju radnih performansi i povećavaju rizik od pojave hroničnih bolesti poput dijabetesa i kardiovaskularnih oboljenja. Takođe, upotreba alkohola i cigareta, koja često predstavlja strategiju za suočavanje sa stresom i nesanicom, dodatno pogoršava zdravstvene probleme.

Studija je sprovedena na uzorku od sto pedeset četiri radnika iz različitih starosnih grupa i radnih okruženja, koji su popunjavali anketu za procenu navika povezanih sa radom, zdravljem, ishranom, pušenjem, alkoholom i BMI-jem. Rezultati pokazuju da starosna grupa i noćni rad značajno utiču na nivo stresa – stariji radnici i radnici u noćnim smenama prijavljuju više nivoe stresa. Pušenje i alkohol dodatno povećavaju stres. I BMI pokazuje značajan uticaj na stres, posebno u noćnim smenama. Došlo se do zaključka da noćni rad, starosna grupa, pušenje, alkohol, BMI i ishrana u znatnoj meri utiču na nivo stresa kod radnika obezbeđenja. Međutim, promene nezdravih navika mogu pomoći u smanjenju nivoa stresa.

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***Ključne reči**: stres, ishrana, noćni rad, alkohol*

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ENZYME REPLACEMENT THERAPY IN PATIENTS WITH TYPE 1 GAUCHER DISEASE: A SINGLE-CENTER EXPERIENCE

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Gaucher disease (GD) is a liposomal storage disorder inherited in an autosomal recessive pattern. The underlying cause of the disease is a mutation within the gene that encodes the enzyme glucocerebrosidase. Accumulation of glucocerebrosidase in macrophages in the liver, spleen, bone marrow, rarely in the lungs and other organs, occurs due to a deficiency of enzyme synthesis, disorder or lack of enzyme function, or a deficiency of saposin C (enzyme activator). Clinical classification of GD is based on the absence (Type 1) or presence (Types 2 and 3) of central nervous system manifestations. Levels of beta-glucocerebrosidase in leukocytes, as well as the levels of serum chitotriosidase, are measured to make the definitive diagnosis of Gaucher disease. Accumulation of beta-glucocerebrosidase causes numerous multi-organ complications (anemia, thrombocytopenia, hepatomegaly, splenomegaly, skeletal and neurological changes). Since 1991, enzyme replacement therapy (ERT) has been used for treating Gaucher disease. Show the treatment results in patients with Type 1 Gaucher disease by administering ERT taliglucerase alfa at the Clinic of Hematology, Allergology and Clinical Immunology, University Clinical Center Niš. Between January 2016 and January 2025, taliglucerase alfa was used to treat 5 patients with Type 1 Gaucher disease who did not respond to previous treatment or because the drug donation was discontinued. All our patients responded well to treatment, and there were no adverse effects (administration of taliglucerase alfa results in significant regression of anemia, thrombocytopenia and organomegaly, along with bone status improvement).

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Key words: *Gaucher disease, enzyme replacement therapy, taliglucerase alfa*

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Introduction

Gaucher disease—Morbus Gaucher (GD) is a liposomal storage disorder inherited in an autosomal-recessive pattern, with the underlying cause being mutations within the gene which encodes enzyme glucocerebrosidase (1–5).

The accumulation of glucocerebrosidase in macrophages occurs in the liver, spleen, bone marrow, and rarely in the lungs and other organs. It results from a deficiency in enzyme synthesis, a disorder or a lack of enzyme function, or a deficiency of saposin C, which acts as an enzyme activator. Accumulation of glucocerebrosidase causes numerous multi-organ complications such as anemia, thrombocytopenia, hepatomegaly, splenomegaly, and skeletal and neurological changes (1, 6–10).

Clinical classification of GD is based on the absence or presence of central nervous system manifestations, and it is differentiated into Type 1 (characterized by the absence of central nervous system manifestations) and Types 2 and 3 (characterized by the presence of central nervous system manifestations) (1, 10).

Type 1 GD is the most common, and clinical manifestations may vary from asymptomatic forms to forms with severe complications in childhood or adulthood. It is manifested with hepato-splenomegaly, leukopenia, thrombocytopenia, skeletal changes and pulmonary disease (1).

Type 2 is characterized as an acute and lethal neuronopathic form involving the nervous system, while Type 3 GD is chronic neuronopathic form involving visceral organs, bones and the heart.

Levels of beta-glucocerebrosidase in leukocytes are measured in order to make the definitive diagnosis of GD. However, a broad spectrum of pathological features observed in GD

is not merely a consequence of accumulation and mechanical activity of glucocerebrosidase, but a consequence of macrophage activation and cytokine secretion as well.

In the serum of patients with GD, the levels of interleukin-1 beta, interleukin-6, THF-alfa, soluble interleukin-2 receptor, and CD14 are elevated. What is specific for patients with GD is the elevation of chitotriosidase activity in serum originating from the Gaucher cells, and it is considered to be a marker of macrophage activation and immune response induction. Chitotriosidase levels serve as a surrogate marker for the total amount of accumulated glucocerbrosidase and for assessing Enzyme replacement therapy (ERT) intervention as well (1, 11–18).

The presence of the Gaucher cells in the bone marrow and other tissues is not pathognomonic of GD, but it can be seen in a number of other diseases (thalassemia, acute and chronic lymphoproliferative disease, granulocytic leukemia).

Since 1991, ERT has been used for treating Gaucher disease by substituting beta-glucocerebrosidase with the enzyme produced by recombinant technology (alglucerase, imiglucerase, velaglucerase, taliglucerase alfa). The therapy has been shown to achieve effective results, corrects the anaemia, thrombocytopenia, organomegaly, improves bone status, and adverse effects are scarce (10, 19–24).

Aim

The paper aimed to show the treatment results in patients with Type 1 Gaucher disease by administering ERT taliglucerase alfa at the Clinic of Hematology, Allergology and Clinical Immunology.

Materials and Methods

Between January 2016 and January 2025, at the Clinic of Hematology, Allergology and Clinical Immunology, taliglucerase alfa was used in treating 5 patients with Type 1 Gaucher disease who did not respond to previous treatment or because the drug donation was discontinued. One patient underwent splenectomy. Since 2016, the patients were treated at the expense of the National Health Insurance Fund.

ERT taliglucerase alfa was administered at a dose of 30 U/kg body weight every other week as a 60–120 minutes intravenous infusion. The dosage of taliglucerase alfa was adjusted on an individual basis to achieve adequate therapeutic response (absence of thrombocytopenia and anemia syndrome, reduced spleen and liver volume, as well as reduction in chitotriosidase levels and improvements in skeletal system) (20–23, 25, 26).

The treatment started after the definitive diagnosis of Type 1 Gaucher disease was made; diagnostic procedure included bone marrow biopsy, confirmation of the presence of Gaucher cells, assessment of chitotriosidase levels, measurement of spleen and liver volume and identification of genotypes based on PCR and direct gene sequencing, while the gold standard for making the diagnosis of Gaucher disease is to determine the beta-glucocerebrosidase levels in leukocytes (1, 11–20).

Results

ERT Taliglucerase alfa was used to treat 5 patients with type 1 Gaucher disease, 3 male patients (60%) and 2 female patients (40%) (Figure 1).

The mean age of all the patients at the time of initiating taliglucerase alfa therapy was 39.2 years. The youngest patient was 17, and the oldest one was 63 years old. Two patients previously received 1 therapeutic line (imiglucerase within the donation programme), one patient received 3 therapeutic lines (recombinant glucocerebrosidase within the clinical study, Protalix from humanitarian aid, imiglucerase), and two patients were treated with taliglucerase alfa in the first therapeutic line.

The time period between establishing GD diagnosis and initiation of ERT with taliglucerase alfa was 44.4 months (range 1–132) (Figure 2).

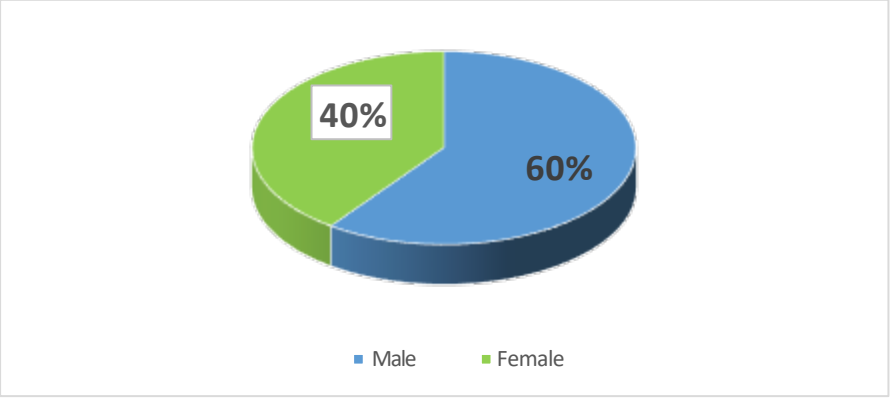

Figure 1. Patients with Gaucher disease by gender

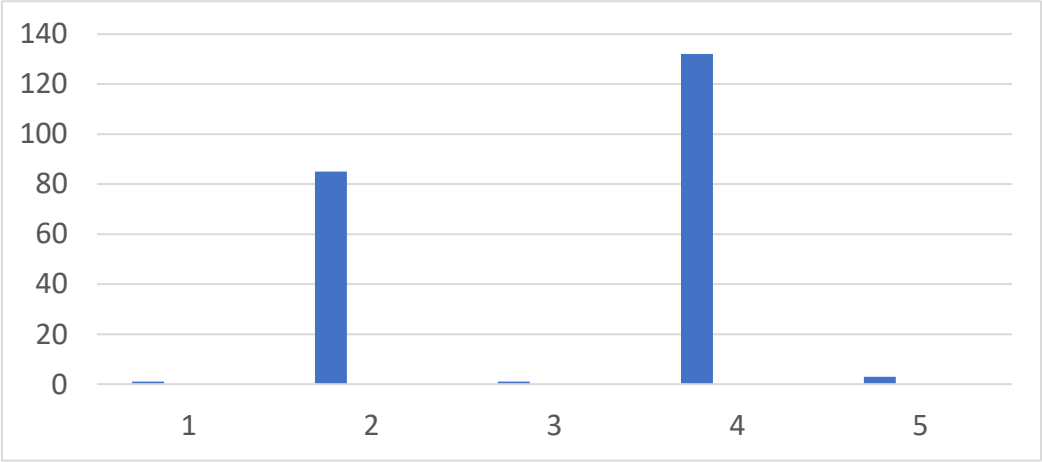

Figure 2. Time in months from diagnosis of GD to initiation of ERT

The time needed for treatment response after initiation of ERT taligluceraze alfa was 6–12 months.

The average duration of treatment with taliglucerase alfa was 61.6 months (range 13–83 months), and the average chitotriosidase level for that period was 3937.5 nmol/h/ml (range 481.4–8299.5 nmol/h/ml).

Chitotriosidase is an enzyme belonging to the family of chitinases that plays a role in innate immunity, and it is a marker of macrophage activation. Activated macrophages secrete chitotriosidase. Chitotriosidase activity level drops 3–6 months after initiation of ERT, and increases 20 weeks after ERT cessation.

Extremely elevated chitotriosidase activity level originating from the Gaucher cells is registered in the plasma of patients with GD. A direct correlation between chitotriosidase concentration and the total amount of non-degraded glucocerebroside substrate was established. That is why one of the parameters of treatment success is still serum chitotriosidase level.

The amount of glucocerebrosidase in patients with GD is increased 10–1.000 times and can make up to 2% body weight of the patient.

The analysis of chitotriosidase average value upon introducing ERT taliglucerase alfa showed the following trend: for the first patient, average chitotriosidase value was 9007.2 nmol/ml/h (range 4202–21226.3); for the second patient, the average chitotriosidase value was 4709.3 nmol/ml/h (range 2992.5–8299.5); for the third patient, the average chitotriosidase value was 683.3 nmol/ml/h (range 534–1137); for the fourth patient, the average chitotriosidase value was 970.7 nmol/ml/h (range 657.4–1443.6); and for the fifth patient, the average chitotriosidase value was 3402.8 nmol/ml/h (range 749.7–6056). Patients responded well to the therapy from its initiation throughout its course, except for two patients who responded at the level of stable disease, meaning that chitotriosidase level was maintained, as well as the volume of the liver and spleen in the previous two years. There were no adverse effects during the administration of the drug (Table 1).

Table 1. Effect of ERT taliglucerase alfa in patients with Gaucher disease

Patients with GD	Chitotriosidase value before ERT administration	Chitotriosidase value after EST application	Side effects
1.	21226.3 nmol/ml/h	4202 nmol/ml/h	No
2.	8229.5 nmol/ml/h	2992.5 nmol/ml/h	No
3.	1137 nmol/ml/h	534 nmol/ml/h	No
4.	1443.6 nmol/ml/h	657.4 nmol/ml/h	No
5.	6056 nmol/ml/h	749.7 nmol/ml/h	No

Apart from the assessment of chitotriosidase levels, our small group of patients was regularly radiologically monitored by performing whole-body NMR imaging once a year (measurements of spleen and liver volume, as well as regular monitoring of skeletal system status).

The most common clinical manifestation of Type 1 GD is painless splenomegaly, mostly detected accidentally. The spleen is sometimes palpated a few centimeters below the left rib arch, or it can be enlarged throughout the abdomen. The liver is slightly enlarged, and portal hypertension is rare.

Magnetic Resonance Imaging of the abdomen performed a year after the initiation of ERT taliglucerase alfa revealed a reduction in the spleen size in two patients: a reduction of 32%, from 201 mm to 136 mm, was registered in the first patient after a year of ERT taliglucerase alfa administration; in the second patient, the spleen volume was reduced from 134 mm to 130 mm. In one patient, the spleen was of regular size both at the time of diagnosis and at the check-up by abdominal NMR imaging. In another patient, the liver and spleen size were maintained despite the administered therapy. Additionally, one patient underwent splenectomy before establishing the diagnosis. The control magnetic resonance imaging was performed every year, aiming at detecting any changes in the liver and spleen volume, and the skeletal system as well.

The presence of anemia and thrombocytopenia is one of the criteria for establishing the diagnosis of GD. Our group of patients had regular laboratory analyses and follow-ups to assess the efficacy of ERT. At the time of diagnosis, two patients had severe anemia syndrome, two patients had moderate thrombo-cytopenia, and one patient had both anemia and thrombocytopenia.

The analysis of average number of platelets upon the introduction of ERT taliglucerase alfa showed the following: for the first patient, the average number of platelets was 122.8 x 10^9/L (range 84–149); for the second patient, the average number of platelets was 233.2 x 10^9/L (range 220–244); for the third patient, the average number of platelets was 149 x 10^9/L (range 125–180); for the fourth patient, the average number of platelets was 271.6x 10^9/L (range 210–330); and for the fifth patient, the average number of platelets was 115 x 10^9/L (range 62–209).

The values of Hgb levels monitored in patients upon ERT taliglucerase alfa introduction were as follows: for the first patient the average HGB value was 137.8 g/l (range 128–147), for the second patient, the average HGB value was 152.4 g/l (range 149–157), for the third patient, the average HGB value was 95.2 g/l (range 81–106), for the fourth patient, the average HGB value was 131.6 g/l (range 109–145), and for the fifth patient, the average HGB value was 140 g/l (range 135–148).

For the assessment of bone involvement, pelvic MRI and long bones MRI were performed. One of the important features of type 1 Gaucher disease is skeletal system involvement, as registered in 60 % of our small group of patients. Skeletal changes are not always in direct correlation with other symptoms; bone involvement is not rare, and it is often the first presenting sign of the disease. Bone involvement most commonly includes the thighbone, vertebrae, pelvis, the upper arm and forearm long bones with the development of osteoporosis, osteolysis, avascular necrosis, and pathological fractures. Episodes of bone crises are manifested as intense pain and are caused by bone microinfarction. At the time of establishing the diagnosis, in 3 out of 5 monitored patients, signal intensity changes in the bones were registered, and the regression of manifestations was observed after the administration ERT Taliglucerase alfa.

Discussion

Gaucher disease is an autosomal recessive disease, more common in females and more prevalent in Ashkenazi Jews than in the general population. At the time of diagnosis, patients' age ranges from birth to 81 years, mean age of 17.4 years, with almost half of the patients diagnosed before 10 years of age. In the current study small group, the mean age was 39.2 years (27–29).

The purpose of treating Gaucher disease is correction of anemia syndrome, thrombocytopenia, and reduction of liver and spleen volume, along with the improvement of bone status. The effects of ERT can be seen 3 to 6 months after the initiation of the treatment, while in this study group of patients, the effects were seen after 6 to 12 months of treatment, which is in accordance with literature data (18, 19, 20–23, 25, 26).

Available literature does not favour any type of ERT, but according to current experience, taliglucerase alfa exhibits lower risk of immunogenicity in comparison to imiglucerase. ERT taliglucerase alfa is well tolerated, and adverse effects are mild to moderate in intensity and transient. The most common adverse effects include headache, nasopharyngitis, hypertension, chest discomfort, nausea, vomiting, itching, and pain in extremities, although no adverse effects were registered in our small group (24, 29, 30).

Getting IV ERT treatment every other week in a hospital environment harms patients' quality of life, because it is a lifelong treatment (31).

All patients included in the present study had a good therapeutic response, except for one patient in whom there was no reduction in the liver and spleen volume over a period of over a year, and treatment modality change has been considered. Taliglucerase alfa dosage was increased in two patients in proportion to their body size. According to literature data, termination of ERT results in disease progression in most patients, as well as in chitotriosidase level increase 20 weeks after treatment discontinuation (22, 23).

Conclusion

Until the 1990s, the only treatment available for Gaucher disease was a symptomatic one, such as erythrocyte transfusion, transfusion of platelets, multivitamin infusions, NSAIDs, androgen and bisphosphonate treatment, and orthopaedic procedures. Splenectomy was accompanied by transient improvement only, and the disease was still progressing, especially in accelerating skeletal structures damage.

A significant advancement in GD treatment was the introduction of replacement therapy with alglucerase (the placenta-derived modified form of beta-glucocerebrosidase) in the 1990s. A recombinant form of alglucerase—imiglucerase, Cerezyme—was introduced as a treatment option in the late 1990s.

Based on previous experience, administration of taliglucerase alfa results in a significant reduction of anemia, thrombocytopenia and organomegaly, as well as in bone status improvement.

Gaucher disease is an example of how modern medicine may alter the course of human life.

References

1. Suvajdžić-Vuković N. Gošeova bolest tipa 1- Klinička slika, hematološki aspekti i supstituciona terapija. Bil Hematol 2004; 32:165-8.
2. Zhao H, Grabowski GA. Gaucher disease: Perspectives on a prototype lyposomal disease. Cell Mol Life Sci 2002; 59(4):694-707. [CrossRef] [PubMed]
3. Beutler E. Lysosomal storage diseases:natural history and ethical and economic aspects. Mol Genet Metab 2006;88(3): 208-15. [CrossRef] [PubMed]
4. Redzić A, Begić F. [Type I Gaucher's disease- a rare genetic metabolic disease]. Med Arh 2003; 57(3):173-6.
5. Beutler E, Gelbart T, Scott CR. Hematologically important mutations:Gaucher disease. Blood Cells Mol Dis 2005;35(3):355-64. [CrossRef] [PubMed]
6. Dokić M. Morbus Gaucher- a report of two cases. Vojnosanit Pregl 2006 ;63(12):1039-44. [CrossRef] [PubMed]
7. Wenstrup RJ, Roca-Espiau M, Weinreb NJ, Bembi B. Skeletal aspects of Gaucher disease: a review. Br J Radiol 2002;75 Suppl 1: A2-12. [CrossRef] [PubMed]
8. Stowens DW, Teitelbaum SI, Kahn AJ, Barranger JA. Skeletal complications of Gaucher disease. Medicine (Baltimore) 1985; 64:310-22. [CrossRef] [PubMed]
9. Maas M, Poll LW, Terk MR. Imaging and quantifying skeletal involvement in Gaucher disease. Br J Radiol 2002; 75(suppl 1): A13-A24. [CrossRef] [PubMed]
10. Mrsić M. [Diagnosis and treatment of Gaucher disease in Croatia]. Lijec Vjesn. 2007 May;129 Suppl 3:38-42.
11. Pandey MK, Grabowski GA. Immunological cells and functions in Gaucher disease. Crit Rev Oncog 2013;18(3):197-220. [CrossRef] [PubMed]
12. Deegan PB, Moran MT, McFarlane I, Schofield JP, Boot RG, Aerts JM, et al. Clinical evaluation of chemokine and enzymatic biomarkers of Gaucher disease. Blood Cells Mol Dis 2005; 35:259-67. [CrossRef] [PubMed]
13. Manolagas SC. The role of IL-6 type cytokines and their receptors in bone. Ann NY Acad Sci 1998;840:194-204. [CrossRef] [PubMed]
14. Barak V, Acker M, Nisman B, Kalickman I, Abrahamov A, Zimran A, et al. Cytokines in Gaucher's disease. Eur Cytokine Netw 1999;10(2):205-10.
15. Cox TM. Biomarkers in lysosomal storage diseases: a review. Acta Paediatr Suppl 2005;94(447):39-42. [CrossRef] [PubMed]
16. Aerts JM, Hollak CE, van Breemen M, Maas M, Groener JE, Boot RG. Identification and use of biomarkers in Gaucher disease and other lysosomal storage diseases. Acta Paediatr Suppl 2005;94(447):43-6. [CrossRef] [PubMed]
17. Korolenko TA, Zhanaeva SY, Falameeva OV, Kaledin VI, Filyushina EE, Buzueva II, et al. Chitotriosidase as a marker of macrophage stimulation. Bull Exp Biol Med 2000;130: 948-50. [CrossRef] [PubMed]
18. Poll IW, Maas M, Terk MR, Roca-Espiau M, Bembi B, Ciana G, et al. Response of Gaucher bone disease to enzyme replacement therapy. Br J Radiol 2002;75 Suppl 1:A25-36. [CrossRef] [PubMed]
19. Grabowski GA, Hopkin RJ. Enzyme therapy for lysosomal storage disease: principles, practice and

prospects. Annu Rev Genomics Hum Genet 2003; 4:403-36. [CrossRef] [PubMed]
20. Brady RO. Enzyme replacement for lysosomal diseases. Annu Rev Med 2006; 57:283-96. [CrossRef] [PubMed]
21. Zimran A. How I treat Gaucher disease. Blood 2011; 118(6):1463-71. [CrossRef] [PubMed]
22. Zimran A, Wajnrajch M, Hernandez B, Pastores GM. Taliglucerase alfa: safety and efficacy across 6 clinical studies in adults and children with Gaucher disease. Orphanet J Rare Dis 2018;13(1):36. [CrossRef] [PubMed]
23. Pastores GM, Shankar SP, Petakov M, Giraldo P, Rosenbaum H, Amato DJ, et al. Enzyme replacement therapy with taliglucerase alfa: 36-month safety and efficacy results in adult patients with Gaucher disease previously treated with imiglucerase. Am J Hematol 2016 Jul;91(7):661-5. [CrossRef] [PubMed]
24. Hollak CE. An evidence-based review of the potential benefits of taliglucerase alfa in the treatment of patients with Gaucher disease. Core Evid 2012;7:15-20. [CrossRef] [PubMed]
25. Charrow J, Andersson HC, Kaplan P, et al. The Gaucher Registry: Demographics and Disease Characteristics od 1698 Patients With Gaucher Disease. Arch Intern Med 2000; 160(18):2835-2843. [CrossRef] [PubMed]
26. Zimran A, Brill-Almon E, Chertkoff R, Petakov M, Blanco-Favela F, Muñoz ET, et al. Pivotal trial with plant cell-expressed recombinant glucocerebrosidase, taliglucerase alfa, a novel enzyme replacement therapy for Gaucher disease. Blood 2011;118(22):5767-73. [CrossRef] [PubMed]
27. Shemesh E, Deroma L, Bembi B, Deegan P, Hollak C, Weinreb NJ, et al. Enzyme replacement and substrate reduction therapy for Gaucher disease. Cochrane Database Syst Rev 2015 Mar 27;2015(3):CD010324. [CrossRef] [PubMed]
28. Nalysnyk L, Rotella P, Simeone JC, et al. Gaucher disease epidemiology and natural history: a comprehensive review of the literature. Hematology 2017;22(2):755–73. [CrossRef] [PubMed]
29. Zimran A, Durán G, Mehta A, Giraldo P, Rosenbaum H, Giona F, et al. Long-term efficacy and safety results of taliglucerase alfa up to 36 months in adult treatment-naïve patients with Gaucher disease. Am J Hematol 2016;91(7):656-60. [CrossRef] [PubMed]
30. Van Rossum A, Holsopple M. Enzyme Replacement or Substrate Reduction? A Review of Gaucher Disease Treatment Options. Hosp Pharm 2016;51(7):553-63. [CrossRef] [PubMed]
31. Revel-Vilk S, Mansfield R, Feder-Krengel N, Machtiger-Azoulay N, Kuter D, Szer J, et al. Real-World Experiences with Taliglucerase Alfa Home Infusions for Patients with Gaucher Disease: A Global Cohort Study. J Clin Med 2023;12(18):5913. [CrossRef] [PubMed]

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PRIMENA ENZIMSKE SUPSTITUCIONE TERAPIJE KOD PACIJENATA SA GOŠEOVOM BOLESTI TIPA 1: ISKUSTVA JEDNOG CENTRA

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Gošeova (*Gaucher*) bolest (GB) jeste lipozomalna bolest nakupljanja koja se nasleđuje autozomno recesivno, a u njenoj osnovi je mutacija gena koji kodira enzim glukocerebrozidazu. Do nagomilavanja glukocerebrozida u makrofazima jetre, slezine, koštane srži, a ređe u plućima i u drugim organima, dolazi usled smanjene sinteze enzima, nedostatka ili poremećaja funkcije enzima ili deficita sapozina C (aktivatora enzima). Klinička podela GB-a zasniva se na odsustvu (tip 1) ili prisustvu (tip 2 i tip 3) manifestacija u centralnom nervnom sistemu. Nivo β-glukozocerebrozidaze u leukocitima i vrednost hitotriozidaze u serumu određuju se radi postavljanja definitivne dijagnoze Gošeove bolesti. Nagomilavanje glukocerebrozida izazviva komplikacije u većem broju organa (anemiju, trombocitopeniju, hepatomegaliju, splenomegaliju, skeletne i neurološke promene). Za lečenje Gošeove bolesti od 1991. godine koristi se enzimska supstituciona terapija. Cilj ovog rada bio je da predstavi rezultate lečenja pacijenata sa Gošeovom bolesti tipa 1 na Klinici za hematologiju, alergologiju i kliničku imunologiju Univerzitetskog kliničkog centra Niš primenom taligluceraze alfa kao enzimske supstitucione terapije. Od januara 2016. godine do januara 2025. godine primenom taligluceraze alfa lečeno je pet pacijenata sa Gošeovom bolešću tipa 1. Kod ovih pacijenata nije bilo odgovora na prethodnu terapiju ili je pak donacija leka bila obustavljena. Svi ovi pacijenti dobro su reagovali na terapiju i nije bilo neželjenih efekata. Zapaženo je da primena taligluceraze alfa dovodi do značajnog smanjenja anemije, trombocitopenije i organomegalije, kao i do poboljšanja statusa kostiju.

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***Ključne reči**: Gošeova bolest, enzimska supstituciona terapija, taligluceraza alfa*

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PREVALENCE OF BURNOUT AND FATIGUE AMONG HEALTHCARE WORKERS OF A SECONDARY HEALTHCARE INSTITUTION IN BELGRADE DURING THE SARS-COV-2 PANDEMIC

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Burnout has been recognized increasingly globally as a major concern, affecting the physical and mental well-being of Healthcare Workers (HCWs). A cross-sectional study was conducted among the healthcare workers in Special Hospital for Rehabilitation and Orthopedic Prosthetics using the following questionnaires: Maslach Burnout Inventory–Human Services Survey (MBI–HSS) for measuring the three aspects of burnout syndrome: emotional exhaustion (EE), depersonalization (DP), and personal accomplishment (PA); Fatigue Severity Scale (FSS) for assessing the presence and degree of fatigue, and sociodemographic characteristics of respondents collected through a general questionnaire. Only fully completed questionnaires, 65 of the total of 79, were included in the study. The majority of participants were females (73.8%), married (61.5%), university degree holders (52.3%), homeowners (66.2%), and those earning above the minimum (81.5%). One third of study participants experienced the death of a close person due to coronavirus disease 2019 (COVID-19). High level of EE was observed in 41.5% of employees. Moderate to high level of DP was recorded in 36.9% of participants, and 40% exhibited a low level of PA. Emotional exhaustion was positively correlated with the FSS. Depersonalization showed a statistically significant positive correlation with the FSS. The length of vacation as the demographic characteristic was also positively correlated with the FSS. Two factors were found to be associated with high levels of EE among HCWs, and those were higher fatigue and lower monthly earnings.

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Key words: *burnout syndrome, coronavirus disease 2019, healthcare workers, fatigue*

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Introduction

The mental health effects of major disasters impact people more profoundly and for longer duration than physical injuries (1). Numerous studies have explored the effect of an infectious disease outbreak on healthcare workers' mental health (2–6). This fact has been proven in studies conducted in the past two decades during viral outbreaks such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and Ebola (7–10). According to the World Health Organization (WHO), healthcare workers (HCWs) are at high risk of physical and mental problems due to their contact with COVID-19 patients (11). Burnout syndrome (BOS) is a psychological syndrome characterized by factors such as emotional exhaustion (EE), mental fatigue, depersonalization (DP) or cynicism in the form of negative feelings and perceptions about the people one works with, and low personal accomplishment (LPA) (12). It develops in 20–80% of HCWs (13). In the 11th version of the International Classification of Diseases (ICD-11), the WHO included BOS as "a syndrome resulting from chronic workplace stress that has not been successfully managed" (12). Burnout has been associated with impaired job performance and poor health, including headaches, sleep disturbances, irritability, marital difficulties, fatigue, hypertension, anxiety, depression, and myocardial infarction, and may contribute to alcoholism and drug addiction (14). According to recent studies, some HCWs have developed psychological distress, fatigue, and burnout while facing COVID-19 (15, 16). Work-related stress among healthcare workers has become a significant health issue not only for employees but also for the entire economy. Undoubtedly, HCWs played a key role in fighting the consequences of the pandemic but, at the same time, their professional and private lives were strongly disrupted (17).

This study aimed to analyze burnout syndrome and fatigue among HCWs in the Special Hospital for Rehabilitation and Orthopedic Prosthetics in Belgrade.

Materials and Methods

A cross-sectional study, conducted from January to February 2024, included the population of respondents represented by HCWs of the Special Hospital for Rehabilitation and Orthopedic Prosthetics in Belgrade. The criteria applied to the research participants included adults (> 18 years), individuals permanently employed in the mentioned sector, and those who voluntarily consented to participate in the study. Exclusion criteria were applied to minors (< 18 years), those who had discontinuity in work for more than a year, and persons who refused to participate. This study was approved by the Ethics Committee of the Special Hospital for Rehabilitation and Orthopedic Prosthetics in Belgrade on January 25, 2024. The data for this study were obtained by voluntary filling of anonymous questionnaires by the respondents. The representative sample size was 79, of which 65 participants filled out all questionnaires. For this research, a general questionnaire was constructed and used along with Maslach Burnout Inventory–Human Services Survey (MBI–HSS) and Fatigue severity scale (FSS). The general questionnaire contained 19 questions and was used to collect the basic sociodemographic data of the respondents (gender, age, marital status, education level, length of service, illness from COVID-19).

Maslach Burnout Inventory–Human Services Survey (MBI–HSS) contains 22 questions with 3 subscales that measure the level of emotional exhaustion (EE), depersonalization (DP) and personal accomplishment (PA). Respondents circled one of the provided answers on a seven-point Likert scale (0—never; 1—few times a year or less; 2—once a month or less; 3—several times a month;4—once a week; 5—several times a week; 6—every day). The border values for EE: low level (0–16), medium level (17–26), high level (≥ 27); DP: low (0–6), medium (7–12), high (≥ 13); PA: low (0–31), medium (32–38), high (≥ 39).

The Krupp Fatigue Scale is a one-dimensional measuring instrument intended to detect fatigue symptoms. It falls under the category of self-ranking measures, meaning that respondents rank each statement on the scale according to the greatest similarity with their personal feelings and perceptions. The scale consists of nine statements that are scored on a seven-point Likert scale, from strong disagreement to streng agreement with the statement offered. The total score can have values from 9 to 63. The total value of FSS is further divided by 9, thus obtaining the average fatigue score, which can have values from 1 (complete absence of fatigue) to 7 (the most pronounced presence of fatigue). Values of the average score higher than 4 were marked by the author as pathological. The average FSS score for individuals with only depression was about 4.5. The score for people with fatigue due to chronic diseases was higher (around 6.5 on average).

Statistical analysis

The descriptive statistics, including means, medians, standard deviations, and percentiles for numerical variables and numbers and percentages for categorical variables, were used to characterize the study sample. The Mann–Whitney U test was used for numerical data to evaluate differences between groups. Spearman's correlation coefficients were calculated to explore the relationship between Maslach Burnout Inventory–Human Services Survey (MBI–HSS), Fatigue severity scale (FSS), and numerical demographic characteristics of the study population. According to Evans' classification (18), a correlation coefficient < 0.20 was considered a very weak correlation, 0.20–0.39 weak, 0.40–0.59 moderate, 0.60–0.79 strong, and > 0.80 very strong correlation. Univariate and multivariate logistic regression analysis were used to determine independent predictors of burnout. MBI–HSS subscales were used as dependent variables in separate regression models. emotional exhaustion was categorized into low/medium vs. high in the regression model. Independent variables were the following: sex, age, marital status, level of education, length of service, profession, executive positions, socioeconomic status, FSS and COVID-19-related characteristics. Variables were included in the multivariate regression analysis if they were significant at the $p < 0.05$ level according to the results of the univariate analysis. For the implementation of multiple logistic regressions, model assumptions were taken into account. All tests were two-tailed. $P < 0.05$ was considered statistically significant. Statistical analysis was done using IBM SPSS Statistics version 25.

Results

A total of 65 employees filled questionnaires, and the response rate was 81.25%. There were more females (73.8%) with an average age of 45.4 ± 12.5 years, within a range from 22 to 64 years. The majority of female participants were married (61.5%), with a university degree (52.3%), homeowners (66.2%), those working in shifts (69.2%), and 81.5% of them earned above the minimum wage. The median years of service were 20, within a range from 7 to 30 years. Additionally, 24.6% of the participants held executive positions (Table 1).

A significant majority (80.0%) reported infection with COVID-19. A 75.4% of participants were vaccinated, and 64.6% experienced a stressful event during the COVID-19 pandemic (Table 2).

Table 1. Demographic characteristics of the study population

Variable	N = 65
Sex, n (%)	
Female	48 (73.8)
Male	17 (26.2)
Age, mean ± SD	45.4 ± 12.5
Marital status, n (%)	
Married	40 (61.5)
Single	13 (20.0)
Divorced	5 (7.7)
Children, n (%)	
None	22 (33.8)
One	14 (21.5)
More	29 (44.6)
Education level, n (%)	
Highschool	20 (30.8)
College	11 (16.9)
Faculty	34 (52.3)
Profession, n (%)	
Nurse	29 (44.6)
Physiotherapist	16 (24.6)
Medical doctor	5 (7.7)
Specialist	15 (23.1)
Years of service	20 (7–30)
Working in shifts, n (%)	45 (69.2)
Shift hours	8 (5.5–12)
Length of annual leave (days)	35 (30–35)
Executive position	16 (24.6)
Duration of executive position (years)	10 (7–20)
Housing, n (%)	
Home owner	43 (66.2)
Renting	5 (7.7)
Other	17 (26.2)
Monthly earnings, n (%)	
Lower than minimal	3 (4.6)
Around minimal	9 (13.8)
Above	53 (81.5)
Sleeping hours during 24 h	6 (6–8)

Data are presented as median (25–75 percentiles)

Table 2. COVID-19-related characteristics of the study population

Variable	n (%)
COVID-19 infection	52 (80.0)
COVID-19 vaccine	49 (75.4)
Loss of close person due to COVID-19	21 (32.3)
COVID-19 as a stressful event	42 (64.6)

Table 3 presents the distribution of participants for all burnout levels alongside median scores with 25th and 75th percentiles for each burnout domain. The EE domain had a median score of 20 (25th–75th percentile: 10.5–36), with 41.5% of participants classified as low burnout, 16.9% under moderate, and 41.5% under high burnout. For the DP domain, the median score was 3 (25th–75th percentile: 0–7.5), with most HCWs (63.1%) experiencing low burnout. personal accomplishment domain scores had a median of 36, with low and high burnout levels closely distributed in 40% and 38.5%, respectively.

The correlation coefficients between FSS and the MBI–HSS domains are presented in Table 4. Emotional exhaustion was positively correlated with the FSS, with a moderate effect size (ρ = 0.570; $p < 0.001$). Depersonalization also showed a statistically significant positive correlation with the FSS, indicated weak effect size (ρ = 0.320; p = 0.009).

Table 5 presents the demographic characteristics of respondents and their associations with burnout levels and the FSS. Median scores for males were 20 for EE, 2 for DP, 35 for PA, and 2.6 for FSS, whereas females had median scores of 21 for EE, 3 for DP, 36 for PA, and 3.4 for FSS. Age correlation coefficients were 0.102 for EE, -0.071 for DP, and 0.099 for PA ($p > 0.05$). Married participants had median scores of 22 for EE, 5 for DP, and 36 for PA, while single or separated participants showed scores of 20 for EE, 3 for DP, and 32 for PA. Participants with children reported median scores of 22 for EE, 3 for DP, and 36 for PA. Educationally, those with high school or college degrees had median scores of 20 for EE and 35 for PA, and university-educated participants had scores of 21.5 for EE and 36.5 for PA ($p > 0.050$). Profession-wise, nurses and physiotherapists had a median EE score of 20, while medical doctors and specialists had a higher median EE score of 26 ($p > 0.050$). The length of vacation was positively correlated with the FSS, with a weak effect size (ρ = 0.267; p = 0.033). Individuals with lower monthly earnings had significantly higher median EE scores of 36 (25th–75th percentile 27–44) than participants with higher monthly earnings (median 20, 25th–75th percentile 10–29) (p = 0.006).

Univariate and multivariate logistic regression analyses were conducted to identify factors associated with burnout. The findings are presented in Table 6. For the EE domain, two variables were identified as significant predictors in the univariate logistic regression model: monthly earnings (p = 0.015) and FSS (p = 0.001). Healthcare workers with higher fatigue and lower monthly earnings were more likely to experience high levels of EE. In multivariate logistic regression analysis, FSS was significantly associated with EE (p = 0.002).

Table 3. Burnout syndrome among healthcare workers

Domain	Median (25th–75th percentile)	Low burnout	Moderate burnout	High burnout
EE	20 (10.5–36)	27 (41.5%)	11 (16.9%)	27 (41.5%)
DP	3 (0–7.5)	41 (63.1%)	14 (21.5%)	10 (15.4%)
PA	36 (29.5–42.5)	26 (40%)	14 (21.5%)	25 (38.5%)

Notes: Cutoffs for low/moderate/high burnout are: EE: low (0–6), moderate (17–26), high (≥ 27); DP: high (≥ 13), moderate (7–12), low (0–6); PA: low (≥ 39), moderate (32–38), high (0–31). The PA subscale is interpreted in the opposite direction as the EE and DP subscales

Table 4. Correlation coefficients between FSS and MBI–HSS domains

Burnout domain	Spearman's Ro correlation coefficient	Effect
EE	0.570*	Moderate
DP	0.320*	Week
PA	-0.175	No correlation

*p < 0.050

Table 5. Demographic characteristics of the study population according to burnout domains and FSS

Variable	EE	DP	PA	FSS
Sex				
Male	20 (11–27)	2 (0–5)	35 (28–42)	2.6 (2–3.6)
Female	21 (11–39)	3 (1–8)	36 (30–43)	3.4 (2.2–4.9)
Age	0.102	-0.071	0.099	0.129
Marital status				
Married	22 (13–36)	5 (2–8)	36 (31–44)	3.6 (2.5–4.8)
Single/Separated	20 (10–31)	3 (0–8)	32 (28–40)	2.6 (2.1–4.2)
Children, yes	22 (11–36)	3 (0–6)	36 (28–44)	3.5 (2–5.1)
Education level				
High school/College	20 (10–35)	5 (1–8)	35 (28–42)	3.6 (2.1–5.3)
University	21.5 (10.8–36.5)	2.5 (0–7.3)	36.5 (29.8–43)	3.1 (2–4.3)
Profession				
Nurse/Physiotherapist	20 (10–31)	3 (0–6)	36 (29–43)	3.2 (2.1–4.5)
Medical doctor/Specialist	26 (18–39)	3 (0–8)	36 (30–42)	3.2 (2.1–4.3)
Years of service	-0.006	-0.054	0.108	0.119
Working in shifts	22 (14–32)	2 (0–7)	35 (30–41)	3.2 (2.1–4.2)
Shift hours	0.041	0.058	0.185	-0.036
Length of vacation per year	0.165	-0.132	0.036	0.267*
Executive position	29 (20–39)	4 (0–7)	35 (31–44)	3.1 (1.9–4.1)
Duration of executive position (years)	0.037	-0.073	-0.434	-0.25
Housing, n (%)				
Home owner	23 (11–38)	3 (0–7)	36 (28–44)	3.2 (2–4.5)

Renting/Other	20 (10–27)	5 (0–8)	35 (30–40)	3.2 (2.3–4.4)
Monthly earnings				
Lower than minimal/Around minimal	36 (27–44)*	6 (1–10)	32 (26–36)	4 (2.7–7)
Above	20 (10–29)	3 (0–6)	38 (30–43)	3 (2–4.3)
Number of sleeping hours during 24h	-0.245	-0.129	0.01	-0.011
COVID-19 infection	21 (11–36)	3 (1–7)	36 (31–43)	3.2 (2.1–4.5)
COVID-19 vaccine	22 (13–36)	3 (0–6)	36 (30–43)	3.2 (2.2–4.5)
Loss of a close person due to COVID-19	23 (16–36)	3 (0–6)	37 (30–44)	3.6 (2.8–5)
COVID-19 as a stressful event	20 (10–31)	4 (1–8)	35 (30–42)	2.9 (2–4.3)

Data are presented as median (25–75 percentiles); ρCorrelation coefficient (rho); *p < 0.050

Table 6. Univariate and multivariate logistic regression models with EE burnout as the dependent variable

Variables	Univariate			Multivariate		
	B	95% CI	p	B	95% CI	p
EE						
Monthly earnings	0.171	0.04–0.71	0.015	0.221	0.05–1.06	0.06
FSS	1.852	1.30–2.65	0.001	1.803	1.24–2.62	0.002

Discussion

In the present study, most respondents were females (73.8%), married (61.5%), holding university degrees (52.3%), homeowners (66.2%), working in shifts (69.2%), earning above the minimum (81.5%), and 64.6% experienced stressful event during the COVID-19 pandemic, similar to the observational study conducted in Verona hospital on HCWs in which 61.3% of participants reported that they had experienced COVID-19-related stressful event (19). A cross-sectional study conducted among Turkish HCWs in 2021 showed that 56.7% have moderate to high EE, 35.8% moderate-to-high DP and, 58% low PA. While in the current study population, 58.4% have moderate to high EE, 36.9% moderate to high DP, and 40% low PA. Almost a third of HCWs in North-West Italy had high EE, DP, and low PA (6). A statistically significant positive correlation was found between the Fatigue Severity Scale and emotional exhaustion; also, depersonalization showed a statistically significant positive correlation with the FSS. Regarding the association between demographic characteristics and FSS, it was found that the length of vacation was positively correlated with fatigue. Many studies have been conducted on burnout syndrome and its associated factors (1, 6, 16, 19), and each of them identified different predictors associated with higher burnout scores. For the EE domain, two significant predictors were identified: monthly earnings and FSS. Healthcare workers with higher fatigue and lower monthly earnings were more likely to experience high levels of emotional exhaustion.

Conclusion

Results of the present study, along with many others concerning burnout syndrome among healthcare workers during COVID-19 pandemic as well as previous pandemics like SARS and MERS, have demonstrated that the mental health of employees in this sector is at risk. Recognizing the potential damage caused by burnout and understanding that humans are the most valuable resource in healthcare delivery, we strongly believe that it is essential to develop and implement a program of preventive mental health care for healthcare workers.

References

1. Irem Akova, Esma Kilic, Mehmet Emin Ozdemir. Prevalence of Burnout, Depression, Anxiety, Stress, and Hopelessness Among Healthcare Workers in COVID-19 Pandemic in Turkey. Inquiry 2022; 59: 469580221079684. [CrossRef] [PubMed]
2. Cabello IR, Echavez JFM, Serrano-Ripoll MJ, Fraile-Navarro D, de Roque MAF, Moreno GP, et al. Impact of viral epidemic outbreaks on mental health of health-care workers: a rapid systematic review. J Affect Disord 2020;277:347–57. [CrossRef] [PubMed]
3. Jovanović N, Podlesek A, Volpe U, Barrett E, Ferrari S, Rojnic Kuzman M, et al. Burnout syndrome among psychiatric trainees in 22 countries: Risk increased by long working hours, lack of supervision, and psychiatry not being first career choice. Eur Psychiatry 2016;32:34–41. [CrossRef] [PubMed]
4. Ozge Ayadin Guclu, MD, Mehmet Karadag, MD, Muhamed Emin Akkoyuniu, MD, Turan Acican, MD, Bunyamin Sertogullarindan, MD, Gokhan Kiribas, MD et al. Association between burnout, anxiety and insomnia in healthcare workers: a cross-sectional study. Psychol Health Med 2022; 27(5):1117-30. [CrossRef] [PubMed]
5. Abdulmajeed A. Alkhamees, Hatem Assiri, Hatim Yousef Alharabi, Abdulah Nasser & Mohammad A. Alkhames. Burnout and depression among psychiatry residents during COVID-19 pandemic. Hum Resour Health 2021; 19(1):46. [CrossRef] [PubMed]
6. Andrea Naldi, Fabrizio Vallelonga, Alessandra Di Liberto, Roberto Cavallo, Monica Agnesone, Marco Gonella et al. COVID-19 pandemic-related anxiety, distress and burnout: prevalence and associated factors in healthcare workers of North-West Italy. BJPsych Open 2021; 7(1):e27. [CrossRef] [PubMed]
7. Ruchira W Khasne, Bhagyashree S Dhakulkar, Hitendra C Mahajan, Atul P Kulkarni. Burnout among Healthcare Workers during COVID-19 Pandemic in India: Results of a Questionnaire-based Survey. Indian J Crit Care Med 2020; 24(8): 664–71. [CrossRef] [PubMed]
8. Li L, Wan C, Ding R, Liu Y, Chen J, Wu Z, Liang C, He Z, Li C. Mental distress among Liberian medical staff working at the China Ebola treatment unit: a cross sectional study. Health Qual Life Outcomes 2015;13:156. [CrossRef] [PubMed]
9. McAlonan GM, Lee A, Cheung V, Cheung C, Tsang K, Sham P, Chua S, Wong J. Immediate and sustained psychological impact of an emerging infectious disease outbreakon health careworkers. Can J Psychiatr 2007;52(4):241–7. [CrossRef] [PubMed]
10. Portero S, Cebrino J, Herruzo J, Vaquero M. Factors related to the probability of suffering mental health problems in emergency care professionals. Rev,Lat Am Enferm 2019;27(e3144):3079–144. [CrossRef] [PubMed]
11. Koh D, Lim M-K, Chia S-E. SARS: health care work can be hazardous to health. Occup Med. 2003;53(4):241-3. [CrossRef] [PubMed]
12. Nicola Magnavita, Francesco Chirico, Sergio Garbarino, Nicola Luigi Bragazzi,Emiliano Santacroce and Salvatore Zaffina. SARS/MERS/SARS-CoV-2 Outbreaks and Burnout Syndrome among HealthcareWorkers. An Umbrella Systematic Review. Int J Environ Res Public Health 2021; 18(8):4361. [CrossRef] [PubMed]
13. Hannah Dobson, Charles B Malpas, Aidan JC Burrell, Caroline Gurvich, Leo Chen, Jayashri Kulkarni et al. Burnout and psychological distress amongst Australian healthcare workers during the COVID-19 pandemic. Australas Psychiatry 2021; 29(1):26-30. [CrossRef] [PubMed]
14. L Abdulla, DM Al-Quhtani, MG Al-Kuwari. Prevalence and determinants of burnout syndrome among primary healthcare physicians in Qatar. South African Family Practice 2011; 53: 380-3. [CrossRef]
15. Lai J, Ma S, Wang Y, Cai Z, Hu J, Wei N, Wu J, Du H, Chen T, Li R, et al. Factors associated with mental health outcomes among health care workers exposed to coronavirus disease 2019. JAMA Netw Open 2020;3(3):1–12. [CrossRef] [PubMed]
16. Zhang S, Wang J, Xie F, Yin D, Shi Y, Zhang M, Yin H, Li F, Yang L, Cao D, Sun T. A cross-sectional study of job burnout, psychological attachment, and the career calling of Chinese doctors. BMC Health Serv Res 2020;20(193):2–11. [CrossRef]
17. Zbigniew Izdebski, Alicja Kozakiewicz, Maciej Białorudzki, Joanna Dec-Pietrowska and Joanna Mazur. Occupational Burnout in HealthcareWorkers, Stress and Other Symptoms of Work Overload during the COVID-19 Pandemic in Poland. Int J Environ Res Public Health 2023; 20(3):2428. [CrossRef] [PubMed]
18. Evans JD. Straightforward Statistics for the Behavioral Sciences. Pacific Grove, CA, USA: Brooks/Cole Publishing, 1996. https://worldveg.tind.io/record/57723
19. Antonio Lasalvila, Francesco Amaddeo, Stefano Porru, Angela Carta, Stefano Tardivo, Chiara Bovo et al. Levels of burn-out among healthcare workers during the COVID-19 pandemic and their associated factors: a cross-sectional study in a tertiary hospital of a highly burdened area of north-east Italy. BMJ Open 2021;11:e04517. [CrossRef] [PubMed]

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PREVALENCIJA SINDROMA SAGOREVANJA I UMORA MEĐU ZDRAVSTVENIM RADNICIMA U JEDNOJ SEKUNDARNOJ ZDRAVSTVENOJ USTANOVI U BEOGRADU U TOKU PANDEMIJE SARS-CoV-2 VIRUSA

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Izgaranje se na globalnom nivou sve više prepoznaje kao glavna briga, budući da utiče na fizičko i mentalno blagostanje zdravstvenih radnika. Sprovedena je studija preseka među zaposlenima u zdravstvenoj službi Specijalne bolnice za rehabilitaciju i ortopedsku protetiku. Korišćeni su sledeći upitnici: *Maslach Burnout Invetory–Human Services Survey* (MBI–HSS) – za merenje triju aspekata sindroma sagorevanja na poslu: emotivne iscrpljenost (engl. *emotional exhaustion* – EE) depersonalizacije (enl. *depersonalization* – DP) lična postignuća (engl. *personal accomplishment* – PA) *Fatigue Severity Scale* (FSS) – za procenu prisustva i stepena zamora; opšti upitnik, kojim su prikupljeni sociodemografski parametri ispitanika. U studiju su uvršteni samo kompletno popunjeni upitnici; takvih je bilo šezdeset pet. Dobijeni rezultati su pokazali da je većina učesnika bila ženskog pola (73,8%); udate (61,5%), sa fakultetskom diplomom (52,3%), vlasnice kuća (66,2%) i sa zaradom koja prevazilazi minimalnu zaradu (81,5%). Trećina učesnika u studiji doživela je smrt bliske osobe usled kovida 19. Visok stepen emocionalne iscrpljenosti primećen je kod 41,5% zaposlenih. Umeren do visok stepen depersonalizacije zabeležen je kod 36,9% zaposlenih, dok je 40% njih ispoljilo nizak nivo ličnog postignuća. U pozitivnoj korelaciji sa FSS-om bio je EE. Depersonalizacija je pokazala statistički značajnu pozitivnu korelaciju sa FSS-om ozbiljnosti umora. Dužina godišnjeg odmora kao demografska karakteristika takođe je bila u pozitivnoj korelaciji sa FSS-om. Uočena su dva faktora povezana sa visokim nivoom EE-ja kod zdravstvenih radnika: viši stepen umora i niže mesečne zarade.

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***Ključne reči**: sindrom sagorevanja, kovid 19, zdravstveni radnici, zamor*

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INFLUENCE OF WORK POSTURE ON THE OCCURRENCE OF DISORDERS OF THE MUSCULOSKELETAL SYSTEM AMONG WORKERS IN THE TOBACCO INDUSTRY

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Musculoskeletal system disordes (MSDs) require special attention because they represent one of the leading causes of absence from work and disability in the world. In addition to affecting the quality of life itself, they greatly reduce the ability to work, and can occur at any age. Their multi-factorial etiologies primarily caused by the interaction of an unhealthy lifestyle and workplace conditions that vary in different occupations.

This research aimed to examine the prevalence of musculoskeletal system disorders among workers of the Niš Tobacco Industry (Niš TI), as well as the influence of the position of workers and other work-related factors on the occurrence of musculoskeletal disorders. Out of a total of 426 workers examined during the 2022 systematic examination, 69 (16.2%) workers were found to have musculoskeletal disorders. Musculoskeletal disorders were more prevalent in workers in the direct production sector (standing position), 61 (88.41%), in female workers (40.6%), and are also associated with overweight and old age of workers.

The high frequency of musculoskeletal disorders among Niš TI employees indicates that it is necessary to take adequate prevention, control, and risk reduction measures for the occurrence of diseases and injuries of the musculoskeletal system, all with the aim of reducing the frequency of diseases, reducing the risk of disability, improving the quality of life, and increasing work ability.

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Key words: *musculoskeletal system disorders, workers, Tobacco industry Niš, working body position*

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Introduction

Musculoskeletal system disorders (MSDs) are one of the most common causes of chronic pain, long-term disability, and inability to work. These disorders are closely related to functional disability and high consumption of health and social resources (1–3), imposing high costs on society for treatment, sick leave, retirement, and significantly reducing productivity in working life (2). In the European Union (EU) member states, MSDs account for a significant proportion of lost working days and affect workers across all sectors and occupations. If MSDs are caused or aggravated primarily by work and the effects of the immediate work environment, they are designated as work-related MSDs (WMSDs) or occupational MSDs (4). Work-related MSDs contribute significantly to reduced work productivity (5, 6), and symptoms usually appear only after years of chronic exposure to risk factors (2).

Musculoskeletal disorders are among the most common health conditions of the working population, affecting millions of workers worldwide (7). The prevalence of occupational MSDs varies between countries, sectors, and demographics, and knowledge of prevalence is crucial to better understand the risks for different sectors and demographics. These data are publicly available and published mostly by developed countries (8). It is estimated that one-third of all absences from work in industrialized countries are related to MSD (9). Thus, according to EU data from 2013, about 60% of workers reported some of the disorders related to MSD, i.e., three out of every five workers, and in 2015, the most common complaint were related to back pain (43%) and pain in the upper limbs (41%). The prevalence of

MSDs also varies depending on sociodemographic factors, so the rates of disorders are higher in women and increase significantly with age (4, 8).

The musculoskeletal system is the fundamental structural component of the tumor body, which includes muscles, bones, joints, and connective tissues. These components are interconnected through the fascial system, which envelops and supports both bones and muscles, enabling them to function as a cohesive unit (3, 10, 11). The health of this system is essential for daily functioning, because disturbances in its parts can lead to various disorders. As many as 150 different types of MSDs have been reported in the literature (12), and include a wide range of inflammatory and degenerative diseases, including some lesser-known conditions causing pain and functional impairment. These include tendon inflammations (tendinitis and tenosynovitis), myalgias, nerve entrapment syndromes, degenerative disorders across different levels of the spinal column, etc. (2).

Musculoskeletal system disorders usually present in the form of pain and temporary or permanent reduced mobility, which can seriously affect the ability to perform daily tasks, work, and social activities (3, 13). They significantly increase the risk of developing chronic conditions such as osteoarthritis, osteoporosis, and sarcopenia, which can affect different parts of the body and cause painful syndromes or inflammatory diseases. Therefore, it is important to preserve the health of the musculoskeletal system, as well as recognize and minimize workplace risks in time to prevent long-term consequences (1, 14).

The occurrence and progression of these disorders are influenced by various factors, such as genetic predisposition, environmental factors, workplace factors, occupational activities, and locations where individuals spend most of their time (3, 15). Work-related MSDs are causally related to physical load resulting from occupational activity (9). It is believed that the intense and sudden action of these factors, such as mechanical injuries, can lead to professional injuries that are classified as traumatism. On the other hand, long-term repetition of the same movements, when their intensity and duration exceed tissue resistance, causes changes in the structure of joints, connective tissue, muscles, and tendons (16). Chronic injuries can occur as a result of long-term workload, which workers often neglect and ignore due to rapid healing and relief of complaints (9). The risk of developing disorders, especially in the lower back, also depends on body posture, working position and movement, i.e., twisting or bending of the trunk. These postural requirements play a particularly important role when working in confined spaces (17). That is why it is not surprising that MSD is high frequency and prevalent among workers who are primarily exposed to manual handling, repetitive and static work, vibrations, as well as poor psychological and social conditions (2). Also playthe key role in the development of these diseases individual characteristics and predispositions (16).

Early diagnosis and effective prevention of MSDs are essential from a socioeconomic perspective, and take place in parallel with the improvement of the working conditions that underlie the occurrence of these conditions (3). An important role in disease recognition and early diagnosis is primarily played by general practitioners, to whom patients first turn for help due to their frequent symptoms, pain, or functional limitations. However, the role of occupational medicine specialists, who, in cooperation with companies and employers, provide preventive services (17), is indispensable. Health promotion and raising awareness among workers during their careers regarding strategies to prevent the of MSDs are crucial for extending their working life (4).

For the effective prevention of work related musculoskeletal disorders (WMSDs), the most important thing is the balance between the mechanical load at work and the carrying capacity of the musculoskeletal system (9), which implies the elimination and/or minimization of risks present at workplaces. Each workplace has unique risk factors that contribute to MSDs, so a careful assessment should be carried out to identify them, and individual factors such as demographic factors should also be taken into account when assessing risk (8). In order to prevent WMSDs and to stimulate the satisfaction and productivity of workers, it is essential to apply the ergonomics of the workplace and work environment (2). Education and training are at a lower level of prevention of MSDs, because they are administrative interventions that change the behavior and competencies of workers in dealing with risks instead of eliminating or reducing job-related risks (8).

Aim

This research aimed to assess the prevalence of MSDs, with a special focus on:

-Assessment of the influence of the body position at work on the incidence of MSDs;

-Assessment of the impact of other factors, such as age, gender, length of service and length of exposure, work experience, and body mass index (BMI);

-Prevalence of MSDs; and

-Definition of preventive measures.

Materials and Methods

A 61-year-old female presented with The workers of the Niš Tobacco Industry (Niš TI) were observed in 2022. The results of systematic medical examinationis of workers employed in TI were used as a source of data. Systematic examinations of workers were carried out in the period from January to December 2022. During 2022, a total of 426 workers were examined. The

analysis was performed according to gender, age, and edukationlevel, length of service, and duration of at the cuarent workplace (work experience spent at the workplace to which the worker was assigned at the time of observation), body position at work, and body mass index (BMI). The assessment of harmful factors of the working environment was obtained in the instructions for the examination of workers, that is, it was derived from the risk assessment act of TI workplaces. The impact of body position during work on the occurrence of MSDs among workers was analyzed based on a detailed work history.

The method of work was retrospective and descriptive. Statistical analyzis was performed using IBM SPSS statistics verision 30.0.0. Relative numbers (structure indicators) were used from descriptive statistical methods, while Student's t-test was used for testing statistical hypotheses, at a statistical significance level of 0.05.

Results

Out of a total of 426 tobacco industry workers examined as part of a regular systematic examination in 2022, 69 workers (16.2%) were diagnosed with MSDs. According to gender and age structure, there were 338 men, aged 18 to 62 mean age (42.21 ± 10.63) and 88 women, aged 28 to 60 (50.82 ± 7.68). In the group of workers who were diagnosed with MSDs, the average age among male respondents was 44.11 ± 8.36, and among female respondents, 51.58 ± 5.77 (Table 1).

Among male workers, the largest number of patients belongs to the age group between 40 and 49 years, while the largest number of women is in the age group of 50 to 59 years (Figure 1).

In men, the total working experience (total WEX) ranged from 2 months to 40 years (17.46 ± 10.55), and the exposed working experience ranged from 2 months to 38 years (10.01 ± 9.43). In women, total WEX ranged from 5 months to 39 years (26.21 ± 8.88), and exposed WEX ranged from 5 months to 39 years (20.24 ± 11.93) (Table 1).

The influence of body position at work on the occurrence of MSDs was observed in workers who predominantly stood for more than 6 hours per workday hours, compared to workers who predominantly sat, as well as in workers who changed their position during work. A statistically significant difference was found in the group of workers who predominantly stand during working hours ($p < 0.05$) (Table 2).

According to the qualification structure, the largest number of workers was distributed the direct production sector, 328 workers, in the technical services sector 68 workers, and 27 workers in the quality control sector. A statistically significant difference was observed in the direct production sector, where there is a higher prevalence of MSDs among female workers (Table 3).

Table 1. Age of employees, total, and exposed work experience

Gender		Number of employees	Age			Total WEX	Exposed WEX
			min	max	mean		
Total	M	338	18	62	42.21 ± 10.63	17.46 ± 10.55	10.01 ± 9.43
	F	88	28	60	50.82 ± 7.68	26.21 ± 8.88	20.24 ± 11.93
MSD	M	41	27	60	44.11 ± 8.36	20.11 ± 8.88	10.06 ± 8.00
	F	28	38	59	51.58 ± 5.77	26.72 ± 7.63	20.35 ± 11.45

**Total WEX—total work experience, Exposed WEX—Exposed work experience*

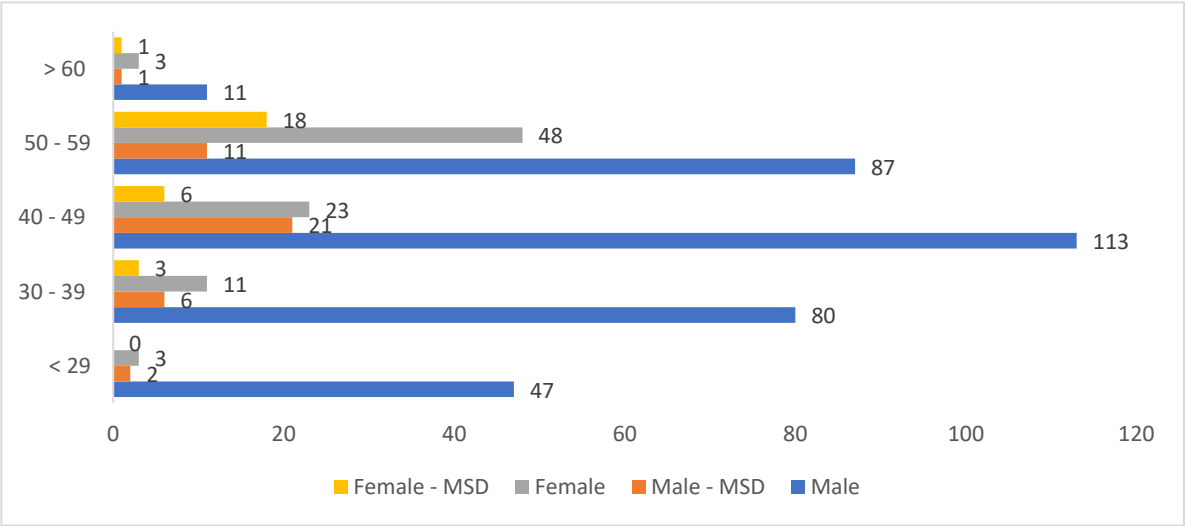

Figure 1. Age structure of all examined workers and workers suffering from MSDs

Table 2. Influence of body position on the occurrence of musculoskeletal diseases

Body position	Number of employees n (%)	Number of people with MSD n (%)	p-value
Standing	328 (77%)	61 (88.41%)	0.0386*
Combined	68 (15.96%)	7 (10.14%)	0.2120
Sitting	27 (6.33%)	1 (1.45%)	0.1033
Total	426	69	

*p < 0.05

Table 3. The difference in the incidence of MSDs according to gender and body position at work

SECTOR/Job position	Body position	Gender	Total number of employees n (%)		MSD n (%)		p-value M/F
SECTOR A—DIRECT PRODUCTION	Standing > 6 h	M	254	59.62%	35	17.78%	0.0001*
		F	74	17.37%	26	35.14%	
SECTOR B—TECHNICAL SERVICES	Combined	M	65	15.26%	6	9.23%	0.1845
		F	3	0.70%	1	33.33%	
SECTOR C—QUALITY CONTROL	Sitting > 6 h	M	8	1.88%	0	0%	0.5270
		F	19	4.46%	1	5.26%	
TOTAL			426	100%	69	16.2%	

*p < 0.05

Analysis of the association between body mass index and musculoskeletal disorders showed that overweight individuals have a higher tendency to develop MSDs. Two-thirds of respondents, 45 (65.22%), were overweight and had a BMI from 25.0 to 29.9, while 14 (20.29%) belonged to the obese category. There were no underweight individuals with a BMI below 18.5 (Table 4).

In male workers, BMI ranged from 20.37 to 35.43 (25.27 ± 2.37), body weight (BW) from 70 to 108 kg (84.49 ± 6.05), body height (BH) from 168 to 192 cm (183.05 ± 4.57). In women BMI ranged, from 20.05 to 31.96 (25.15 ± 3.2), BW from 56 to 92 kg (72.32 ± 8.7), BH from 157 to 182 cm (69.71 ± 4.83). In the group of workers suffering from MSDs, the average BMI was 27.86 ± 2.63 for male workers and 27.27 ± 3.1 for female workers (Table 5).

Musculoskeletal disorders were most prevalent among workers who worked in a sitting position, and complained of lower back pain 24 (34.78%), cervical spine poin 18 (26.09%), and joint pain 10 (14.49%) (Table 6).

According to gender, the most common diagnoses according to the International Classification of Diseases, 10th Revision (ICD-10) in men were lower back pain (51.22%), followed by spondylosis of the cervical spine (21.95%) and arthralgia (14.63%), while in women the most frequent diagnosis was cervical spondylosis (39.29%), lumboischialgia (21.43%) and arthralgia (14.29%) (Table 7).

An inspection of the Risk Assessment Act for workplaces where workers are assigned found that rot all workplaces pose an increased risk and that the dynamics of work activities are of such intensity that the work is characterized as moderate physical effort. Data obtained from the occupational history show that workers in the direct production sector spend more than 6 hours standing during working hours, while workers in the quality control sector spend more than 6 hours in a sitting position. Workers in the technical sector have a combination of standing and siting positions.

Table 4. Influence of BMI on incidence of musculoskeletal disorders

BMI Body position	18.5–24.9 (normal)	25.0–29.9 (overweight)	> 30 (obese)	Total
Standing (n = 61)	8 (72.73%)	42 (93.33%)	11 (78.57%)	61 (88.41%)
Combined (n = 7)	2 (27.27%)	3 (6.67%)	2 (14.29%)	7 (10.14%)
Sitting (n = 1)	0	0	1 (7.14%)	1 (1.45%)
Total	10 (14.49%)	45 (65.22%)	14 (20.29%)	69 (100%)

Table 5. Body mass index of workers suffering from MSDs

	Gender	Number	BMI			
			min	max	mean	SD
TOTAL	M	338	20.37	35.43	25.27	2.37
	F	88	20.05	31.96	25.15	3.2
	M	41	23.37	35.43	27.86	2.63
	F	28	20.08	31.96	27.27	3.1

Table 6. Distribution of MSDs according to body position at work

Body position / Diagnosis	Standing (n = 61)	Combined (n = 7)	Sitting (n = 1)
M54.5 Dolor sacralis	24 (34.78%)	3 (4.35%)	
M47 Spondylosis vert. cervicalis	18 (26.09%)	2 (2.9%)	
M53.1 Sy cervicobrachialis	4 (5.8%)	1 (1.45%)	
M06.9 Arthritis reumatoides			1 (1.45%)
M25.5 Arthralgia	10 (14.49%)		
M81.9 Osteoporosis	3 (4.35%)		
M17 Gonarthrosis	2 (2.9%)	1 (1.45%)	

Table 7. Distribution of MSDs according to ICD 10 and by gender

Diagnosis	Male (n = 41)	Female (n = 28)
M54.5 Dolor sacralis	21 (51.22%)	6 (21.43%)
M47 Spondylosis vert. cervicalis	9 (21.95%)	11 (39.29%)
M53.1 Sy cervicobrachialis	3 (7.32%)	2 (7.14%)
M06.9 Arthritis reumatoides	/	1 (3.57%)
M25.5 Arthralgia	6 (14.63%)	4 (14.29%)
M81.9 Osteoporosis	/	3 (10.71%)
M17 Gonarthrosis	2 (4.88%)	1 (3.57%)

Discussion

A total of 426 employees of the Niš Tobacco Industry responded to the regular systematic examination in 2022. Musculoskeletal disorders were identified in 69 (16.2%) cases, namely in 41 (12.13%) of 338 men and in 28 (31.82%) of 88 examined women. The mean age of male workers who were diagnosed with MSDs was 44.11 ± 8.36, of which 51.22% belonged to the age group between 40 and 49 years old, while the average age of women was 51.58 ± 5.77, and the highest percentage was between 50 and 59 years old (64.29%). Statistically, there was a significant difference in the exposed working experience. On average, women spent 20.35 ± 11.45 years in the workplace, and men significantly less, 10.06 ± 8.00 years. There was a statistically significant difference in the prevalence of MSDs in the group of workers who predominantly stood during working hours ($p < 0.05$), which indicates the importance of the influence of body position during

work on the expression of MSDs. A statistically significant difference was also present in the direct production sector, where there was a higher prevalence of MSDs among female workers ($p < 0.0001$), which can be explained by the difference in anatomical and physiological structure, as well as the difference in physical endurance between the men and women. Among the MSDs in men, lower back pain was the most common (51.22%), followed by spondylosis of the cervical spine (21.95%) and arthralgia (14.63%), while in women, the most common diagnosis was spondylosis of the cervical spine (39.29%), followed by lower back pain (21.43%) and arthralgia (14.29%). Analyzing the significance of the influence of body mass index, it is noted that overweight people have a greater tendency to suffer from MSDs. Two-thirds of respondents 45 (65.22%) were overweight, while almost 20% belonged to the obese group. All workplaces, according to the Risk Assessment Act, are characterized as workplaces without increased risk with moderate physical effort.

According to data from the European Agency for Safety and Health at Work (EU-OSHA), in France, for example, postural and joint loads (74.6% of men and 73.9% of women), followed by standing or upright work in place (48.6% of men and 42.9% of women), walking during work (47.5% of men and 34.5% of women) and manual handling of loads (44.1% of men and 29.0% of women) were cited as the most common occupational risks for MSDs. In addition to work involving high physical demands, both organizational and psychosocial risk factors can also affect the health of the musculoskeletal system of workers (18). In a large number of European countries, pain in the lower back is not classified as an occupational disease, except in cases where it is caused by an injury at work. For example, in the Czech Republic in 2012, MSDs accounted for 20% of disability cases, white in Germany they accounted for 23.3% of all sick leave in 2010. Given the prevalence of this diagnosis in the working population and the numerous pieus of evidence that physical exertion can not only cause but also worsen MSDs, these disorders represent a global social problem and require prevention in the early stages, which primarily includes technical prevention and good work organization (19). According to the EU Report from 2013, in 22 of the 29 countries that participated in the survey, risk prevention is considered a priority. Of these, in 16 countries, MSDs were singled out as a key focus in disease prevention (20).

A review study by Jacques-Bret et al. showed that the highest prevalence of MSDs was recorded in the lower back (over 60%), as well as in the shoulders and upper extremities (35–55%) in surgeons and dentists, which is associated with prolonged maintenance and repetition of incorrect body positions (21). Previous studies have showen that risk factors, such as working posture, long working hours, repetitive and intense movements, work experience, age, gender, and worning stressful conditions, are significantly associated with the occurrence of MSDs, which emphasizes the need for preventive measures and ergonomic adaptation of the work environment (22). The situation is particularly pronounced in low- and middle-income countries, where limited reporting systems make it difficult to monitor and solve this problem (23).

Monitoring the prevalence of work-related diseases and defining the risk factors that favor their occurrence provides us with valuable data that we can use to create and implement adequate prevention measures to reduce the risk of illness and/or injuries at work. Occupational health professionals should consider the implementation of appropriate ergonomic measures that can play a key role in the prevention and management of MSDs in this occupational group. Education of workers, implementation of programs to raise the awareness among workers about healthy lifestyles, and organizing sports activities are just some of the measures that can significantly affect the improvement of the physical and psychological well-being of workers. Therefore, it is important to carry out further research in practice, in order to examine all specific causative factors and to develop intervention strategies to improve occupational health.

Conclusion

Workers employed in the direct production sector of the Tobacco industry in Niš have a greater tendency to develop musculoskeletal disorders, which indicates the influence of non-physiological body positions, i.e., prolongded standing at work. A statistically significant difference in the incidence of MSDs also exists between the sexes, as indicated by our results, where the prevalence of MSDs is significantly higher in women. Women are more likely to suffer from spondylosis of the cervical spine, which is probably caused by long-term non-physiological body posture during work, while male workers are more prone to lower back pain, which can be related to lifting heavy loads. Overweight and older age may also be related to the high rate of MSDs in tobacco workers.

References

1. Weyh C, Pilat C, Krüger K. Musculoskeletal disorders and level of physical activity in welders. Occupational Medicine 2020;70(8):586-92. [CrossRef] [PubMed]
2. Kilbom Å, Armstrong T, Buckle P, Fine L, Hagberg M, Haring-Sweeney M, et al. Musculoskeletal disorders: Work-related risk factors and prevention. Int J Occup Environ Health 1996;2(3):239-46. [PubMed]
3. Greggi C, Visconti VV, Albanese M, Gasperini B, Chiavoghilefu A, Prezioso C, et al. Work-Related Musculoskeletal Disorders: A Systematic Review and Meta-Analysis. *Journal of Clinical Medicine* 2024;13(13):3964. [CrossRef] [PubMed]
4. European Agency for Safety and Health at Work. Work-related musculoskeletal disorders: prevalence, costs and demographics in the EU. Luxembourg: Publications Office of the European Union; 2019. Available from: URL: https://osha.europa.eu/en/publications/msds-facts-and-figures-overview-prevalence-costs-and-demographics-msds-europe
5. Prütz F, Seeling S, Ryl L, Scheidt-Nave CE, Ziese T, Lampert T. Welche Krankheiten bestimmen die Zukunft?. Fehlzeiten-Report 2014: Erfolgreiche Unternehmen von morgen–gesunde Zukunft heute gestalten 2014:113-26. [CrossRef]
6. Ariëns GA, van Mechelen W, Bongers PM, Bouter LM, van der Wal G. Physical risk factors for neck pain. Scand J Work Environ Health. 2000;26:7–19. [CrossRef] [PubMed]
7. Chen HF, Lee CH, Chang RE. Workload of Attending Physicians at an Academic Center in Taiwan. J Chin Med Assoc 2010;73:425–30. [CrossRef] [PubMed]
8. Tang KH. The prevalence, causes and prevention of occupational musculoskeletal disorders. Glob Acad J Med Sci 2022;4(2):56-68. [CrossRef]
9. Luttmann A, Jàger M, Griefahn B, Caffier G, Liebers F. Preventing musculoskeletal disorders in the workplace. WHO; 2003. Available from: URL: https://iris.who.int/handle/10665/42651
10. Vilella RC, Reddivari AKR. Musculoskeletal Examination. In: StatPearls. Treasure Island (FL): StatPearls Publishing; September 4, 2023. [CrossRef] [PubMed]
11. Bordoni B, Mahabadi N, Varacallo MS. StatPearls Publishing; Treasure Island, FL, USA: 2022. Anatomy, Fascia. [PubMed]
12. Punnett L, Wegman DH. Work-related musculoskeletal disorders: the epidemiologic evidence and the debate. Journal of electromyography and kinesiology 2004;14(1):13-23. [CrossRef] [PubMed]
13. Capone AC, Parikh PM, Gatti ME, Davidson BJ, Davison SP. Occupational injury in plastic surgeons. Plastic and reconstructive surgery 2010;125(5):1555-61. [CrossRef] [PubMed]
14. Epstein S, Sparer EH, Tran BN, Ruan Q, Dennerlein J, Singhal D, et al. Prevalence of Work-Related Musculoskeletal Disorders Among Surgeons and Interventionalists: A Systematic Review and Meta-analysis. JAMA Surg 2018;153(2):e174947. [CrossRef] [PubMed]
15. ChChan FK, Hsu CC, Lin HJ, Wang JJ, Su SB, Huang CC, et al. Physicians as well as nonphysician health care professionals in Taiwan have higher risk for lumbar herniated intervertebral disc than general population. Medicine (Baltimore) 2018;97(1):e9561. [PubMed]
16. Vuković S, Krstev S, Maksimović MŽ. Diseases of the locomotor system in forestry workers. Serbian archive for all medicine. 2004;132(7-8):246-9.
17. Hagberg M, Violante FS, Bonfiglioli R, Descatha A, Gold J, Evanoff B, et al. Prevention of musculoskeletal disorders in workers: classification and health surveillance - statements of the Scientific Committee on Musculoskeletal Disorders of the International Commission on Occupational Health. BMC Musculoskelet Disord 2012;13:109. [CrossRef] [PubMed]
18. Isusi I. Work-related musculoskeletal disorders: facts and figures—synthesis report of 10 EU member states reports. European Agency for Safety and Health at Work, Luxembourg: Publications Office of the European Union. 2020. Avaliable from: URL: https://osha.europa.eu/en/publications/work-related-musculoskeletal-disorders-facts-and-figures-synthesis-report-10-eu-member
19. Laštovková A, Nakládalová M, Fenclová Z, Urban P, Gaďourek P, Lebeda T, et al. Low-back Pain Disorders as Occupational Diseases in the Czech Republic and 22 European Countries: Comparison of National Systems, Related Diagnoses and Evaluation Criteria. Cent Eur J Public Health 2015;23(3):244-51. [CrossRef] [PubMed]
20. EU Commission. Report on the Current Situation in Relation to Occupational Diseases' Systems in EU Member States and EFTA/EEA Countries. Particular Relative Commission Recommendation 2003/670/EC Concerning the European Schedule of Occupational Diseases and Gathering of Data on Relevant Related Aspects. 2003;670. Available from: URL: https://ec.europa.eu/social/BlobServlet?docId=9982&langId=en
21. Jacquier-Bret J, Gorce P. Prevalence of body area work-related musculoskeletal disorders among healthcare professionals: a systematic review. Int J Environ Res Public Health 2023;20(1):841. [CrossRef] [PubMed]
22. Das D, Kumar A, Sharma M. A systematic review of work-related musculoskeletal disorders among handicraft workers. Int J Occup Saf Ergon 2018;26(1):55–70. [CrossRef] [PubMed]
23. Louw QA, Morris LD, Grimmer-Somers K. The prevalence of low back pain in Africa: a systematic review. BMC Musculoskelet Disord 2007;8(1):105. [CrossRef] [PubMed]

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UTICAJ POLOŽAJA TELA U TOKU RADA NA POJAVU BOLESTI MIŠIĆNO-SKELETNOG SISTEMA KOD RADNIKA U DUVANSKOJ INDUSTRIJI

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Bolesti mišićno-skeletnog sistema zahtevaju posebnu pažnju, s obzirom na to da predstavljaju jedan od vodećih uzroka odsustva sa posla i invaliditeta u svetu. Pored toga što utiču na sam kvalitet života, u velikoj meri umanjuju radnu sposobnost. Mogu se javiti u bilo kojoj životnoj dobi. Mada su uzroci njihove pojave različiti, nastaju prvenstveno usled interakcije nezdravog stila života i uslova na radnom mestu, koji se razlikuju u zavisnosti od zanimanja.

Cilj ovog istraživanja bio je da ispita zastupljenost bolesti mišićno-skeletnog sistema među radnicima Duvanske industrije Niš (DIN), kao i uticaj položaja tela radnika i drugih faktora u vezi sa radom na pojavu oboljenja mišićno-skeletnog sistema. Od ukupno četiristo dvadeset šest radnika pregledanih na sistematskom pregledu 2022. godine, kod šezdeset devet (16,2%) radnika registrovani su poremećaji mišićno-skeletnog sistema. Bolesti mišićno-skeletnog sistema bile su zastupljenije kod radnika u sektoru direktne proizvodnje (stojeći položaj) – 61 (88,41%) i kod žena (40,6%). Takođe, dovode se u vezu i sa prekomernom telesnom težinom i sa starošću radnika.

Visok stepen učestalosti mišićno-skeletnih poremećaja među zaposlenima u DIN-u ukazuje na to da je potrebno preduzeti adekvatne mere prevencije, kontrole i redukcije rizika za nastanak oboljenja i povreda mišićno-skeletnog sistema kako bi se smanjila učestalost oboljevanja i rizik od nastanka invaliditeta, ali i poboljšao kvalitet života i povećala radna sposobnost.

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***Ključne reči**: poremećaji mišićno-skeletnog sistema, radnici, Duvanska industrija Niš, položaj tela u toku rada*

Case report

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PRIMARY VAGINAL MELANOMA

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Primary vaginal melanoma is a rare type of mucosal melanoma accounting for 0.3% to 0.8% of all melanomas in women and less than 3% of all vaginal malignancies.

A 78-year-old female presented with complaints of vaginal tingling, vaginal watery vaginal discharge, and dysuria lasting for four months, with a gradual increase in symptom frequency over time. The patient underwent complete tumor excision. Immunohistochemical analysis showed that the tumor cells were strongly positive for SOX10, HMB-45, and S100 proteins. Histopathological examination of the entire tumor revealed epithelioid melanoma cells in the vertical (invasive) growth phase with no evidence of a preceding radial growth phase. Most tumor cells contained dark-brown intracellular pigment. Superficial microscopic ulceration was present. The Breslow tumor thickness was 12 mm. Tumor-infiltrating lymphocytes were non-brisk, and the mitotic rate was 15 mitoses per mm^2. Lymphovascular invasion was present, while perineural and intraneural infiltration were not noticed. Tumor regression and microscopic satellites were absent. The deep resection margin was free of tumor cells. According to the AJCC 8th edition melanoma staging system, the tumor was classified as stage IIC (T4b, N0, M0).

Surgical resection with wide local excision remains the mainstay in the treatment of this aggressive disease with poor overall survival. Mucosal melanoma arising in the vaginal wall is often clinically indiscernible; therefore, prompt biopsy and timely, accurate diagnosis are of crucial importance.

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Key words: *vaginal melanoma, melanocytes, mucosal melanomas*

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Introduction

Primary vaginal melanoma (PVM) arises from aberrant melanocytes, whose precursors migrate during gastrulation from the neural crest to the ectodermal mucosa (1). It is a rare type of mucosal melanoma, accounting for 0.3%–0.8% of all melanomas in women and less than 3% of vaginal malignancies (2). Primary vaginal melanoma may present as a pigmented plaque, ulceration, or amelanotic lesion, and may be multifocal in approximately 20% of cases. The most common localization is on the anterior wall in the lower third of the vagina (3). It predominantly occurs in postmenopausal women, with a median age ranging from 54 to 76 years (4).

The clinical outcome remains poor with a 5-year overall survival rate of 13–32.3% in patients (5). Surgical resection with clear margins is fundamental, while adjuvant radiotherapy contributes to local disease control. Currently, there is no established evidence-based systematic therapy recommended in either the adjuvant setting or for metastatic disease.

Nevertheless, immune checkpoint inhibitors have emerged as a justified systemic treatment option, regarding their documented clinical benefit in other mucosal melanomas, while targeted therapies are much less effective (6).

Case Presentation

A 78-year-old female presented with complaints of vaginal tingling, watery vaginal discharge, and dysuria lasting for four months with a gradual increase in symptom frequency over time.

Visual examination of the vulva revealed no skin lesions. Combined vaginal speculum examination and palpation identified a polypoid,

blackish lesion arising from the anterior wall in the lower third of the vagina, measuring 2.5 x 2 cm (Figure 1). The tumor mass had a smooth surface and was associated with vaginal discharge, without bleeding. The remaining parts of the vaginal walls and uterine cervix were unremarkable. Bimanual examination disclosed a mobile cervix without parametrium thickening, while the uterus was in anteversion and anteflexion and normal in size. The fallopian tubes and ovaries were not palpable. Rectal examination revealed no abnormalities in the Douglas pouch.

An incisional biopsy was performed, and histopathological analysis confirmed the diagnosis of mucosal melanoma (Figure 1). Immunohistochemical analysis demonstrated strong positivity of tumor cells for the SOX10, HMB-45, and S100 proteins (Figure 2).

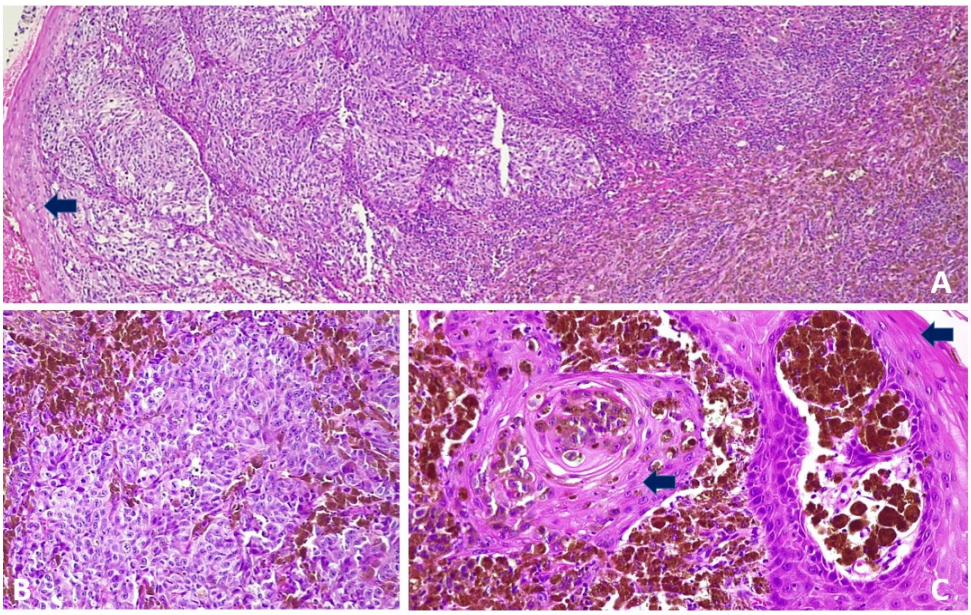

Figure 1. Micromorphology of primary vaginal melanoma. A) The vaginal neoplasm is composed of alveolar nests of spindled and epithelioid tumor cells, with extensive ulceration of the overlying squamous epithelium. The squamous epithelium is marked by an arrow. B) Tumor cells show a variable degree of atypia and melanin pigment content. Large, epithelioid melanocytes with amelanotic cytoplasm are admixed with heavily pigmented cells. C) Melanocytic proliferation shows pagetoid involvement of the squamous epithelium.

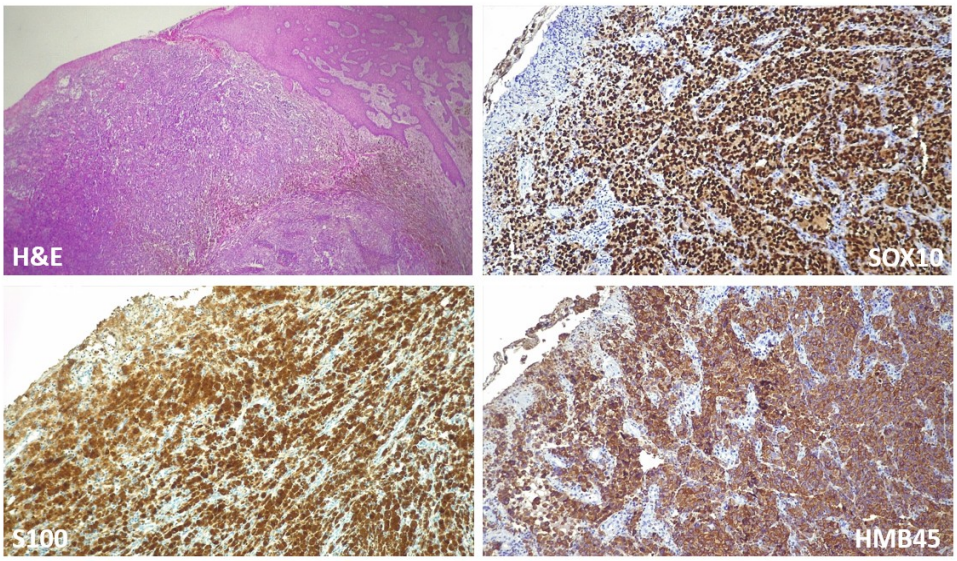

Figure 2. Immunophenotype of primary vaginal melanoma. Strong and diffuse expression of SOX10, S100, and HMB-45 confirms the melanocytic differentiation in both pigmented and non-pigmented tumor cells.

A complete clinical evaluation was performed following the histopathological diagnosis. Computed tomography of the abdomen and pelvis, as well as chest radiography, showed no evidence of metastatic disease. Laboratory blood and urine tests were also within normal limits. Bilateral iliac, cervical, and axillary lymph nodes showed no evidence of enlargement.

The multidisciplinary team suggested dermoscopic examination and cystoscopy before the surgical tumor removal. Extensive evaluation did not detect any suspicious hyperpigmented skin or urinary tract mucosal lesions, and the patient underwent complete tumor excision without the sentinel lymph node (SLN) biopsy.

Gross examination revealed no visible ulceration on the tumor surface. The cut surfaces were homogeneous and dark-colored on serial sectioning. All tissue samples were processed using standard techniques, cut into 4-μm sections, and stained with hematoxylin and eosin.

Histopathological analysis of the entire tumor revealed epithelioid and spindled melanoma cells in the vertical (invasive) growth phase with no evidence of the preceding radial growth phase (Figure 1). Most tumor cells contained dark-brown intracellular pigment. Superficial microscopic ulceration was present. The Breslow tumor thickness was 12 mm. Tumor-infiltrating lymphocytes (TILs) were non-brisk, and the mitotic rate was 15 mitoses per mm^2. Lymphovascular invasion was present, while perineural and intraneural infiltration were not noticed. Tumor regression and the microscopic satellites were absent. The deep resection margin was free of tumor cells. According to the AJCC 8th edition melanoma staging system, the tumor was classified as stage IIC (T4b, N0, M0).

Adjuvant therapy with either pembrolizumab or nivolumab for 12 months should be considered in patients with stage IIB-IIC disease, according to ESMO 2025 guidelines (7–13).

Discussion

Mucosal melanomas belong to a heterogeneous group of extracutaneous melanomas. Primary melanomas arising on the mucosal surfaces include those of the head and neck, anorectal region, urinary tract, and vulvovaginal area, all of which are aggressive tumors associated with challenging clinical diagnosis due to their often inconspicuous localization. Following biopsy of a suspicious lesion, pathological diagnosis often requires adjuvant diagnostic methods, most commonly immunohistochemical analysis of S100 protein, SOX10, and HMB-45, to confirm the melanocytic nature of the lesions. Primary vaginal melanomas are among the most frequently ulcerated forms with a pronounced mitotic activity. In the present case, tumor localization on the anterior wall of the distal vagina, along with histological features such as epithelioid cell morphology, vertical growth phase, and high mitotic rate, are consistent with previously described findings (14, 15).

Histopathological parameters necessary to determine the stage of the disease and the prognostic profile of melanoma include Breslow melanoma thickness, ulceration, microsatellite metastases or in-transient metastases, mitotic rate, Clark level of invasion (Chung modification for mucosal melanoma), TILs, lymphovascular invasion, neurotropism, resection margin status, and the presence and extent of tumor regression (16). However, mucosal melanomas are associated with a significantly poorer clinical outcome compared to their cutaneous counterparts, and there are no standardized protocols for assessing histopathological prognostic parameters in primary mucosal melanomas. Improved understanding of mucosal melanoma indicates that traditionally established histologic parameters, such as tumor thickness and ulceration, correlate less strongly with prognosis in mucosal melanoma than in cutaneous melanoma (17). Moreover, molecular analyses revealed that mucosal melanoma is rarely associated with BRAF mutations, which are often detected in cutaneous neoplasms. The most common genetic alterations in primary mucosal melanoma, including vulvovaginal cases, involve activating mutations in the receptor tyrosine kinase c-KIT (KIT) and, particularly, in SF3B1 (14, 17–19).

The standard approach to the treatment of vaginal melanoma is surgical resection.

Wide local excision (WLE), total vaginectomy, or total hysterectomy with vaginectomy and vulvectomy may be performed depending on tumor extent and location. Assessment of regional lymph nodes (LNs) is recommended, with lymphadenectomy indicated in cases of a positive sentinel lymph node (SLN) biopsy (20, 21). Wide local excision with the surgical safety margin of 1 cm is executed for tumors with a Breslow thickness of ≤ 2 mm, and 2 cm for tumors > 2 mm, with consideration of adjuvant pelvic radiotherapy (21). The European Organization for Research and Treatment of Cancer protocol includes longitudinal sectioning of SLNs and examination of both halves using hematoxylin and eosin staining and immunohistochemistry (22). The revised American Joint Committee on Cancer staging system 2017 is applied in vaginal melanoma and incorporates tumor thickness, regional LNs involvement, and distant metastases. However, some authors suggest that tumor size (< 3 cm vs. ≥ 3 cm) may also serve as an important prognostic factor (23). The recommended treatment is a WLE with adequate margins (approximately 1 cm circumferentially), while radical procedures (e.g., vaginectomy or pelvic exenteration) may be considered based on tumor location (6). Sentinel lymph node biopsy is challenging in vaginal melanoma, but the excision of clinically or radiologically suspicious LNs is rather suggested (24). Adjuvant radiotherapy may be considered in patients with positive or close surgical margins, tumor size > 3 cm, nodal involvement, or unresectable disease, as well as in

those who are not candidates for surgery. However, radiotherapy primarily contributes to local disease control, with no clear evidence supporting its role in systemic disease control (12). Regarding systemic treatment, immunotherapy with pembrolizumab, nivolumab, or ipilimumab is an evidence-based option for mucosal melanomas, including PVM (19–24). Targeted therapies may be considered in selected cases with identified activating mutations (e.g., c-KIT, BRAF/MEK, or NRAS), although their efficacy remains variable.

Conclusion

Surgical resection with a WLE remains the mainstay of treatment for this aggressive disease, which is associated with poor overall survival. Vaginal mucosal melanoma that arises in vaginal walls is often clinically inconspicuous; therefore, prompt biopsy and timely, accurate diagnosis are of crucial significance.

References

1. Mihajlovic M, Vlajkovic S, Jovanovic P, Stefanovic V. Primary mucosal melanomas: a comprehensive review. Int J Clin Pathol 2012; 5(8): 739-53. [PubMed]
2. Schmidt M, Honig A, Schwab M, Adam P, Dietl J. Primary vaginal melanoma: a case report and literature review. Eur J Gynaecol Oncol 2008; 29(3): 285-8. [PubMed]
3. Chen I, Xiong Y, Wang H, Liang L, Shang H, Yan X. Malignant melanoma of the vagina: a case report and review of the literature. Oncol Lett 2014; 8: 1585-8. [CrossRef] [PubMed]
4. Aulmann S, Sinn HP, Penzel R, Gilks CB, Schott S, Hassel CJ, et al. Comparison of molecular abnormalities in vulvar and vaginal melanomas. Mod Pathol 2014; 27(10): 1386-93. [CrossRef] [PubMed]
5. Huang Q, Huang H, Wan T, Deng T, Lui J. Clinical outcome of 31 patients with primary malignant melanoma: a rare and aggressive tumor. Case Rep Obstet Gynecol 2013; 24(4): 330-5. [CrossRef] [PubMed]
6. Gadducci A, Carinelli S, Guerrieri ME, Aletti GE. Melanoma of the lower genital tract: Prognostic factors and treatment modalities. Gynecol Oncol 2018; 150(1): 180–9. [CrossRef] [PubMed]
7. Amaral T, Ottaviano M, Arance A, Blank C, Chiarion-Sileni V, Donia M, et al. Cutaneous melanoma: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 2025;36(1):10-30. [CrossRef] [PubMed]
8. Knight A, Karapetyan L, Kirkwood JM. Immunotherapy in Melanoma: Recent Advances and Future Directions. Cancers 2023; 15(4): 1106-24. [CrossRef] [PubMed]
9. Fernandez MF, Choi J, Sosman J. New Approaches to Targeted Therapy in Melanoma. Cancers 2023; 15(12): 3224-39. [CrossRef] [PubMed]
10. Jamaer E, Liang Z, Stag B. Primary malignant melanoma of the vagina. BMJ Case Rep 2020;13(1):e232200. [CrossRef] [PubMed]
11. Villani A, Potestio L, Fabbrocini G, Troncone G, Malapelle U, Scalvenzi M. The Treatment of Advanced Melanoma: Therapeutic Update. Int J Mol Sci 2022; 23(12): 6388-405. [CrossRef] [PubMed]
12. Natarelli N, Aleman SJ, Mark IM, Tran JT, Kwak S, Botto E, et al. A Review of Current and Pipeline Drugs for Treatment of Melanoma. Pharmaceuticals 2024; 17(2): 214-27. [CrossRef] [PubMed]
13. Seth R, Agarwala S, Messersmith H, Alluri KC, Ascierto PA, Atkins MB, et al. Systemic Therapy for Melanoma: ASCO Guideline Update. ASCO Special Articles 2023;41(30):4794- 820. [CrossRef] [PubMed]
14. Tacastacas JD, Bray J, Cohen YK, et al. Update on primary mucosal melanoma. J Am Acad Dermatol 2014; 71(2):366-75. [CrossRef] [PubMed]
15. Gupta D, Malpica A, Deavers MT, Silva EG. Vaginal melanoma. A clinicopathologic and immunohistochemical study of 26 cases. Am J Surg Pathol 2002; 26(11): 1450-7. [CrossRef] [PubMed]
16. Ivan D, Prieto VG. An update on reporting histopathologic prognostic factors in melanoma. Arch Pathol Lab Med 2011; 135(7): 825-9. [CrossRef] [PubMed]
17. Williams MD. Update from the 4th Edition of the World Health Organization Classification of Head and Neck Tumours: Mucosal Melanomas. Head Neck Pathol 2017; 11(1): 110-7. [CrossRef] [PubMed]
18. Tyrrell H, Payne M. Combatting mucosal melanoma: recent advances and future perspectives. Melanoma Manag 2018;5(3): MMT11. [CrossRef] [PubMed]
19. Michielin O, van Akkooi ACJ, Ascierto PA, Dummer R, Keilholz U. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2019; 30(12): 1884-901. [CrossRef] [PubMed]

20.Androutsopoulos G, Terzakis E, Ioannidou G, Tsamandas A, and Decavalas G. Vaginal primary malignant melanoma: a rare and aggressive tumor. Case Rep Obstet Gynecol 2013; 2013: 137908. [CrossRef] [PubMed]
21.Kalampokas E, Kalampokas T, Damaskos C. Primary Vaginal Melanoma, A Rare and Aggressive Entity. A Case Report and Review of the Literature. In vivo 2017; 31(1): 133-40. [CrossRef] [PubMed]
22.Gokaslan H, Sismanoglu A, Pekin T, Kaya H, Ceyhan N. Primary malignant melanoma of the vagina: a case report and review of the current treatment options. Eur J Obstet Gynecol Reprod Biol 2005; 121(2): 243-8. [CrossRef] [PubMed]
23.B. Piura. Management of primary melanoma of the female urogenital tract Lancet Oncol 2008; 9: 973-81. [CrossRef] [PubMed]
24.Miner TJ, Delgado R, Zeisler J, Busam K, Alektiar K, Barakat R, et al. Primary vaginal melanoma: a critical analysis of therapy. Ann Surg Oncol 2004; 11(1): 34-9. [CrossRef] [PubMed]

Prikaz slučaja

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PRIMARNI MELANOM VAGINE

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Primarni melanom vagine predstavlja redak tip melanoma sluzokože, koji čini od 0,3% do 0,8% svih melanoma kod žena, a manje od 3% maligniteta vagine.

Sedamdesetosmogodišnja žena je kao tegobe navela peckanje u vagini, vodenasti iscedak iz vagine i dizuriju, napominjući da je u poslednja četiri meseca to postalo češće. Pacijentkinji je urađena potpuna ekscizija tumora. Tumorske ćelije su imunohistohemijski bile snažno pozitivne na SOX10, HMB-45 i S100 protein. Histopatološka analiza tumora pokazala je ćelije epitelioidnog melanoma u vertikalnoj (invazivnoj) fazi rasta. Većina tumorskih ćelija sadržala je tamnobraon intracelularni pigment, a zabeleženo je i prisustvo površinske mikroskopske ulceracije. Debljina tumora je iznosila 12 mm. Mitotička stopa je bila 15 mitotičkih figura po 1 mm^2, sa limfovaskularnom invazijom koja je bila pozitivna. Nisu primećene ni perineuralna ni intraneuralna infiltracija. Nije bilo ni regresije tumora ni mikroskopskih satelita. Na margini duboke resekcije nije bilo tumorskih ćelija. Prema grupi AJCC 8. stadijuma za melanom, ovo je bio slučaj stadijuma IIC (T4b, N0, M0).

Hirurška resekcija za koju se koristi VLE (engl. *volumetric laser endomicroscopy* – VLE) ostaje glavni standard u lečenju ove agresivne bolesti, kod koje je ukupno preživljavanje pacijentkinja kraće. Melanom sluzokože koji nastaje u zidovima vagine često je klinički teže dijagnostikovana lezija, tako da su brza biopsija i pravovremena i tačna dijagnoza od presudnog značaja.

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***Ključne reči**: melanom vagine, melanociti, mukozalni melanom*

Original article

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ACUTE DISTAL TIBIOFIBULAR SYNDESMOTIC INJURIES IN ANKLE FRACTURES

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A stable tibiofibular syndesmosis articulation maintains the tibiofibular alignment and is necessary for normal ankle function. An ideal syndesmotic reduction and stabilization strategy should replicate the orientation and stabilizing forces of the syndesmotic ligaments. The aim of this study is to evaluate the outcomes of operative treatment for tibiofibular syndesmosis rupture associated with malleolar fractures.

This study presents patients with tibiofibular syndesmosis disruption treated at the Orthopedic Clinic of the Clinical Center in Niš from January 2017 to January 2019. The subjects included individuals with malleolarfractures accompanied by tibiofibular syndesmosis injuries. Treatment involved malleolar fixation and transfixation of the tibiofibular syndesmosis. Radiographic, clinical, operative and statistical methods were used to evaluate the study objectives.

In the group of 46 subjects with tibiofibular syndesmosis rupture, there were 28 (61%) men and 18 (39%) women, with age ranging from 18 to 79 years (mean age = 43.6 ± 9.6 years). The predominant cause of injury was slipping and falling in 32 patients (70%), followed by falls from a height in 9 patients (19%), and traffic accidents in 5 patients (11%). According to the Lauge−Hansen classification, 27 (59%) patients sustained supination-external rotation type injuries. Based on the final functional treatment outcome assessed using the Olerud-Molander scoring system, the average ankle score was 87 points (range 55 to 100). Among the patients, 34 (74%) achieved excellent or good results: 24 (52%) had excellent outcomes (score from 90 to 100 points), and 10 (22%) had good outcomes (80 to 89 points). Satisfactory outcomes were observed in 7 (15%) patients, while poor outcomes were recorded in 5 patients (11%).

Our results indicate that stable and timely surgical reconstruction and fixation of the malleolus and tibiofibular syndesmosis enable functional restoration and recovery of the ankle joint with a low rate of complications.

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Key words: *syndesmosis injury, ankle fracture, joint repair, syndesmosis fixation*

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Introduction

The incidence of ankle fractures is 187 per 100,000 inhabitants (1:800) annually (1), accounting for 3.92% of all body fractures and ranking first among intra-articular fractures. It is estimated that 13−20% of all ankle fractures are accompanied by syndesmosis injuries. Such injuries can also occur in ankle sprains, affecting up to 18% of cases, particularly in sports activities (2, 3). Stable and precise articulation of the distal tibiofibular syndesmosis (DTFS) is essential for maintaining the tibiofibular alignment and enabling normal ankle movement. Anatomically, the syndesmosis is formed by the medial rough, convex surface of the distal fibula, which articulates with a triangular, depressed area on the distal tibia. The anterior edge of the tibial incisura is significantly more developed, and extends across the medial two-thirds of the fibula, thereby providing support and preventing anterior displacement of the fibula (4).

Syndesmosis is a fibrous joint in which two adjacent bones are connected by a strong membrane and ligaments. The syndesmosis ligament complex resists axial, translational, and rotational forces and thus provides syndesmotic stability. It consists of four components: the anterior inferior tibiofibular ligament (AITFL), posterior inferior tibiofibular ligament (PITFL), interosseous ligament (IOL), and transverse tibiofibular ligament (TTFL) (5, 6). The anatomical syndesmotic unit also includes the posterior

malleolus, to which the PITFL is attached. In cases of injury where the posterior malleolus is intact, the PITFL is torn by a type of avulsion from its attachment site on the malleolus. In posterior malleolus fractures, posterior syndesmosis ligaments may remain intact and attached to the fragment. In that case, malleolus fixation is required, because reduction of the ankle joint and greater syndesmotic stability can be achieved (6, 7). The lateral malleolus and syndesmosis are key structures for the anatomical reduction of dislocated ankle fractures. Restoring the integrity of the lateral malleolus is essential for reestablishing the overall stability of the ankle joint (8, 9).

Precise ankle joint congruence is essential for its movement, and malposition after trauma leads to significant adverse consequences that alter joint biomechanics and cause pathological compressive stress (10, 11). The aim of this paper is to evaluate the functional outcomes and possible anatomical changes in ankle joint cartilage after operative treatment of tibiofibular syndesmosis rupture associated with malleolar fractures.

Materials and Methods

Patients with disruption of distal tibiofibular syndesmosis treated at the Orthopedic Clinic of the Clinical Center in Niš during the period from January 2017 to January 2019 are presented. In a two-year period, there were 383 ankle injuries, and this study includes 46 patients (12% of the total number of injuries) with closed ankle fractures and associated syndesmotic rupture. Patients were regularly followed up at six and twelve months after surgery. Forclassificaton of ankle fractures, the Dennis−Weber classification (12) and the Lauge-Hansen classification (13) were used. Inclusion criteria for the study were acute syndesmotic injuries associated with ankle malleolar fractures (Dennis−Weber classification type B and C). Exclusion criteria were fibular fractures below the syndesmosis – (Dennis−Weber type A), open ankle fractures, and patients younger than 18 years.

Radiographic method

For the purpose of accurate assessment of fractures and evaluation of treatment outcomes, radiography was performed in two standard projections. Standard anteroposterior (AP) radiographs were obtained with the foot in 20° internal rotation in men, and 15° in women. To obtain a true assessment of the ankle joint condition, the following parameters were measured: tibiofibular clear space (TCS) and tibiofibular overlap (TFO). These parameters reflect tibiofibular alignment, the degree of syndesmotic injury and fibular displacement, as well as the quality of postoperative reduction, and were used in our analysis. Radiographic evaluation was performed at admission, intraoperatively, postoperatively, and at six- and twelve-month follow-up. The Kellgren−Lawrence (KL) scale was used to assess the severity of post-traumatic osteoarthritis (PTOA) (14).

Operative method

Surgery was performed immediately after admission and preoperative preparation, on average after 30 ± 8.6 hours. If adequate fibula length, a reduced talocrural angle, and a centered talus were achieved, fibular fracture fixation was performed. A tibiofibular transfixation screw was placed either independently or through one of the distal plate holes, above the tibiofibular syndesmosis, i.e., 1.5 to 3.5 cm above the joint line.The transsyndesmotic screw was placed after previous radiological confirmation of syndesmotic reduction in order to avoid fibular malreduction within the incisura. In some cases, the transfixation screw involved four cortices, i.e., passed through the fibula and tibia, and in others through three cortices and the entire tibial metaphysis (Figure 1). The axis of the transfixation screw formed an angle of 30° with the line of the articular surface. It is not necessary to tighten it excessively, nor to maintain the foot at a 90° angle relative to the lower leg during screw insertion. In addition to rigid syndesmotic fixation, we also used dynamic fixation. For this type of syndesmotic fixation, we used a system consisting of two polyester strips and two titanium endobuttons (Figure 2). If internal fixation stability was adequate and wounds were satisfactory, passive and active mobilization of the ankle joint inoperated patients was initiated on the second operative day under the supervision of a physiotherapist. Patients stayed at the Clinic for 5 days (range 4−8) on average and were discharged home after being trained to walk with crutches. Partial weight bearing and support of the operated extremity with axillary crutches were allowed for up to 8−10 weeks, while full weight-bearing was permitted after 8−12 weeks.

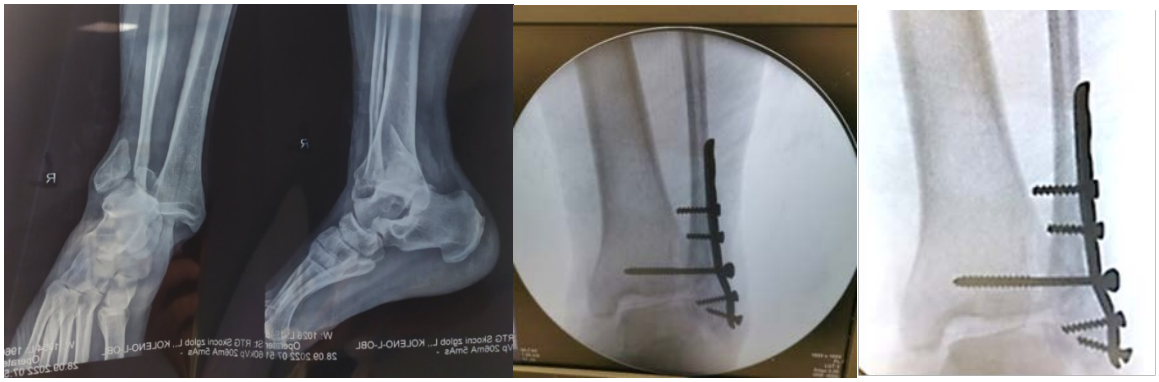
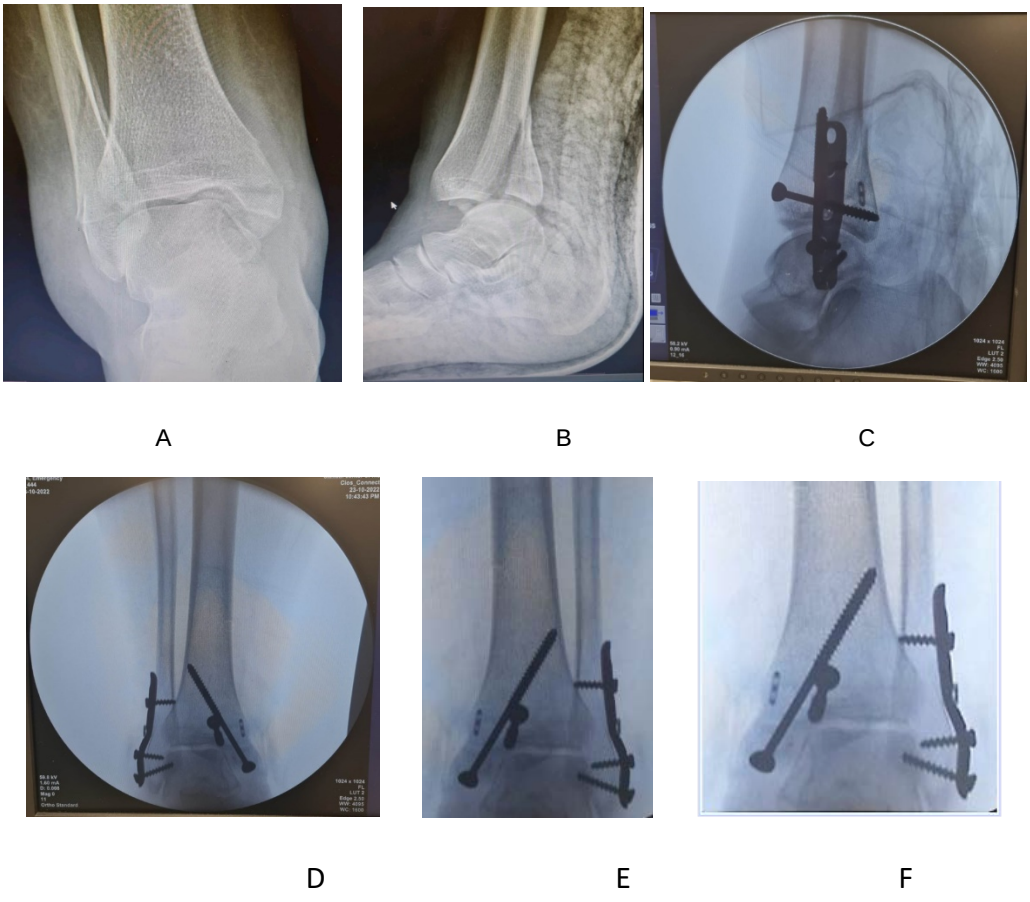


Figure 1. Supination-external rotation (SER) type ankle fracture. A – Preoperative radiograph. B – Postoperative radiograph after surgical treatment with plate and screw fixation of the malleolus and rigid distal tibiofibular syndesmosis fixation. C – Anteroposterior radiograph six months postoperatively



Clinical method

After 12 months of follow-up, the functional outcomes of patients were evaluated. Assessment of subjective and objective symptoms was performed using the Olerud–Molander scoring system (15). Movements in the talocrural joint were measured with the patient in a prone position. The axis of the goniometer was placed on the lateralside of the heel, so that one arm followed the longitudinal axis of the foot, i.e., the fifth metatarsal bone, and the other arm followed the longitudinal axis of the lower leg along the fibular side. The final position of the foot in plantar flexion and dorsi flexion was measured. Osteosynthetic material was removed after an average of 7.5 months (range 5–14 months). Isolated removal of the syndesmotic screw and elastic fixation was not performed, except in two cases of syndesmotic screw loosening and skin infection.

Ethical Considerations

The research protocol of this study were reviewed and approved by the Ethics Committee and Professional Board of our Clinic.

Statistical Method

The statistical power was set at 0.80, with a significance level of 5% ($p < 0.05$) and a confidence interval of 95%. The normality of the data distribution was assessed using the Shapiro–Wilk test. The Mann–Whitney U-test was used for non-normally distributed numerical data. Values of the Olerud–Molander scoring system were analyzed using the χ^2 test, comparing observed and expected frequencies.

Results

The statistical results of the study are presented in tabular form (Table 1). In a group of 46 subjects with distal tibiofibular syndesmosis rupture, there were 28 (61%) men and 18 (39%) women, and patients' age ranged from 18 to 79 years ($\dot{x}$ = 43.6 ± 9.6 years). The most common cause of injury was slipping and falling reported in 32 patients (70%) followed by falls from height in 9 patients (19%) and traffic accidents in 5 patients (11%). According to the Lauge–Hansen classification, 27 (59%) patients sustained SER type injuries. The second most common was the pronation-abduction (PAB) type, observed in 14 (30%) patients, while 5 (11%) injuries were classified as the pronation-external rotation (PER) type.

Table 1. Schapiro–Wilk test of normality; Mean ($\bar{x}$) – sample mean; SD – standard deviation; SE – standard error; CI – 95% confidence interval; U-test (Wilcoxon rank-sum test). DFL – dorsi flexion (20°), PFL – plantar flexion (50°), TCS – tibiofibular clear space (< 6 mm), TFO – tibiofibular overlap (> 6 mm).

	DFL	**DFL**	**PFL**	**PFL**	**TCS**	**TCS**	**TFO**	**TFO**
	After 6 m.	After 12 m.	After 6 m.	After 12 m.	Before op.	After op.	Before op.	After op.
Shapiro–Wilk p > 0,05	0.000018	0.000011	0.016	0.34 x 10^{-7}	0.000091	1.85 x 10^{-7}	0.00085	0.00024
Average ($\bar{x}$)	14.15	15.73	36.04	39.34	8.76	4.96	2.74	8.65
SD	3.34	4.56	5.35	11.15	1.52	0.59	1.22	1.37
SE	0.493	0.672	0.789	1.644	0.224	0.087	0.18	0.20
Effect size	**small** (0.2)		**medium** (0.31)		**large** (0.88)		**large** (0.87)	
CI (95%)	14.45 ± 0.99	15.73 ± 1.3542	36.04 ± 1.5888	39.34 ± 3.3111	8.76 ± 0.4514	4.96 ± 0.175	2.74 ± 0.3623	8.65 ± 0.4068
Power	0.828		0.979		1.0		1.0	
U-test p < 0.05	**0.04969**, p (x ≤ Z) = 0.02484		**0.003412**, p (x ≤ Z) = 0.001706		**4.441e-16**, p (x ≤ Z) = 1		**0**, p (x ≤ Z) = 0	

All tested numerical data were not normally distributed (Shapiro–Wilk test: $p < 0.05$) (Table 1). A statistically significant difference was found in the values of dorsi flexion and plantar flexion of the foot between two measurements, at 6 months and 12 months ($p < 0.001$). Likewise, a statistically highly significant difference was observed TCS and TFO values before and after surgery ($p < 0.0001$). According to the Olerud–Molander scoring system, the average ankle score was $\bar{x}$ = 86.85 ± 3.73 points (95% CI: 83.13–90.59; SE: 1.85; range, 55–100 points). The obtained values were not normally distributed (Shapiro–Wilk test, $p = 0.0000314$). Thirty four (74%) patients had excellent or good results: 24 (52%) patients had excellent outcomes (90–100 points), and 10 (22%) patients had good outcomes (80–89 points). In these patients, the ankle joint was painless, physiological movements were preserved, and no visible deformities were observed. They returned to their usual work and sports activities. Satisfactory results were observed in 7 (15%) patients. These patients had occasional pain that increased during work, prolonged standing, and walking. Swelling of the ankle and foot was also present. At 12 months after surgery, they were unable to perform the same work as before the injury. Poor results were

recorded in 5 (11%) patients. These patients had constant pain and swelling of the ankle and foot. Movements in the ankle joint were reduced by more than 60%. They had difficulty ambulating with the use of aids and were unable to work or participate in sports 12 months after the injury. We compared the results of the Olerud–Molander test using the χ^2 test (Chi-square test for variance, χ^2 distribution, df = 45, two-tailed) in relation to the expected standard values of the overall population and found a statistically highly significant difference between the observed and expected values ($p < 1.709e-29$) with a small effect size (0.14). In patients with satisfactory (7; 15%) and poor results (5; 11%), postoperative complications were observed, including displacement of the fibular fracture and syndesmosis due to a short fibular plate and an inadequately long syndesmotic screw. In 2 (4.5%) patients, a transsyndesmotic screw fracture was identified. In 6 (13%) patients, bone loss with subsequent screw loosening was observed. In 9 (21.7%) patients, poor syndesmotic reduction persisted. In 10 (24%) patients, synostosis at the level of the tibiofibular syndesmosis was observed, and in one patient (2.2%), inadequate reduction and fixation of the medial malleolus were found. Prolonged wound healing and local infection were present in 3 (6.5%) patients. In 7 (16%) patients, post-traumatic osteoarthrosis of the ankle joint was observed 12 months after surgery: 5 patients had grade 1 changes according to the KL scale, and 2 patients had grade 2 ankle osteoarthritis.

Discussion

This study showed that rupture of the ligaments forming the syndesmosis results in the separation of the tibia and fibula, i.e., widening of the ankle mortise. Lateralization of the fibula occurs, with an increase in TCS and a decrease in TFO, thus creating the conditions for ankle joint instability. The study also showed that surgical intervention achieves adequate reconstruction of the tibiofibular syndesmosis and the ankle joint.

Khambete et al. (16) showed that normal ankle joint movement depends on a precise relationship determined by the syndesmosis. If the syndesmosis is disturbed, the tibiofibular joint space may widen and the talus may shift laterally, which alters the biomechanics of the tibiotalar joint, including contact surface ratio and pressure distribution. Burns et al. (17) showed that complete syndesmotic disruption combined with rupture of the deltoid ligament causes a 39% decrease in tibiotalar contact area and a 42% increase in pressure. There is no consensus on the optimal method for syndesmotic fixation. The two main methods are rigid fixation using a metallic syndesmotic screw, still considered "gold standard", and flexible dynamic stabilization using a suture-button system with endobuttons (2, 3, 18, 19). The transfixation screw is placed either independently or through the plate used for fibular fracture osteosynthesis. There are many controversies regarding syndesmotic screw fixation. Should the screw be placed through three or four cortices? It is considered that tricortical fixation is less rigid than quadricortical fixation and allows some degree of syndesmotic micromotion (20). Flexible dynamic stabilization is performed using a suture-button system, and consists of creating a nylon loop around the tibia and fibula at the level of the syndesmosis. The strip is placed in an anterior-posterior direction along the course of AITFL and PITFL fibers, i.e., following their anatomical axis. This is a relatively new method of fixation; it does not require ideal anatomical reduction of the syndesmosis and allows a certain degree of syndesmotic motion enabling potential self-reduction if the reduction is not anatomical (18, 19, 21).

The PITFL complex is an anatomical unit consisting of the ligament and the posterior malleolus and represents the core component of ankle joint stability, accounting for 42% of syndesmotic stability. The posterior malleolus should be considered key to anatomical syndesmosis reduction, and open reduction and direct fixation should be performed as the first step. Fixation of the posterior malleolus restores articular surface of the tibia, corrects fibular shortening, enables early weight-bearing and rehabilitation, and thus reduces the occurrence of post-traumatic osteoarthritis (6, 16, 22, 23).

Complications in tibiofibular syndesmosis treatment are possible and depend on the extent of soft tissue injury, fracture type, and the degree of cartilage damage to the talus and tibial plafond at the time of injury, as well as the quality of anatomical reduction. In our series, we observed complications that have also been reported by other authors. Hovis et al. (24) reported postoperative fibular fracture displacement in 9 (8.7%) and postoperative wound complications in 6 (5.9%) of 102 patients. Yablon et al. (8), in a study of 53 patients, reported infection in 2 (3.7%) cases and painful contact between talus and lateral maleolus, the so-called impingement, in 3 (5.6%) patients. They also reported talar instability predisposing to degenerative arthritis. Malreduction is a very common complicationand is defined by deviations in radiological parameters (TCS, TFO). It is manifested as malposition of the medial malleolus. In our series, malreduction was present in 10 (24%) patients. Ovaska et al. (25) reported malreduction in 9% of cases, while in 59% it referred specifically to syndesmosis malreduction. Sanders et al. (26) reported malreduction in 39% cases.

In their systematic review, Desouky et al. evaluated the outcomes of removing versus retaining syndesmotic screws in open and closed ankle fractures associated with unstable syndesmosis, based on functional, clinical, and radiological outcomes (27). Overall, the current literature provides no evidence to support routine removal of syndesmotic screws. Considering the potential complications and financial burden, syndesmotic screw removal should not be

performed unless there is a clear clinical indication (27).

A recent study by Qin Wang et al. conducted a meta-analysis synthesizing multiple sources of literature to investigate whether differences exist between elastic and rigid fixation in the treatment of acute tibiofibular syndesmosis injuries (28). The aim was to provide evidence-based guidelines for clinical management. The outcome measures included the American Orthopaedic Foot and Ankle Society (AOFAS) scores at 3, 6, and 12 months postoperatively; TBCS and TBOL at early postoperative assessment and at 12-month follow-up; intraoperative blood loss; operative time; time to full weight-bearing postoperatively; and postoperative complications. The meta-analysis was performed using Review Manager 5.4. A total of 35 studies were included, comprising 16 randomized controlled trials and 19 retrospective cohort studies. The study population included 2120 cases, with 1044 in the elastic fixation group and 1076 in the rigid fixation group. The elastic fixation group had higher AOFAS scores at 3, 6, and 12 months postoperatively compared with the rigid fixation group. The authors concluded that, compared with rigid fixation, elastic fixation in the treatment of acute tibiofibular syndesmosis injuries offers several advantages, including better postoperative ankle function recovery, more precise anatomical reduction of the syndesmosis, a lower incidence of postoperative complications, and shorter time to full weight-bearing. These findings provide useful guidelines for clinical practice (28).

In our study, we demonstrated favourable outcomes in the treatment of tibiofibular syndesmosis rupture and its impact on the consequences of ankle injury.

The limitations of this study include a small sample size for statistical analysis, which is why not all data were normally distributed; the influence of malleolar fractures on the development of PTOA was not considered; and no comparative measurements were performed with the contralateral healthy ankle. The study recommends further research focused on identifying factors contributing to ankle PTOA and exploring potential preventive measures. These may include the use of arthroscopy during surgical intervention to assess the condition of articular cartilage, removal of loose cartilage fragments, and early administration of chondroprotective agents.

Conclusion

This study demonstrated that surgical reconstruction of the ankle joint yields favourable outcomes. An almost anatomical position of the tibiofibular syndesmosis is achieved, resulting in stability and restoration of normal ankle biomechanics, which in turn reduces the incidence of complications.

References

1. Xing W, Wang Y, Sun L, Wang L, Kong Z, Zhang C, et al. Ankle joint dislocation treating dislocated trimalleolar fractures accompanied with the complex posterior malleolus fracture without separation of the tibiofibular syndesmosis. Medicine (Baltimore) 2018;97(37):e12079. [CrossRef] [PubMed]
2. Schepers T. Acute distal tibiofibular syndesmosis injury: a systematic review of suture-button versus syndesmotic screw repair. Int Orthop 2012;36(6):1199-206. [CrossRef] [PubMed]
3. Gan K, Zhou K, Hu K, Lu L, Gu S, Shen Y. Dynamic Fixation Versus Static Fixation for Distal Tibiofibular Syndesmosis Injuries: A Meta-Analysis. Med Sci Monit 2019;18(25):1314-22. [CrossRef] [PubMed]
4. Yuen CP, Lui TH. Distal Tibiofibular Syndesmosis: Anatomy, Biomechanics, Injury and Management. Open Orthop J 2017; 31(11):670-7. [CrossRef] [PubMed]
5. Hermans JJ, Beumer A, de Jong TA, Kleinrensink GJ. Anatomy of the distal tibiofibular syndesmosis in adults: a pictorial essay with a multimodality approach. J Anat 2010;217(6):633-45. [CrossRef] [PubMed]
6. Ogilvie-Harris DJ, Reed SC, Hedman TP. Disruption of the ankle syndesmosis: biomechanical study of the ligamentous restraints. Arthroscopy 1994;10(5):558-60. [CrossRef] [PubMed]
7. Mladenović M, Stojiljković P, Mladenović D, Krstić A, Anđelković V. Uloga i značaj fiksacije zadnjeg maleolusa kod trimaleolarnih preloma. Timočki medicinski glasnik 2021; 46(2):79-85. [CrossRef]
8. Yablon IG, Heller FG, Shouse L. The key role of the lateral malleolus in displaced fractures of the ankle. J Bone Joint Surg Am 1977;59(2):169-73. [CrossRef] [PubMed]
9. Lampridis V, Gougoulias N, Sakellariou A. Stability in ankle fractures: Diagnosis and treatment. EFORT Open Rev 2018;3(5):294-303. [CrossRef] [PubMed]
10. Pretterklieber ML. Anatomie und Kinematik der Sprunggelenke des Menschen. Der Radiologe 1999;39:1–7. [CrossRef] [PubMed]
11. Thordarson DB, Motamed S, Hedman T, Ebramzadeh E, Bakshian S. The effect of fibular malreduction on contact pressures in an ankle fracture malunion model. J Bone Joint Surg Am 1997;79(12):1809-15. [CrossRef] [PubMed]
12. Danis R. Les fractures malleolaires. In: Danis R, ed. Theorie et Pratique de l'Osteosynthese. Paris, France: Masson et Cie; 1949:133–65.
13. Lauge Hansen N. Ligamentous ankle fractures.Diagnosis and treatment. Acta Chir Scand 1949; 97: 544 – 50. [PubMed]
14. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16(4):494-502. [CrossRef] [PubMed]
15. Olerud C, Molander H. A scoring scale for symptom evaluation after ankle fracture. Arch Orthop Trauma Surg 1984; 103: 190 – 4. [CrossRef] [PubMed]
16. Khambete P, Harlow E, Ina J, Miskovsky S. Biomechanics of the Distal Tibiofibular Syndesmosis: A Systematic Review of Cadaveric Studies. Foot Ankle Orthop 2021; 6(2): 24730114211012701. [CrossRef] [PubMed]
17. Burns WC 2nd, Prakash K, Adelaar R, Beaudoin A, Krause W. Tibiotalar joint dynamics: indications for the syndesmotic screw--a cadaver study. Foot Ankle 1993;14(3):153-8. [CrossRef] [PubMed]
18. O'Daly AE, Kreulen RT, Thamyongkit S, Pisano A, Luksameearunothai K, Hasenboehler EA, Helgeson MD, Shafiq B. Biomechanical Evaluation of a New Suture Button Technique for Reduction and Stabilization of the Distal Tibiofibular Syndesmosis. Foot Ankle Orthop 2020;5(4):2473011420969140. [CrossRef] [PubMed]
19. Regauer M, Mackay G, Nelson O, Böcker W, Ehrnthaller C. Evidence-Based Surgical Treatment Algorithm for Unstable Syndesmotic Injuries. J Clin Med 2022;11(2):331. [CrossRef] [PubMed]
20. Olerud C. The effect of the syndesmotic screw on the extension capacity of the ankle joint. Arch Orthop Trauma Surg 1985;104(5):299–302. [CrossRef] [PubMed]
21. Weber AC, Hull MG, Johnson AJ, Henn RF 3rd. Cost analysis of ankle syndesmosis internal fixation. J Clin Orthop Trauma 2019;10(1):173-7. [CrossRef] [PubMed]
22. Miller MA, McDonald TC, Graves ML, Spitler CA, Russell GV, Jones LC, Replogle W, Wise JA, Hydrick J, Bergin PF. Stability of the Syndesmosis After Posterior Malleolar Fracture Fixation. Foot Ankle Int 2018;39(1):99-104. [CrossRef] [PubMed]
23. Solan MC, Sakellariou A. Posterior malleolus fractures: worth fixing. Bone Joint J 2017;99-B (11):1413-9. [CrossRef] [PubMed]
24. Hovis WD, Kaiser BW, Watson JT, Bucholz RW. Treatment of syndesmotic disruptions of the ankle with bioabsorbable screw fixation. J Bone Joint Surg Am 2002;84(1):26-31. [CrossRef] [PubMed]
25. Ovaska MT, Mäkinen TJ, Madanat R, Kiljunen V, Lindahl J. A comprehensive analysis of patients with malreduced ankle fractures undergoing re-operation. Int Orthop 2014;38(1):83-8. [CrossRef] [PubMed]
26. Sanders D, Schneider P, Taylor M, Tieszer C, Lawendy A R. Canadian Orthopaedic Trauma Society Improved Reduction of the Tibiofibular Syndesmosis With Tight Rope Compared with Screw Fixation: Results of a Randomized Controlled Study. J Orthop Trauma 2019;33:531–7. [CrossRef] [PubMed]
27. Desouky O, Elseby A, Ghalab AH. Removal of Syndesmotic Screw After Fixation in Ankle Fractures: A Systematic Review. Cureus 2021;13(6):e15435. [CrossRef] [PubMed]
28. Wang Q, Liu S, Wang Z, Li A, Ding J. Meta-analysis of elastic versus rigid fixation in the treatment of acute tibiofibular syndesmosis injury. *Syst Rev* 2024;13,51. [CrossRef] [PubMed]

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AKUTNE POVREDE DISTALNE TIBIOFIBULARNE SINDEZMOZE U PRELOMIMA SKOČNOG ZGLOBA

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Stabilna artikulacija tibiofibularne sindezmoze održava odnos između tibije i fibule i neophodna je za normalnu funkciju skočnog zgloba. Idealna strategija redukcije i stabilizacije sindezmoze treba da replicira orijentaciju i stabilizacione sile ligamenata. Cilj ove studije bio je da proceni rezultate operativnog lečenja rupture tibiofibularne sindezmoze udružene s prelomom maleolusa.

U radu su prikazani slučajevi pacijenata sa disrupcijom tibiofibularne sindezmoze koji su lečeni na Ortopedskoj klinici Kliničkog centra u Nišu od januara 2017. godine do januara 2019. godine. Pomenuti pacijenti su imali prelom maleolusa, koji je bio praćen povredama tibiofibularne sindezmoze. Pacijenti su lečeni fiksacijom maleolusa i transfiksacijom tibiofibularne sindezmoze. Za evaluaciju postavljenih ciljeva rada korišćene su radiografska, klinička, operativna i statistička metoda.

U grupi od 46 pacijenata sa rupturom tibiofibularne sindezmoze bilo je 28 (61 %) muškaraca i 18 (39%) žena, starih od 16 do 79 godina ($\bar{x}$ = 43,6 ± 9,6 godina). Dominanti uzroci povreda pacijenata bili su klizanje i pad (kod 32 pacijenta, odnosno kod 70%), pad sa visine (kod devet pacijenata, odnosno kod 19%) i saobraćajni udes (kod pet pacijenata, odnosno kod 11%). Prema Lauge–Hansen klasifikaciji, tip povreda kod 27 (59%) pacijenata bio je spoljašnje rotacioni. Na osnovu konačnog funkcionalnog rezultata lečenja po Olerud–Molander sistemu bodovanja, prosečna ocena skočnog zgloba za ove pacijente bila je 87 poena (opseg: od 55 do 100 poena). U grupi pacijenata sa odličnim i dobrim rezultatima bila su 34 (74%) pacijenta; od toga, 24 (52%) pacijenta imala su odličan rezultat (skor od 90 do 100 poena), a njih deset (22%) dobar rezultat (skor od 80 do 89 poena). Zadovoljavajući rezultati zabeleženi su kod sedam (15%) pacijenata, a loši rezultati kod pet (11%) pacijenata.

Dobijeni rezultati ukazuju na to da stabilna i pravovremena hirurška rekonstrukcija i fiksacija maleolusa i tibiofibularne sindezmoze dovodi do vraćanja funkcije i oporavka skočnog zgloba s minimalnim posledicama.

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***Ključne reči**: povreda sindezmoze, prelom skočnog zgloba, reparacija zgloba i sindezmoze*

Case report

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THORACIC EPIDURAL AS THE SOLE ANESTHETIC TECHNIQUE FOR COLORECTAL SURGERY IN AWAKE PATIENTS WITH SEVERE RESPIRATORY DYSFUNCTION

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The report presents a case of major abdominal surgery performed in an awake patient with a high surgical risk due to severe chronic obstructive pulmonary disease. Elective sigmoid colon resection was successfully performed under thoracic epidural anesthesia as the sole anesthetic technique. The patient remained awake and moderately sedated throughout the procedure, which was well tolerated. The applied anesthetic approach itself, which has been proven to reduce the intraoperative and postoperative risks of cardiac, respiratory, and gastrointestinal complications, significantly contributed to a faster postoperative recovery with minimal complications. In a situation where the risk of general endotracheal anesthesia outweighs its benefits, thoracic epidural anesthesia was opted for as the sole anesthetic technique. This approach avoided potentially severe complications, while the additional advantages of epidural anesthesia and analgesia accelerated recovery, especially in a high-risk patient.

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Key words: *thoracic epidural anesthesia, chronic obstructive pulmonary disease, colorectal surgery*

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Introduction

This paper presents a case of elective sigmoid colon resection in a patient with severely compromised pulmonary function, placing the patient in a high-risk group for the development of postoperative complications and raising concerns regarding the feasibility of surgery. Current literature indicates that thoracic epidural anesthesia may be considered a viable alternative to general endotracheal anesthesia in such high-risk patients. Accordingly, high thoracic epidural anesthesia was used as the sole anesthetic technique.

Case Presentation

Several days before the surgery, a 63-year-old male patient scheduled for elective sigmoid colon resection was evaluated at the Clinic for Anesthesia, Rheumatology and Intensive Care of the University Clinical Center Niš. The patient was a long-term smoker with a history of chronic obstructive pulmonary disease (COPD). Spirometry indicated severe obstructive ventilatory disorder, with FEV of 820 ml (23% of predicted value) and an FEV1/FVC ratio of 34.43% (Table 1). Preoperative arterial blood gas analysis indicated mild partial respiratory insufficiency: pH 7.39, pCO2—41.9 mmHg, pO2—69 mmHg, HCO3—25 mmol/L, SpO2—93% (Table 2). Auscultation revealed bilateral diffuse wheezing, both low- and high-pitched. Pulmonary therapy included dual inhaled therapy with fluticasone/salmeterol and tiotropium bromide, and oral prednisone and aminophylline. Among other comorbidities, the patient's surgical history included essential hypertension, angina pectoris, a previous myocardial infarction (one year before surgery), and hyperlipoproteinemia, as well as the implantation of a knee endoprosthesis. The patient used nebivolol, ramipril, acetylsalicylic acid, molsidomine, trimetazidine, with nitroglycerin used as needed. Preoperative chest radiography and laboratory results were within normal limits. Both surgical and anesthetic techniques were considered preoperatively, and it was decided to proceed with open surgery, using thoracic epidural anesthesia as the sole anesthetic option to minimize possible respiratory complications in this high-risk patient. Immediately before surgery, an epidural catheter was inserted using an 18-gauge Tuohy needle, with the patient in a sitting position.

The procedure was performed under strict aseptic conditions at the Th8–Th9 level. The patient was given 2 ml of 2% lidocaine for local infiltration of the puncture site and 2 ml as a test dose. After that, a mixture of 15 ml of 0.5% bupivacaine with 100 mcg of fentanyl was administered to the patient as a bolus dose for the epidural block. Ten minutes after the application, a sensory block up to the Th4 level was achieved and confirmed by pinprick testing. Analgesic and surgical conditions, including adequate muscle relaxation, were satisfactory. Due to increased cardiovascular risk, in addition to the standard monitoring (including five-lead ECG and SpO2), a radial arterial cannula was placed in the right arm for continuous arterial pressure monitoring and advanced hemodynamic assessment, if needed. The patient was lightly sedated with 1 mg of midazolam, and a continuous infusion of remifentanil in target-controlled infusion (TCI) mode at 1ng/ml. Standard respiratory function monitoring was performed during procedural sedation, including capnography (Capnostream) and bispectral index monitoring to assess sedation depth. The patient maintained spontaneous ventilation throughout the procedure, with oxygen saturation values of 98–100% under oxygen supplementation via a nasal cannula at 4–6 l/min. The patient was hemodynamically stable during the entire procedure. Capnography values (EtCO2) and SpO2 were satisfactory, with a respiratory rate of 14 breaths per minute. The patient remained responsive to verbal stimuli and interacted with the surgical and anesthesiology team, corresponding to Ramsay Sedation Scale level 3 (1). BIS values ranged between 70 and 80, consistent with moderate sedation. The operation lasted 2 hours and 15 minutes, after which the patient was transferred to the intensive care unit for postoperative monitoring. Total intraoperative blood loss was 100 ml. Continuous epidural analgesia was maintained using 0.1% bupivacaine with fentanyl (2 mcg/ml) at an infusion rate of 4–8 ml/h. Supplemental analgesic therapy was not required during the epidural analgesia, which was discontinued 48 hours after admission to the intensive care unit and subsequently replaced with intravenous non-opioid analgesic therapy. During the entire stay in the intensive care unit, the patient maintained spontaneous ventilation. However, despite avoiding general anesthesia, the optimization of pulmonary function was required, including oxygen therapy, bronchodilators, antibiotics, and respiratory physiotherapy. On the first postoperative day, arterial blood gas analysis showed a more severe degree of respiratory insufficiency compared to preoperative values (pH 7.48, pCO2 35 mmHg, pO2 53 mmHg, HCO3 26.1 mmol/L, SpO2 90%) (Table 2). By the fourth postoperative day, pulmonary function was optimized to the level before surgery, so the patient was transferred to the Department of Digestive Surgery. Seven days after surgery, the patient was discharged from the hospital seven days after surgery in good general condition.

Table 1. Interpretation of spirometry values

Spirometry test	Normal	Abnormal	Patient
FEV 1	Equal to or greater than 80%	Mild 70–79% Moderate 60–69% Severe < 60%	**23%**
FEV1/FVC	Equal to or greater than 80%	Mild 60–69% Moderate 50–59% Severe < 50%	**34.43%**

Table 2. Preoperative and postoperative arterial blood gas analyses

Arterial blood gas analyses	Normal range	Preoperative	First postoperative day
SpO2	> 94%	93%	90%
pO2	80–100 mmHg	69 mmHg	53 mmHg
pCO2	35–45 mmHg	41.9 mmHg	35 mmHg
HCO3	22–26 mmol/L	25 mmol/L	26.1 mmol/L
pH	7.35–7.45	7.39	7.48

Discussion

General endotracheal anesthesia (GETA) in surgical patients with high risk of pulmonary is associated with numerous adverse effects, including ventilator-induced lung injury, ventilator-associated pneumonia, cardiac dysfunction, neuromuscular complications such as critical illnesses, polyneuropathy, and myopathy, as well as intraoperative and postoperative hypoxemia (2). Although absolute contraindications to GETA are not clearly defined in the literature, it is often considered a last-resort option in cases when regional anesthesia techniques are unsuccessful. Thoracic epidural anesthesia has been reported to be a safe and effective technique in patients with COPD. Given the presence of advanced COPD and significant comorbidities in this patient, the benefits of thoracic epidural anesthesia (TEA) likely made a vital contribution to a satisfactory outcome and recovery. Thoracic epidural anesthesia provides superior analgesia and has been associated with a reduced risk of venous thromboembolism, myocardial infarction, intraoperative blood loss, and renal failure (3, 4). For these reasons, TEA is considered the gold standard for open colorectal surgery and is recommended within Enhanced Recovery After Surgery (ERAS) protocols. Reviews of the available literature on the use of TEA in major emergency abdominal surgeries have consistently shown a clinically significant reduction in postoperative mortality reported to be as high as 35% (4). Two review articles indicate that epidural anesthesia, with or without postoperative epidural analgesia, reduces postoperative pulmonary infections compared to general anesthesia, regardless of the type of postoperative systemic analgesia (5, 6). Ballantine et al. confirmed that postoperative epidural analgesia significantly reduces the incidence of pulmonary morbidity (7). On the other hand, there are concerns regarding the use of TEA in patients with severe COPD due to its potential effects on auxiliary respiratory muscles, including respiratory muscle impairment and alterations in bronchial tone. In a study involving patients with severe COPD, thoracic epidural analgesia with 0.25% bupivacaine did not adversely affect ventilatory mechanics, gas exchange, or inspiratory muscle strength (8). Moreover, a study by van Lier et al. demonstrated that the theoretical effect of TEA on respiratory muscle function, particularly the reduction of intercostal nerve conduction, is not clinically relevant (9). In our patient, the epidural technique proved to be safe with stable and satisfactory SpO2 levels maintained throughout the procedure. Compared to spinal anesthesia, TEA causes significantly fewer changes in inspiratory capacity and expiratory reserve volume (10). Furthermore, TEA does not affect airway resistance and respiratory gas exchange. In addition, it has been shown to improve left ventricular function in high-risk patients by preserving ventriculo-pulmonary coupling, thereby enhancing myocardial oxygen balance (10–12). Conversely, GETA through endotracheal intubation and altered respiratory mechanics may lead to changes in right ventricular function. This may be further exacerbated by the direct cardiodepressive action of anesthetic agents on myocardial function (12). Moreover, GETA reduces functional residual capacity, worsens ventilation-perfusion mismatch, and impairs diaphragmatic function (10). Therefore, in high-risk patients, such as those with COPD, who are at increased risk of right ventricular dysfunction and adverse respiratory effects associated with GETA, locoregional anesthesia techniques should be considered. Taking into account the cardiovascular status of our patient and previous damage to the myocardium, the use of thoracic epidural anesthesia and analgesia was completely justified. Nevertheless, caution is required when applying central neuraxial techniques in patients at risk of hemodynamic instability or with significant organ dysfunction. In the present case, stable hemodynamic and metabolic parameters supported the use of TEA for this procedure.

Technical challenges associated with epidural needle insertion, as well as the risk of inaccurate or unsafe catheter placement — especially in high and mid-thoracic epidural space — may pose certain difficulties for the anesthesiologists. In addition, persistent perioperative hypotension and potential neurological complications must be taken into account. Placement of the epidural catheter at the lumbar level with cephalad spread of the block may represent a simpler alternative. Unfortunately, the expected benefit for the patient may be decreased compared to well-managed thoracic epidural anesthesia.

When the risks of general anesthesia outweigh its benefits, surgery is often deferred, leaving the patient without definitive or even palliative treatment options. However, if the overall risk–benefit ratio is in favor of surgery and in the absence of contraindications, performing thoracic epidural anesthesia for awake abdominal surgery could contribute to a reasonable quality of life even after surgery. In this context, several studies have shown the advantages of epidural anesthesia in awake patients compared to orotracheal endotracheal anesthesia (OETA)(13–16).

Conclusion

General anesthesia in patients with severe respiratory disease is associated with an increased risk of prolonged mechanical ventilation, along with higher morbidity and mortality. It also presents an ethical dilemma regarding the appropriateness of surgical intervention for patients with advanced terminal chronic respiratory disease. Improving outcomes after any type of surgery relies on reducing the stress response and preventing organ dysfunction. In this context, TEA in the high-risk patient undergoing awake abdominal surgery may represent a valuable alternative, avoiding potential severe complications and accelerating recovery.

References

1. Ramsay MAE, Savege TM, Simpson BRJ, Goodwin R. Controlled sedation with alphaxalone–alphadolone. Br Med J 1974;2(5920):656–9. [CrossRef] [PubMed]
2. Jin F, Chung F. Minimising perioperative adverse events in the elderly. Br J Anaesth 2001;87:608–24. [CrossRef] [PubMed]
3. Romanzi A, Boleso N, Di Palma G, Ferrara F, Rossi M, Esposito C, et al. Awake major abdominal surgeries in the COVID-19 era. Pain Res Manag 2021;2021:8763429. [CrossRef] [PubMed]
4. Vester-Andersen M, Lundstrøm LH, Møller MH. The association between epidural analgesia and mortality in emergency abdominal surgery: a population-based cohort study. Acta Anaesthesiol Scand 2020;64:104–11. [CrossRef] [PubMed]
5. Jayr C, Thomas H, Rey A, Farhat F, Lasser P, Bourgain JL. Postoperative pulmonary complications: epidural analgesia using bupivacaine and opioids versus parenteral opioids. Anesthesiology 1993;78:666–76. [CrossRef] [PubMed]
6. Rodgers A, Walker N, Schug S, McKee A, Kehlet H, van Zundert A, et al. Reduction of postoperative mortality and morbidity with epidural or spinal anaesthesia: results from an overview of randomised trials. BMJ 2000;321:1493–7. [CrossRef] [PubMed]
7. Ballantyne JC, Carr DB, deFerranti A, Suarez T, Lau J, Chalmers TC, et al. The comparative effects of postoperative analgesic therapies on pulmonary outcome: cumulative meta-analysis of randomized controlled trials. Anesth Analg 1998;86:598–612. [CrossRef] [PubMed]
8. Gruber EM, Tschernko EM, Kritzinger M, Deviatko E, Wisser W, Zurakowski D, et al. The effects of thoracic epidural analgesia with bupivacaine 0.25% on ventilatory mechanics in patients with severe chronic obstructive pulmonary disease. Anesth Analg 2001;92:1015–9. [CrossRef] [PubMed]
9. Van Lier F, van der Geest PJ, Hoeks SE, van Gestel YRBM, Lenzen MJ, Stolker RJ, et al. Epidural analgesia is associated with improved health outcomes of surgical patients with chronic obstructive pulmonary disease. Anesthesiology 2011;115:315–21. [CrossRef] [PubMed]
10. Corcione N, Karim HM, Mina B, Esquinas AM. Non-invasive ventilation during surgery under neuraxial anaesthesia: a pathophysiological perspective and systematic review. Anaesthesiol Intensive Ther 2019;51:289–98. [CrossRef] [PubMed]
11. Rigg JR, Jamrozik K, Myles PS, Silbert BS, Peyton PJ, Parsons RW, et al. Epidural anaesthesia and analgesia and outcome of major surgery: a randomised trial. Lancet 2002;359:1276–82. [CrossRef] [PubMed]
12. Wink J, Veering BT, Aarts LPHJ, Wouters PF. Effects of thoracic epidural anesthesia on neuronal cardiac regulation and cardiac function. Anesthesiology 2019;130:472–91. [CrossRef] [PubMed]
13. Yen CR, Tsou MY, Lin SM, Chan KH, Chu YC. Thoracic epidural anaesthesia for a polymyositis patient undergoing awake mini-thoracotomy and unroofing of a huge pulmonary bulla. Acta Anaesthesiol Taiwan 2008;46:42–5. [CrossRef] [PubMed]
14. Mineo TC, Pompeo E, Mineo D, Tacconi F, Marino M, Sabato AF. Awake nonresectional lung volume reduction surgery. Ann Surg 2006;243:131–6. [CrossRef] [PubMed]
15. Pompeo E, Mineo TC. Awake operative videothoracoscopic pulmonary resections. Thorac Surg Clin 2008;18:311–20. [CrossRef] [PubMed]
16. Noda M, Okada Y, Maeda S, Sado T, Sakurada A, Hoshikawa Y, et al. Is there a benefit of awake thoracoscopic surgery in patients with secondary spontaneous pneumothorax? J Thorac Cardiovasc Surg 2012;143:613–6. [CrossRef] [PubMed]

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PRIMENA TORAKALNE EPIDURALNE ANESTEZIJE NA BUDNOM PACIJENTU SA TEŠKIM POREMEĆAJEM RESPIRATORNE FUNKCIJE U TOKU OPERATIVNOG KOLOREKTALNOG ZAHVATA

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U ovom radu se prikazuje slučaj koji je podrazumevao izvođenje velike operacije abdomena na budnom pacijentu, kod kojeg je, zbog teške forme hronične opstruktivne bolesti pluća, zahvat bio visokorizičan. Obavljena je elektivna operacija resekcije sigmoidnog kolona. Operativni zahvat je uspešno izveden u torakalnoj epiduralnoj anesteziji, koja je predstavljala jedinu anesteziološku tehniku. Pacijent je u toku cele operacije bio budan, umereno sediran, i dobro je podneo proceduru. Sama tehnika anestezije, koja dokazano smanjuje intraoperativni i postoperativni rizik od nastanka srčanih, respiratornih i gastrointestinalnih komplikacija, značajno je doprinela bržem oporavku posle operacije, i to sa minimalnim komplikacijama. Budući da je rizik za primenu opšte endotrahealne anestezije bio veći od koristi koje bi ona mogla imati, doneta je odluka da torakalna epiduralna anestezija bude jedina anesteziološka tehnika; na taj način izbegnute su potencijalne ozbiljne komplikacije. Takođe, dodatne prednosti epiduralne anestezije i analgezije uticale su na to da oporavak bude brži, naročito kod pacijenta kod kojeg je postojao ovako visok rizik od izvođenja operacije.

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***Ključne reči**: torakalna epiduralna anestezija, hronična opstruktivna bolest pluća, kolorektalna hirurgija*

Review article

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FIRST-LINE PHARMACOTHERAPY OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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Chemotherapy-induced peripheral neuropathy (CIPN) is a very common adverse event iatrogenically caused by the administration of neurotoxic chemotherapy protocols in oncology patients. Of the chemotherapeutics—oxaliplatin, cisplatin, carboplatin, docetaxel, paclitaxel, vinblastine and vincristine—bortezomib shows the greatest neurotoxicity. Recommendations for the first line of pharmacotherapy of this side effect of chemotherapy are gabapentinoids (pregabalin and gabapentin), antidepressants (amitriptyline, duloxetine, venlafaxine, desvenlafaxine), as well as topical application of lidocaine. Adequate pharmacotherapy of CIPN implies an individualized approach for each patient and compliance with guidelines for recommended first-line pharmacotherapy, first, in the form of monotherapy and later, depending on tolerability and achieved analgesic response, in the form of polytherapy with first-line drugs. If there is no adequate analgesic response to polytherapy with first-line drugs, only then is it necessary to switch to the second line of therapy. Treatment of CIPN leads to an adequate quality of life due to the reduction of the neuropathic component of pain, a reduction in depression and anxiety and the expected completion of the planned chemotherapy cycles, and consequently, a better prognosis of the underlying oncological disease of these patients.

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Key words: *chemotherapy, peripheral neuropathy, pharmacotherapy*

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Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a very common side effect of chemotherapy, iatrogenically induced in oncology patients. It is known which chemotherapeutic agents cause this side effect, such as platinum derivatives (oxaliplatin, cisplatin, carboplatin) and taxanes (docetaxel, paclitaxel, vinblastine and vincristine, bortezomib) (1).

There are numerous factors that are associated with a higher incidence of CIPN. In a large number of cases, given that it occurs during chemotherapy sessions, it is necessary to reduce the doses of cytostatics in subsequent cycles of therapy and, of course, to implement supportive pharmacotherapy of this side effect (SE) of chemotherapy. Adequate pharmacotherapy of this SE is directly proportional to the completion of the intended chemotherapy sessions in the intended dose regimen and consequently to the survival rate of these patients (2).

In some patients, this SE occurs even after chemotherapy sessions—1 month after completing the first cycle in 68% of patients, 3 months after the end of chemotherapy in 60% of patients, and in 30% of patients after 6 or more months of neurotoxic chemotherapy (3).

Clinical Status and Diagnosis of CIPN

Peripheral neuropathic pain of these patients is characterized by sensations—tingling, pricking, burning, stabbing, cold, electric shock or stabbing sensations that patients state when taking anamnestic data about their neuropathy. This neuropathy is chronic, of significant intensity and responds extremely poorly to the standard analgesic therapy application. The majority of

sufferers also mention other comorbidities such as sleep disorder, anxiety, depression, cognitive disorders, all of which significantly affect the reduction of work ability and quality of life of these patients (4).

In order to be able to diagnose the neuropathy of these patients with certainty, we use some quantitative tools that later serve us to reassess the neuropathy in the controls when we also monitor the therapeutic effect of the prescribed antipain therapy in this group of patients.

In daily clinical practice, multidimensional scales are used to survey patients, and based on the obtained score, we can confidently claim that the patient has a neuropathic component of pain, and during the controls, resurvey and rescore the patients, and thus monitor the effects of the applied pharmacotherapy (5).

One of the most commonly used questionnaires is the painDETECT Questionnaire, which assesses several aspects of pain—nature of pain, pattern of occurrence, strength, spread, and anatomical localization of pain, and based on the obtained score, we can determine the presence of a neuropathic pain component in the surveyed patient. It was translated and validated into the Serbian language. The results obtained with this questionnaire in terms of the neuropathy presence of the surveyed patients include "unlikely" score less than 13 points, "possible" score 13–18 points and "probable" score over 18 points (6). In addition to this questionnaire, there are other validated and translated questionnaires, such as the DN4 questionnaire (Douleur Neuropathique 4 Questionnaire) (7).

First-line Pharmacotherapy of CIPN

Before starting pharmacotherapy, it is necessary to determine the presence of comorbidities, since depression, anxiety and insomnia can be treated simultaneously with this pharmacotherapy. In addition, the combination of this therapy with cardiology or nephrology therapy can lead to potential interactions and SE, so it is necessary to adjust the dose or revise the choice of drug.

It is necessary to familiarize the patient with the nature of their disease and the detailed pharmacotherapy plan, and to set realistic expectations of the therapy and to familiarize the patient in detail with all aspects of the therapy that will be applied in order to have adequate compliance (8).

Based on the European Federation of Neurological Societies (EFNS) recommendations, the drugs used in the present study for this indication were divided into those that were first therapeutic choice and those that were second therapeutic choice, as shown in Table 1 (9).

Table 1. Recommendations for pharmacotherapy of peripheral neuropathic pain.

First-line pharmacotherapy	pregabalin, gabapentin, amitriptyline, duloxetine, venlafaxine, lidocaine patches
Second-line pharmacotherapy	tramadol, oxycodone, lamotrigine, capsaicin patches

When choosing the drug of the first therapeutic line for the treatment of CIPN, it is necessary to take a good pharmacological anamnesis of the patient in terms of the existence of comorbidities and taking some chronic therapy of the patient, then to familiarize the patient with the expected efficiency and safety of the therapy.

European Federation of Neurological Societies recommendations for the first-line treatment of peripheral neuropathic pain, which includes CIPN, include gabapentinoids, antidepressants and topical analgesics (9).

Gabapentinoids—pregabalin and gabapentin—have found their place in the pharmacotherapy of neuropathy from the group of anticonvulsant drugs. They work with the same mechanism of action, i.e., by binding to the alpha-2-delta subunit of voltage-dependent calcium channels, leading to depolarization, i.e. to a decrease in the influx of calcium ions into the presynaptic neuron. As a result, the release of excitatory neurotransmitters (glutamate) is reduced, and postsynaptic excitability is reduced. In this way, by reducing sensitization, an analgesic effect is achieved (10). These drugs, in addition to their primary indications, namely adjuvant therapy of partial convulsions in adults with or without secondary generalization and generalized anxiety disorder, have also found their role in the pharmacotherapy of peripheral neuropathic pain, such as CIPN.

The dose titration of these two drugs is different. Namely, pharmacotherapy with any of these drugs should be started in the evening for a few days due to possible dizziness and fainting, and then after a few days, start with the morning dose. It should evaluate and start with the lowest effective dose. The maximum daily dose (MDD) of

pregabalin is 600 mg, and gabapentin is 3600 mg. It is possible to titrate the doses and divide the daily dose of these drugs into 3, when it is necessary for higher dose regimens (11).

Pregabalin has been shown to be effective in all investigated types of neuropathic pain (diabetic polyneuropathy, cancer pain and back pain) with a favorable safety profile and adequate patient adherence. It is structurally similar to the main inhibitory neurotransmitter—gaba-aminobutyric acid (GABA). It is eliminated from the systemic circulation via renal excretion in an unchanged form (< 2% of the dose is detected in the urine in the form of metabolites). Pregabalin clearance is directly proportional to creatinine clearance. Based on this, its use in patients with renal insufficiency is contraindicated. Considering the route of elimination of this drug as well as its biotransformation, drug-drug interactions are very rare because it is excreted unchanged by renal excretion, so it is possible to combine it with other drugs that act on the CNS, e.g. opioid analgesics in the treatment of mixed malignant pain syndrome. The initial dose is usually 150 mg divided into two equal daily doses. Given that it has been proven that pregabalin has a dose-dependent effect, i.e. its effectiveness increases directly proportionally with the applied dose, in cases of inadequate analgesic response, it is advised to increase the dose to 300 mg during one week of therapy. The favorable pharmacokinetic profile of pregabalin is reflected in the rapid reaching of the maximum drug level in plasma (0.7–1.3 h), good tolerability, bioavailability (90%), short elimination half-life (4.6–6.8 h), absence of drug-drug interactions and hepatic side effects (12).

Pharmacotherapy with gabapentin usually starts with a dose of 300 mg per day with gradual titration as with pregabalin, and the MDD is 3600 mg. As with pregabalin, with larger doses, it is possible to divide the entire dose into three equal doses, i.e., that in addition to the morning and evening doses, the midday dose of the drug should also be introduced, given that its elimination half-life is 5–7 hours. The path of biotransformation and excretion of gabapentin is the same as that of pregabalin, and renal insufficiency is also a contraindication for the use of this drug. The most common SEs that occur during the use of these two drugs are dizziness, drowsiness, headache, dry mouth, appearance of peripheral edema and weight gain. They are usually mild to moderate in intensity and disappear when the dose is reduced (13).

Antidepressants also occupy their important place in CIPN pharmacotherapy. Today, it is avoided by the use of tricyclic antidepressants (TCAs). From this group of drugs, the only one that has found its place for these therapeutic purposes is amitriptyline. Tricyclic antidepressants achieve their analgesic effect completely independently of their antidepressant effect. They can have an antidepressant and analgesic effect equally well. The analgesic effect is achieved by inhibiting serotonin uptake into nerve endings, blocking sodium channels, modulating cholinergic and histaminergic transmission and reducing central sensitization. Pharmacotherapy with amitriptyline is started gradually, usually 10 mg or up to 25 mg in the evening with a gradual titration of the dose on a weekly basis to a maximum effective dose of 75–150 mg per day. At the same time, amitriptyline improves sleep and reduces anxiety in these patients. A contraindication for the use of TCAs and the reason why many clinicians avoid prescribing them to their patients is cardiotoxicity, especially in patients with conduction disorders and arrhythmias. Among the SEs, anticholinergic effects that can be quite pronounced (confusion, urine retention, constipation, orthostatic hypotension), prolongation of the QT interval, and excessive sedation are significant (14).

Today, in daily clinical work, most often for the treatment of peripheral neuropathies and also CIPN, antidepressants are drugs from the group of selective serotonin and noradrenaline reuptake inhibitors (SNRIs), namely duloxetine and venlafaxine. By inhibiting the uptake of serotonin and noradrenaline in the descending pathways for pain, they achieve their analgesic effect in addition to the basic antidepressant effect. Their advantage over TCAs is a better safety profile in terms of very minor and mild SEs, and they can be used more safely in high-risk populations such as the elderly and co-medicated with other drugs.

Duloxetine has its primary indication area, such as major depression, obsessive-compulsive disorder, generalized anxiety disorder. In addition to these psychiatric indications, today it is successfully used in the pharmacotherapy of neuropathic pain and fibromyalgia. Its effectiveness has been proven in the treatment of neuropathic pain caused by chemotherapeutic agents, diabetic polyneuropathy, radiculopathy, and neuropathy in multiple sclerosis. Recommended doses of duloxetine are 60–120 mg per day. The recommendation for the use of antidepressants is to take the daily dose in the morning due to the energizing effect. The advantage of duloxetine over venlafaxine is that it is safer in cardiovascular patients, while SE patients can expect nausea, vomiting, increase in liver enzymes, and fainting (15).

Venlafaxine is also a drug from the SNRI group that is used in the pharmacotherapy of major depression, panic disorders, social phobias and generalized anxiety disorder. This drug can be used in CIPN with initial doses of 37.5–75 mg per day with gradual titration to a therapeutically effective dose of 150–225 mg per day, and the maximum daily dose is 375 mg per day. In lower doses, up to 100 mg per day, it inhibits the reuptake of serotonin, while the reuptake of noradrenaline increases in the range of 100–375 mg per day. It has been proven to be the most effective in the treatment of diabetic polyneuropathy and postherpetic neuralgia. The most common SEs of using this drug are arterial hypertension, hyponatremia and disorders of the gastrointestinal tract, as well as sexual dysfunctions (16).

Today, in addition to venlafaxine, desvenlafaxine is also available as an adequate therapeutic substitute with a better safety profile than venlafaxine. Desvenlafaxine is an active metabolite of venlafaxine, belonging to the SNRI group, which is used in the treatment of major depressive disorder, as well as in the treatment of certain forms of chronic pain. The usual starting dose is 50 mg, which can be increased up to 200 mg per day, depending on the patient's individual needs and response to therapy. It is used in the treatment of peripheral neuropathic pain, including pain associated with diabetic polyneuropathy, fibromyalgia, and chronic pain in patients with depression, as well as in CIPN (17). The mechanism of action in pain therapy is similar to its antidepressant effect, because it increases the concentration of noradrenaline and serotonin in the central nervous system, which contributes to the analgesic effect. The most common SEs are nausea, diarrhea, dry mouth and dizziness, which mostly occur during the introduction of therapy and are transient. Compared to venlafaxine, it has more stable pharmacokinetics, a lower risk of hypertension onset and easier dosing without the need for titration. Additionally, it undergoes minimal first-pass metabolism through the liver, which reduces the possibility of interaction with other drugs (18).

Topical analgesics, in first-line therapy, include lidocaine patches. The advantage of topical application of lidocaine to painful areas is that there is no systemic resorption and therefore no possible systemic therapy SEs. The only SE is local mild skin irritation at the application site. Lidocaine is used in the form of 5% lidocaine patches (700 mg lidocaine). Up to 4 patches per day can be applied with a therapeutic effect that can be maintained for 18 to 24 hours. Side effects of systemic resorption is manifested in the form of central nervous system (CNS) excitation (trembling, shaking, convulsions) and/or CNS depression (respiratory depression, respiratory arrest), cardiovascular manifestations, such as myocardial depression, atrioventricular block and vasodilatation leading to hypotension (19).

Lidocaine acts by reducing neuronal excitability at the level of sodium channels and by inhibiting the propagation of action potentials, and by reducing sodium permeability depolarization as a manifestation of nerve conduction is prevented. Lidocaine first shows its effect on smaller diameter fibers (Aδ and C fibers), and then on motor axons. It is effective as an adjunct to oral pharmacotherapy of peripheral neuropathies. Caution is required in patients who are on co-medication with antiarrhythmics (mexiletine) and in those with impaired hepatic function (20).

EMLA cream contains 20% pure lidocaine and an 80% lidocaine/prilocaine mixture. There is evidence of the effectiveness of this topical agent in relieving pain in peripheral neuropathies, especially during the first 2 hours after application (21).

Dexamethasone and betamethasone are long-acting corticosteroids that can be co-administered in patients with chronic peripheral neuropathy. These drugs can be prescribed with a limit of use of up to 15 days and a gradual de-escalation of the dose within that time period due to possible systemic SEs. Use longer than 3 weeks can lead to metabolic side effects such as: hyperglycemia, iatrogenic Cushing's syndrome, immunosuppression and greater susceptibility to infections, osteoporosis and the occurrence of pathological fractures. They achieve their antipain effect, especially in the acute phase of painful neuropathies, with their strong anti-inflammatory and antiedematous effect. Thanks to the long half-life of elimination (36–54 hours), dosing is very comfortable. Dexamethasone can be administered both parenterally and orally, while betamethasone is administered as depot formulations on a weekly basis (22, 23).

Second-line therapy is applied only if all therapeutic possibilities of monotherapy or combined therapy with first-line drugs have been exhausted. Medicines of this category mainly suppress the nociceptive component of pain, and do not have an adequate analgesic effect on the neuropathic component, and therefore belong to the alternative therapy of peripheral neuropathies and thus CINP (9).

If the introduction of first-line pharmacotherapy for the treatment of peripheral neuropathic pain, which includes CIPN, achieves a reduction in pain with an intensity equal to or less than 3 on the pain intensity scale, with tolerable SE, it is advised to continue the started pharmacotherapy due to an adequate analgesic response. If, after the introduction of the therapy, partial pain control is achieved, i.e. pain intensity is still greater than 4 on the scale of pain intensity, it is necessary to add another drug from the group of drugs of the first therapeutic choice. If the pain control is inadequate to the original therapy, with pain intensity reduced by less than 30% and in addition to taking the optimal dose of the drug of the first therapeutic choice for a long enough time, it is advised to stop the first-introduced drug and switch to an alternative drug of the first therapeutic choice (24). In daily clinical practice, the fact that chronic neuropathic pain, such as CIPN, cannot be cured by monotherapy is very often encountered, so it is necessary to introduce rational polytherapy, which involves combining drugs with different mechanisms of action, which should lead to an adequate analgesic response through a synergistic effect. The combination of one of the gabapentinoids with opioids/antidepressants/topical analgesics showed the greatest effectiveness. With such combinations, it is always necessary to keep in mind the safety and tolerance of the prescribed polytherapy and not just the efficiency. When combining these drugs, it is possible to have additive SE, drug-drug interaction, which leads to a decrease in patient compliance. If monotherapy with drugs of first choice or their combination within polytherapy does not lead to adequate relief of peripheral neuropathy, it is recommended to introduce drugs of second therapeutic choice and

non-pharmacological methods of treatment within the framework of a multidisciplinary assessment of the patient (25).

Conclusion

Chemoterapy-induced peripheral neuropathy is a very common adverse event iatrogenically caused by the administration of neurotoxic chemotherapy protocols in oncology patients. This SE of chemotherapy should not be ignored, but it is necessary to implement adequate pharmacotherapy respecting the individualized approach to each patient and the guidelines for recommended first- and second-line pharmacotherapy, first in the form of monotherapy and later, depending on tolerability and the achieved analgesic response, in the form of polytherapy with first-line drugs. If there is no adequate analgesic response to polytherapy with first-line drugs, only then is it necessary to switch to the second line of therapy. Adequate treatment of this neuropathy enables oncology patients to have an adequate quality of life due to the reduction of the neuropathic pain component, a reduction in depression and anxiety, and also the completion of planned chemotherapy cycles that lead to a better prognosis of the underlying oncological disease, which is why CIPN pharmacotherapy is extremely important.

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References

1. Colvin LA. Chemotherapy-induced peripheral neuropathy: where are we now? Pain 2019;160(Suppl 1):S1-S10. [CrossRef][PubMed]
2. Desforges AD, Hebert CM, Spence AL, Reid B, Dhaibar HA, Cruz-Topete D, et al. Treatment and diagnosis of chemotherapy-induced peripheral neuropathy: An update. Biomed Pharmacother 2022;147:112671. [CrossRef][PubMed]
3. Hu LY, Mi WL, Wu GC, Wang YQ, Mao-Ying QL. Prevention and Treatment for Chemotherapy-Induced Peripheral Neuropathy: Therapies Based on CIPN Mechanisms. Curr Neuropharmacol 2019;17(2):184-96. [CrossRef][PubMed]
4. Burgess J, Ferdousi M, Gosal D, Boon C, Matsumoto K, Marshall A, et al. Chemotherapy-Induced Peripheral Neuropathy: Epidemiology, Pathomechanisms and Treatment. Oncol Ther 2021;9(2):385-450. [CrossRef][PubMed]
5. Zhang S. Chemotherapy-induced peripheral neuropathy and rehabilitation: A review. Semin Oncol 2021;48(3):193-207. [CrossRef][PubMed]
6. König SL, Prusak M, Pramhas S, Windpassinger M. Correlation between the Neuropathic PainDETECT Screening Questionnaire and Pain Intensity in Chronic Pain Patients. Medicina (Kaunas) 2021;57(4):353. [CrossRef][PubMed]
7. Krtinic D, Rankovic GN, Petkovic I, Cvetanovic A, Conic I, Mitic MT, et al. DN4 questionnaire as a useful tool for evaluating the pharmacotherapeutic response to opioid pharmacotherapy in malignant neuropathy. Pharmazie 2024;79(6):109-13. [CrossRef][PubMed]
8. Staff NP, Grisold A, Grisold W, Windebank AJ. Chemotherapy-induced peripheral neuropathy: A current review. Ann Neurol 2017;81(6):772-81. [CrossRef][PubMed]
9. Joint Task Force of the EFNS and the PNS. European Federation of Neurological Societies/Peripheral Nerve Society guideline on management of multifocal motor neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society--first revision. J Peripher Nerv Syst 2010;15(4):295-301. [CrossRef][PubMed]
10. Chang TW, Yang FY, Liu YC, Hung CH. Gabapentinoids for chemotherapy-induced peripheral neuropathy: systematic review and meta-analysis. BMJ Support Palliat Care 2024;14(3):269-78. [CrossRef][PubMed]
11. Jordan B, Jahn F, Jordan K. Peripheral neuropathy: from guidelines to clinical practise. Curr Opin Oncol 2025;37(2):168-74. [CrossRef][PubMed]
12. Han FY, Kuo A, Nicholson JR, Corradinni L, Smith MT. Comparative analgesic efficacy of pregabalin administered according to either a prevention protocol or an intervention protocol in rats with cisplatin-induced peripheral neuropathy. Clin Exp Pharmacol Physiol 2018;45(10):1067-75. [CrossRef][PubMed]
13. Wang C, Chen S, Jiang W. Treatment for chemotherapy-induced peripheral neuropathy: A systematic review of randomized control trials. Front Pharmacol 2022;13:1080888. [CrossRef][PubMed]
14. Loprinzi CL, Lacchetti C, Bleeker J, Cavaletti G, Chauhan C, Hertz DL, et al. Prevention and Management of Chemotherapy-Induced Peripheral Neuropathy in Survivors of Adult Cancers: ASCO Guideline Update. J Clin Oncol 2020;38(28):3325-48. [CrossRef][PubMed]
15. Chow R, Novosel M, So OW, Bellampalli S, Xiang J, Boldt G, et al. Duloxetine for prevention and treatment of chemotherapy-induced peripheral neuropathy (CIPN): systematic review and meta-analysis. BMJ Support Palliat Care 2023;13(1):27-34. [CrossRef][PubMed]
16. Aziz MT, Good BL, Lowe DK. Serotonin-norepinephrine reuptake inhibitors for the management of chemotherapy-induced peripheral neuropathy. Ann Pharmacother 2014;48(5):626-32. [CrossRef][PubMed]
17. Gurba KN, Haroutounian S. Antidepressant analgesics in the management of chronic pain. In: Lynch ME, Craig KD, Peng PW, editors. Clinical Pain Management: A Practical Guide. 2nd ed. Hoboken (NJ): John Wiley & Sons Ltd.; 2022. P. 171-80. [CrossRef]
18. Norman TR, Olver JS. Desvenlafaxine in the treatment of major depression: An updated overview. Expert Opin Pharmacother 2021;22(9):1087-97. [CrossRef][PubMed]
19. Gudin J, Nalamachu S. Utility of lidocaine as a topical analgesic and improvements in patch delivery systems. Postgrad Med 2020;132(1):28-36. [CrossRef][PubMed]
20. Landmann G, Stockinger L, Gerber B, Benrath J, Schmelz M, Rukwied R. Local hyperexcitability of C-nociceptors may predict responsiveness to topical lidocaine in neuropathic pain. PLoS One 2022;17(7):e0271327. [CrossRef][PubMed]
21. Junputipong N, Rojhirunsakool S, Deewongkij P, Kamanamool N, Udompataikul M. Comparison of the onset, depth, and duration of cutaneous anesthesia between topical 10% lidocaine and EMLA creams: a randomized, intraindividual, comparative trial. J Dermatolog Treat 2022;33(7):3047-52. [CrossRef][PubMed]
22. Haywood A, Good P, Khan S, Leupp A, Jenkins-Marsh S, Rickett K, et al. Corticosteroids for the management of cancer-related pain in adults. Cochrane Database Syst Rev 2015;2015(4):CD010756. [CrossRef][PubMed]
23. Leppert W, Buss T. The role of corticosteroids in the treatment of pain in cancer patients. Curr Pain Headache Rep 2012;16(4):307-13. [CrossRef][PubMed]
24. Chen CS, Hertz DL. Chemotherapy-Induced Peripheral Neuropathy. Handb Exp Pharmacol 2023;277:299-337. [CrossRef][PubMed]
25. Molinares D, Kurtevski S, Zhu Y. Chemotherapy-Induced Peripheral Neuropathy: Diagnosis, Agents, General Clinical Presentation, and Treatments. Curr Oncol Rep 2023;25(11):1227-35. [CrossRef][PubMed]

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PRVA LINIJA FARMAKOTERAPIJE PERIFERNE NEUROPATIJE INDUKOVANE HEMIOTERAPIJOM

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Periferna neuropatija indukovana hemioterapijom (engl. *chemotherapy-induced peripheral neuropathy* – CIPN) predstavlja veoma čestu neželjenu pojavu, koja je jatrogeno izazvana sprovođenjem neurotoksičnih hemioterapijskih protokola na onkološkim pacijentima. Najveću neurotoksičnost među hemioterapeuticima pokazuju oksaliplatin, cisplatin, karboplatin, docetaksel, paklitaksel, vinblastin i vinkristin, boretzomib. Preporučuje se da se za prvu liniju farmakoterapije ovog neželjenog efekta hemioterapije koriste gabapentionoidi (pregabalin i gabapentin) i antidepresivi (amitrptilin, duloksetin, venlafaksin, desvenlafaksin). Takođe, preproučuje se topikalna primena lidokaina. Adekvatna farmakoterapija CIPN-a podrazumeva individualizovani pristup pacijentu i poštovanje smernica za preporučenu farmakoterapiju prve linije, najpre korišćenjem jednog leka, a potom, u zavisnosti od tolerabilnosti i postignutog analgetskog odgovora, i korišćenjem većeg broja lekova iz prve linije farmakoterapije. Treba preći na drugu liniju terapije jedino ako nema adekvatnog analgetskog odgovora na politerapiju lekovima iz prve linije. Zbrinjavanje CIPN-a omogućava bolji kvalitet života, budući da dolazi do kupiranja neuropatske komponente bola, smanjenja stepena depresivnosti i anksioznosti i predviđenog završetka planiranih hemioterapijskih ciklusa; samim tim, prognoza lečenja osnovnog onkološkog oboljenja ovih pacijenata postaje bolja.

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***Ključne reči**: hemioterapija, periferna neuropatija, farmakoterapija*

Review article

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THE SIGNIFICANCE OF QUANTITATIVE SENSORY TESTING IN PATIENTS WITH CHRONIC MUSCULOSKELETAL PAIN

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Quantitative Sensory Testing (QST) provides objective, reproducible data, which can help confirm a diagnosis or monitor treatment outcomes in patients with chronic musculoskeletal pain (CMSP). Quantitative Sensory Testing has the potential to become an integral part of routine clinical evaluations in a variety of chronic musculoskeletal conditions. Quantitative Sensory Testing can detect early sensory changes before they are clinically obvious. Quantitative Sensory Testing procedures are non-invasive, making them suitable for repeated assessments and monitoring over time. The standardization of testing protocols is essential for obtaining reliable results. Impaired Conditioned pain modulation could serve as a biomarker for central sensitization in chronic pain conditions, helping clinicians identify patients whose pain is centrally mediated. Quantitative Sensory Testing has the potential to become an integral part of routine clinical evaluations in a variety of chronic musculoskeletal conditions. Quantitative Sensory Testing is particularly useful for evaluating the impact of physical therapies, exercise programs, and psychological interventions.

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Key words: *central sensitization, chronic pain, musculoskeletal pain*

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Introduction

Chronic musculoskeletal pain (CMSP) is a widespread condition that affects millions globally, contributing significantly to disability and diminishing quality of life. The International Association for the Study of Pain (IASP) defines musculoskeletal pain as stemming from occupational injuries, repetitive stress, or overuse, encompassing disorders that induce pain in muscles, bones, joints, or adjacent tissues, such as lumbar and cervical pain, tendinopathy, neuropathic syndromes, muscular pain, and stress fractures (1). The IASP further categorizes chronic pain as persistent discomfort that is present for over three months. However, in cases like fibromyalgia or nonspecific low-back pain, it may be reclassified as a standalone condition termed “chronic primary pain”, reflecting its complexity and recognition as a neurological disorder (2). CMSP, conversely, is termed “chronic secondary pain”, where discomfort initially arises as a symptom of an underlying pathology (2). Current clinical frameworks advocate for a mechanismbased management approach, distinguishing between adaptive and physiological pain, such as nociceptive or inflammatory pain, and maladaptive and pathological pain, such as neuropathic or nociplastic pain, each necessitating tailored therapeutic strategies (3). Notably, research highlights central sensitization, marked by abnormal pain processing, as a critical predictor of poor clinical outcomes in CMSP (4).

Quantitative sensory testing (QST) has emerged as a promising diagnostic tool to objectively evaluate the sensory functions of the nervous system, enhance diagnostic accuracy, and inform treatment strategies in patients with CMSP. Over recent decades, QST has gained significant traction in clinical practice, offering valuable insights into sensory disturbances linked to diverse chronic pain conditions, including osteoarthritis, fibromyalgia, chronic low back pain (CLBP), myofascial pain syndrome, and complex regional pain syndrome (CRPS) (5–7). Quantitative sensory testing can help clinicians tailor interventions to the specific pain mechanisms present in a patient, such as nociceptive pain, neuropathic pain, or central sensitization (8). Central to chronic pain are the phenomena of peripheral sensitization and central sensitization.

Peripheral sensitization arises from heightened excitability of nociceptors, resulting in hyperalgesia. Quantitative sensory testing detects peripheral sensitization through reduced thermal (heat and cold) pain thresholds (9). Central sensitization is a condition where the central nervous system becomes hyperresponsive to stimuli, which results in increased pain perception and chronic pain states in patients with chronic musculoskeletal pain (osteoarthritis, lumbar and cervical syndrome, fibromyalgia, rheumatoid arthritis, complex regional pain syndrome, tendinopathies, etc.) (10–12). Central sensitization represents a mechanism for the development of nociplastic pain. In patients with CMSP, persistent inflammation causing prolonged nociceptive pain increases the risk of nociplastic pain, which may manifest in isolation or alongside other types of pain (13). Quantitative sensory testing identifies central sensitization via temporal summation, where administering rapid, repeated mechanical or thermal stimuli intensifies perceived pain. Importantly, QST-measured central sensitization differs from symptom-based assessments, such as those assessed by the Central sensitization inventory (CSI) (14).

QST Measurement Principles

The QST method allows for the assessment of sensory processing in both large and small afferent nerve fibers, as well as other ascending pathways, by modulating various sensory stimuli (15, 16).

Quantitative sensory testing involves the systematic application of controlled sensory stimuli to assess the integrity of sensory pathways, both peripheral and central. Developed by the German Research Network on Neuropathic Pain, QST serves as a standardized framework for assessing thermal and mechanical sensory functions (Rolke). This approach involves applying a battery of standardized tests that enable clinicians to objectively evaluate somatosensory responses mediated by both large-diameter (Aβ) and small-diameter (Aδ and C) nerve fibers (17).

These stimuli can include mechanical (touch or pressure), thermal (temperature changes), and electrical (nerve conduction) stimuli. The responses are quantitatively measured, typically via threshold detection or response amplitude, allowing for a detailed, objective evaluation of reduced sensory capacity, such as hypoesthesia and hypoalgesia, or increased sensitivity, such as hyperesthesia, hyperalgesia, and allodynia (18).

QST and Central Sensitization

Quantitative sensory testing is a crucial tool for identifying central sensitization through the use of tests like Conditioned Pain Modulation (CPM), Temporal Summation of Pain (TSP), and the wind-up phenomenon.

1. Conditioned Pain Modulation (CPM)

Blunted CPM responses are a key indicator of central sensitization, which is often found in chronic musculoskeletal pain conditions like fibromyalgia or chronic low back pain. A reduced CPM response can signal that the patient's body is not able to inhibit pain signals as it should, leading to increased pain perception. Conditioned Pain Modulation is a reliable test, but its reliability varies depending on the protocols and methodology used. The most reliable test stimulus is the pressure pain threshold, while among the conditioning stimuli, cold water is the most effective (19). Literature data show that the combination of determining the pressure pain threshold with an algometer as the test stimulus and cold water as the conditioning stimulus has demonstrated the highest reliability (20).

Conditioned Pain Modulation testing is significant in determining the pain modulation profile, where individuals with a pronociceptive profile exhibit pain facilitation, indicating a less effective CPM and a higher risk for developing pain conditions (9). Pain modulation profiles are variable throughout life and represent a dynamic phenomenon that can become pronociceptive during pain conditions and later return to normal (21, 22).

2. Temporal Summation and the Wind-Up Phenomenon

Quantitative sensory testing can measure temporal summation (the increasing pain response over time), which can help identify patients who are particularly sensitive to repeated or prolonged stimuli. The wind-up phenomenon refers to an exaggerated pain response when stimuli are presented in rapid succession, which can occur in conditions like myofascial pain syndrome and fibromyalgia. Quantitative sensory testing consists of tests that assess pain thresholds and temporal summation. Using thermal and mechanical modalities, QST can identify patients who are particularly sensitive to repeated or prolonged stimuli. Pain thresholds represent the transition point at which a sensation, such as pressure, becomes painful, while temporal summation describes the progressive escalation in perceived pain following repeated application of stimuli such as heat or pressure (23). Studies suggest that temporal summation reflects endogenous facilitatory pain pathways, characterized by amplified pain perception despite constant stimulus intensity during repeated or prolonged noxious input (24). Wind-up is a phenomenon connected to central sensitization. It is “a frequency-dependent increase in the excitability of spinal cord neurones, evoked by electrical stimulation of afferent C-fibers”. In humans, temporal summation of second pain reflects the wind-up phenomenon (25).

Clinical Significance of Quantitative sensory testing

Quantitative sensory testing has significant applications in determining personalized treatment, as well as in monitoring its effectiveness (9, 24).

Quantitative sensory testing can also help clinicians decide whether pharmacological treatments are appropriate (25). If QST indicates the presence of peripheral nociceptive pain, therapies with local anti-inflammatory effects are recommended, such as NSAIDs (nonsteroidal anti inflammaory drugs), local injections, or physical therapy (26).

In patients with central sensitization or reduced CPM, medications are recommended that are effective in modulating central sensitization and improving pain perception (duloxetine, gabapentin, or pregabalin). If the pain is centrally mediated, opioid analgesics might be less effective, and non-opioid therapies like antidepressants or anticonvulsants (e.g., gabapentinoids) could be better choices (26).

Before initiating treatment, a baseline QST can help identify pain thresholds, sensory deficits, and central sensitization markers. Monitoring QST after therapy is essential for assessing treatment effectiveness through evaluating pain modulation (e.g., improvement in CPM response, reduced temporal summation, increased pressure pain thresholds) (27, 28).

Some studies show that patients with less effective CPM respond better to certain medications, such as SNRIs (serotonin-noradrenaline reuptake inhibitors), which enhance descending inhibition, compared to patients with effective CPM (21, 29).

If conditioned pain modulation is impaired, interventions that focus on modulating central pain processing, such as exercises, graded motor imagery (GMI), cognitive-behavioral therapy (CBT), biofeedback, or peripheral nerve stimulation, should be included (30–34). In patients with central sensitization (hypersensitivity or heightened pain perception), it is necessary to apply treatments targeting pain modulation (e.g., mindfulness-based stress reduction, medication management, exercise therapy targeting central sensitization) (35–37).

In patients with normal pain thresholds, conservative treatments like manual therapy, stretching exercises, or strengthening programs can be effective (38). If a high pain threshold is identified in patients with CMSP, physical therapies and rehabilitation that improve mobility and motor control are highly significant, as well as techniques that enhance sensory processing (e.g., sensory discrimination training) (39).

If a patient undergoes a multidisciplinary program combining exercise therapy and CBT, QST can reveal whether the CPM response improves, suggesting that the treatment is addressing central sensitization effectively. The improvements in mechanical or thermal pain thresholds can indicate recovery or responsiveness to interventions, while worsening thresholds can suggest disease progression (17).

Conclusion

Quantitative Sensory Testing provides a comprehensive tool for understanding the pain processing mechanisms in patients with CMP. By providing insights into how a patient's nervous system processes pain, QST can play an essential role in diagnosing, monitoring, and tailoring treatment strategies. As part of a multidisciplinary approach, QST can significantly enhance the management of chronic musculoskeletal pain.

References

1. IASP. Global year against musculoskeletal pain: epidemiology of musculoskeletal pain [IASP Web site]. 2009. Available at: http://www.iasp-pain.org/AM/
2. Treede RD, Rief W, Barke A, Aziz Q, Bennett MI, Benoliel R, et al. Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the International Classification of Diseases (ICD-11). Pain 2019;160(1):19-27. [CrossRef] [PubMed]
3. Pavlakovic G, Petzke F. The role of quantitative sensory testing in the evaluation of musculoskeletal pain conditions. Curr Rheumatol Rep 2010;12(6):455–61. [CrossRef] [PubMed]
4. Lim EC, Sterling M, Stone A, Vicenzino B. Central hyperexcitability as measured with nociceptive flexor reflex threshold in chronic musculoskeletal pain: A systematic review. Pain 2011;152(8):1811–20. [CrossRef] [PubMed]
5. Nijs J, George SZ, Clauw DJ, Fernández-de-Las-Peñas C, Kosek E, Ickmans K, et al. Central sensitisation in chronic pain conditions: latest discoveries and their potential for precision medicine. *The Lancet Rheumatology* 2021; 3.5: e383-e392. [CrossRef] [PubMed]
6. Trouvin, Anne-Priscille, Nadine Attal, and Serge Perrot. "Assessing central sensitization with quantitative sensory testing in inflammatory rheumatic diseases: a systematic review." *Joint Bone Spine* 2022; 89(5): 105399. [CrossRef] [PubMed]
7. Clark J, Nijs J, Yeowell G, Goodwin PC. What Are the Predictors of Altered Central Pain Modulation in Chronic Musculoskeletal Pain Populations? A Systematic Review. Pain Physician 2017;20(6):487-500. [CrossRef] [PubMed]
8. Jensen OK. On the Use of Quantitative Sensory Testing to Estimate Central Sensitization in Humans. Comment on Schuttert et al. The Definition, Assessment, and Prevalence of (Human Assumed) Central Sensitisation in Patients with Chronic Low Back Pain: A Systematic Review. J Clin Med 2022;11(7):1982. [CrossRef] [PubMed]
9. Granovsky Y, Yarnitsky D. Personalized pain medicine: the clinical value of psychophysical assessment of pain modulation profile. Rambam Maimonides Med J 2013;4(4):e0024. [CrossRef] [PubMed]
10. Neelapala YVR, Bhagat M, Frey-Law L. Conditioned Pain Modulation in Chronic Low Back Pain: A Systematic Review of Literature. Clin J Pain 2020;36(2):135-41. [CrossRef] [PubMed]
11. Kılıçaslan HÖ, Genç A, Tuncer S. Central sensitization in osteoarthritic knee pain: A cross-sectional study. Turk J Phys Med Rehabil 2022;69(1):89-96. [CrossRef] [PubMed]
12. Aleksandar Knezevic, Milena Kovacevic, Milica Jeremic-Knezevic, Zeljka Nikolasevic, Snezana Tomasevic-Todorovic, Zeljko Zivanovic, et al. Patients with neuropathic pain from lumbosacral radiculopathy demonstrate similar pressure pain thresholds and conditioned pain modulation to those with fibromyalgia. Neurophysiologie Clinique 202353;53(4): 102841 [CrossRef] [PubMed]
13. Tomašević-Todorović S, Spasojević T. Central Sensitization in Patients with Chronic Musculoskeletal Pain. Acta clinica Croatica 2023;62(Suppl4):102-6. [CrossRef] [PubMed]
14. Neblett R. The central sensitization inventory: A user's manual. *J Appl Biobehav Res* 2018; 23:e12123. [CrossRef]
15. Arendt-Nielsen L, Yarnitsky D. Experimental and clinical applications of quantitative sensory testing applied to skin, muscles and viscera. J Pain 2009; 10:556–72. [CrossRef] [PubMed]
16. Chong PST, Cros DP. Technology literature review: Quantitative sensory testing. Muscle Nerve 2004;29:734–47. [CrossRef] [PubMed]
17. Rolke R, Baron R, Maier C, Tölle TR, Treede -DR, Beyer A, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. Pain 2006;123(3):231-43. [CrossRef]
18. Cruz-Almeida Y, Fillingim RB. Can quantitative sensory testing move us closer to mechanism-based pain management? Pain Med 2014;15(1):61-72. [CrossRef] [PubMed]
19. Kennedy DL, Kemp HI, Ridout D, Yarnitsky D, Rice AS. Reliability of conditioned pain modulation: a systematic review. Pain 2016;157(11):2410-9. [CrossRef] [PubMed]
20. Imai Y, Petersen KK, Morch CD, Arendt Nielsen L. Comparing test-retest reliability and magnitude of conditioned pain modulation using different combinations of test and conditioning stimuli. Somatosens Mot Res 2016;33(3-4):169-77. [CrossRef] [PubMed]
21. Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y. Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. Pain 2012;153(6):1193-8. [CrossRef] [PubMed]
22. Kosek E, Ordeberg G. Lack of pressure pain modulation by heterotopic noxious conditioning stimulation in patients with painful osteoarthritis before, but not following, surgical pain relief. Pain 2000;88(1):69-78. [CrossRef] [PubMed]
23. Middlebrook N, Heneghan NR, Evans DW, Rushton A, Falla D. Reliability of temporal summation, thermal and pressure pain thresholds in a healthy cohort and musculoskeletal trauma population. PLoS One 2020;15(5):e0233521. [CrossRef] [PubMed]
24. Castelo-Branco L, Cardenas-Rojas A, Rebello-Sanchez I, Pacheco-Barrios K, de Melo PS, Gonzalez-Mego P, et al. Temporal Summation in Fibromyalgia Patients: Comparing Phasic and Tonic Paradigms. Front Pain Res (Lausanne) 2022;3:881543. [CrossRef] [PubMed]
25. Petersen KK, Kilic K, Hertel E, Sejersgaard-Jacobsen TH, Jørgensen MK, Troelsen A, et al. Quantitative sensory testing as an assessment tool to predict the response to standard pain treatment in knee osteoarthritis: a systematic

review and meta-analysis. Pain Rep 2023;8(4): e1079. [CrossRef] [PubMed]
26. Queremel Milani DA, Davis DD. Pain Management Medications. 2023 Jul 3. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. [PubMed]
27. Santiago V, Janal MN, Cook DB, Raphael KG. Temporal Summation and Aftersensations of Second Pain in Women with Myofascial Temporomandibular Disorder Differ by Presence of Temporomandibular Joint Pain. J Pain Res. 2022 Oct 19;15:3275-3286. [CrossRef] [PubMed]
28. Leone C, Truini A. The CPM Effect: Functional Assessment of the Diffuse Noxious Inhibitory Control in Humans. J Clin Neurophysiol 2019;36(6):430-6. [CrossRef] [PubMed]
29. Damien J, Colloca L, Bellei-Rodriguez CÉ, Marchand S. Pain Modulation: From Conditioned Pain Modulation to Placebo and Nocebo Effects in Experimental and Clinical Pain. Int Rev Neurobiol 2018;139:255-96. [CrossRef] [PubMed]
30. Alsouhibani A, Hoeger Bement M. Impaired conditioned pain modulation was restored after a single exercise session in individuals with and without fibromyalgia. Pain Rep 2022;7(3): e996. [CrossRef] [PubMed]
31. Riquelme-Aguado V, Di-Bonaventura S, González-Álvarez ME, Zabarte-Del Campo A, Fernández-Carnero J, Gil-Crujera A, et al. How Does Conditioned Pain Modulation Influence Motor Imagery Processes in Women with Fibromyalgia Syndrome? A Cross-Sectional Study Secondary Analysis. J Clin Med 2024;13(23):7339. [CrossRef] [PubMed]
32. Lim JA, Choi SH, Lee WJ, Jang JH, Moon JY, Kim YC, et al. Cognitive-behavioral therapy for patients with chronic pain: Implications of gender differences in empathy. Medicine (Baltimore) 2018;97(23): e10867. [CrossRef] [PubMed]
33. Sielski R, Rief W, Glombiewski JA. Efficacy of Biofeedback in Chronic back Pain: a Meta-Analysis. Int J Behav Med 2017: 24(1): 25–41. [CrossRef] [PubMed]
34. Luna D, Hettie G, Pirrotta L, Salmasi V, Hah JM. Real-world long-term outcomes of peripheral nerve stimulation: a prospective observational study. Pain Manag 2025;15(1):37-44. [CrossRef] [PubMed]
35. Zeidan F, Vago DR. Mindfulness meditation-based pain relief: a mechanistic account. Ann N Y Acad Sci 2016;1373(1):114-27. [CrossRef] [PubMed]
36. Tao ZY, Wang PX, Wei SQ, Traub RJ, Li JF, Cao DY. The Role of Descending Pain Modulation in Chronic Primary Pain: Potential Application of Drugs Targeting Serotonergic System. Neural Plast 2019; 2019:1389296. [CrossRef] [PubMed]
37. Verbrugghe J, Agten A, Stevens S, Vandenabeele F, Roussel N, Verbunt J, et al. High intensity training improves symptoms of central sensitization at six-month follow-up in persons with chronic nonspecific low back pain: Secondary analysis of a randomized controlled trial. Braz J Phys Ther 2023;27(2):100496. [CrossRef] [PubMed]
38. Domingues L, Pimentel-Santos FM, Cruz EB, Sousa AC, Santos A, Cordovil A, et al. Is a combined programme of manual therapy and exercise more effective than usual care in patients with non-specific chronic neck pain? A randomized controlled trial. Clin Rehabil 2019;33(12):1908-18. [CrossRef] [PubMed]
39. Kälin S, Rausch-Osthoff AK, Bauer CM. What is the effect of sensory discrimination training on chronic low back pain? A systematic review. BMC Musculoskelet Disord 2016;17:143. [CrossRef] [PubMed]

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ZNAČAJ PRIMENE KVANTITATIVNOG SENZORNOG TESTIRANJA KOD PACIJENATA SA HRONIČNIM MUSKULOSKELETNIM BOLOM

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Kvantitativno senzorno testiranje (engl. *quantitatory sensory testing* – QST) pruža objektivne, ponovljive podatke koji mogu pomoći u potvrđivanju dijagnoze ili u praćenju ishoda lečenja kod pacijenata sa hroničnim muskuloskeletnim bolom (engl. *chronic musculoskeletal pain* – CMSP). QST može otkriti rane senzorne promene pre nego što one postanu klinički očigledne. Budući da QST procedure nisu invazivne, pogodne su za ponovljene procene i dugotrajno praćenje. Standardizacija protokola testiranja od ključnog je značaja za dobijanje pouzdanih rezultata. Oslabljena modulacija bola uslovljena kondicioniranjem (engl. *conditioned pain modulation* – CPM) mogla bi poslužiti kao biomarker za centralnu senzitizaciju u hroničnim bolnim stanjima i pomoći kliničarima da identifikuju pacijente čiji je bol centralno posredovan. QST ima potencijala da postane sastavni deo rutinskih kliničkih evaluacija u različitim hroničnim mišićno-skeletnim stanjima. QST je posebno značajan u proceni efekta fizikalnih terapija, programa vežbi i psihološke terapije.

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***Ključne reči**: centralna senzitizacija, hronični bol, muskuloskeletnim bol*

Case report

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DIGITAL WORKFLOW FOR THE CORRECTION OF A GUMMY SMILE

Ana Jevremović, Danimir Jevremović

Excessive gingival display during smiling, known as a "gummy smile," can negatively affect a patient's aesthetic appearance and self-confidence. The digital workflow for correcting this condition involves the use of technologies such as intraoral scanning, Cone Beam Computed Tomography (CBCT) diagnostics, and Computer-Aided Design (CAD) and Computer-Aided Manufacturing (CAM) systems for the fabrication of surgical guides. This paper presents a clinical case in which a digital protocol was used to plan and execute gingivoplasty with the aid of a surgical guide. The results demonstrated high precision, minimal invasiveness, and predictable aesthetic correction. The application of digital technologies enhances communication with patients, reduces treatment time, and increases procedural safety, making it an effective approach in aesthetic dentistry.

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Key words: *digital planning, computer-aided design/computer-aided manufacturing, gummy smile, gingivoplasty, surgical guide, aesthetics*

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Introduction

A perfect smile is not solely the result of harmony between white and pink aesthetics but rather a balance between teeth, gingiva, and lips. Excessive gingival display, commonly referred to as a "gummy smile" (GS), is an aesthetic concern that may also have psychosocial implications for the patient. The overall prevalence of GS in the general population is estimated at approximately 10–15%, with a higher incidence reported in females (1). The etiology of GS is multifactorial and includes various anatomical and functional causes such as vertical maxillary excess, anterior dentoalveolar extrusion, altered passive eruption, hyperactive upper lip, short upper lip, and excessive compensatory tooth eruption (2). In many cases, a combination of these etiological mechanisms is present, complicating both diagnosis and treatment planning (3).

The correction of GS can be achieved through a variety of therapeutic modalities depending on the underlying etiology and the degree of severity. Available options include orthodontic treatment, periodontal surgery (gingivectomy and gingivoplasty), clinical crown lengthening, lip repositioning surgery, botulinum toxin application, orthognathic surgery, digitally guided periodontal surgery, and piezosurgery (1–4).

Contemporary approaches integrate digital planning aided by Cone Beam Computed Tomography (CBCT), intraoral and extraoral scanning, as well as the use of piezosurgical instruments for precise and minimally invasive intervention. These techniques facilitate the development of a personalized treatment plan with high aesthetic predictability (4).

Given the increasing demand for aesthetic and minimally invasive procedures, the concept of digital workflow enables improved communication with both the patient and the dental technician. For the clinician, it serves as a diagnostic tool to select the most appropriate therapeutic approach and to plan treatments with greater precision and predictability (5–7). Digital planning relies on diagnostic tools such as digital photography, intraoral and facial scanning, and CBCT imaging, which allow for the assessment of ideal relationships between hard and soft tissues in the context of surgical planning for GS correction using surgical guides (8, 9).

Clinical crown lengthening is a periodontal surgical procedure designed to expose a greater portion of the tooth surface, either for aesthetic or

functional purposes. This intervention involves the removal of excess gingival tissue, and if necessary, alveolar bone, in order to reveal more of the clinical crown (10).

The goals of crown lengthening include achieving optimal biological width, preserving periodontal health, and ensuring both the functional and aesthetic stability of future prosthetic restorations (11). The procedure may be performed using traditional surgical methods, lasers, or piezoelectric devices, and often depends on the visual assessment and manual precision of the clinician, factors that can lead to unpredictable results and prolonged recovery (12).

With the application of modern digital technologies, including CBCT imaging, CAD/CAM planning, and the fabrication of surgical guides, greater accuracy and predictability in clinical outcomes can be achieved (13, 14).

Aim

The aim of this article is to present a clinical case of guided periodontal surgery for the correction of excessive gingival display using a digitally planned intervention.

Case report

A 24-year-old female patient presented to the dental clinic dissatisfied with the aesthetics of her smile due to excessive gingival display. Clinical examination revealed short maxillary anterior teeth with an excess of gingival tissue (Figure 1). Periodontal examination revealed probing depths of 3–5 mm with adequate periodontal health (no plaque, bleeding, or periodontal pockets). Extraoral and intraoral photographs were taken, along with intraoral scans. Cone-beam computed tomography was requested, and the patient signed an informed consent form.

Digital treatment planning began with the analysis of digital photographs and intraoral scans using the 3Shape system (Copenhagen, Denmark). A proposal for the future appearance of the teeth was created within the software (virtual diagnostic wax-up). The design accounted for the visibility of the anterior teeth, as well as the ideal height-to-width ratio of the central incisors (approximately 80%). The virtual design (Figure 2) was then translated into a physical model using 3D printing technology (Figure 3), followed by the fabrication of a diagnostic mock-up using silicone impression material (Virtual, Ivoclar Vivadent, Liechtenstein) and dual-cure composite resin (Protemp 4, 3M, Germany) (Figure 4).

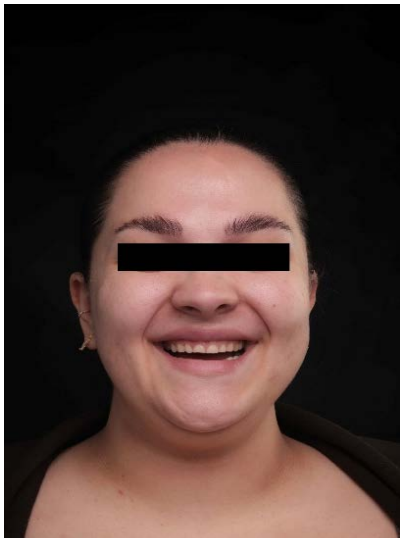

Figure 1a. Initial smile (extraoral)

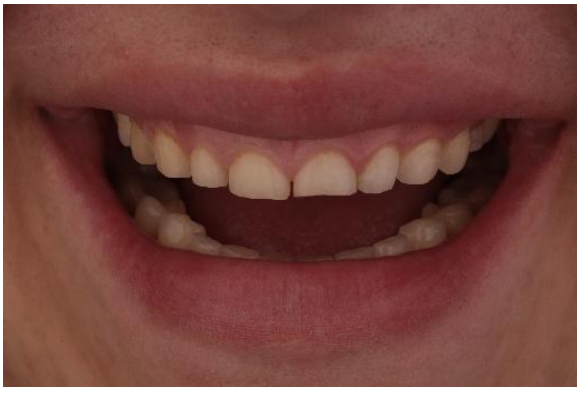

Figure 1b. Initial smile (intraoral)

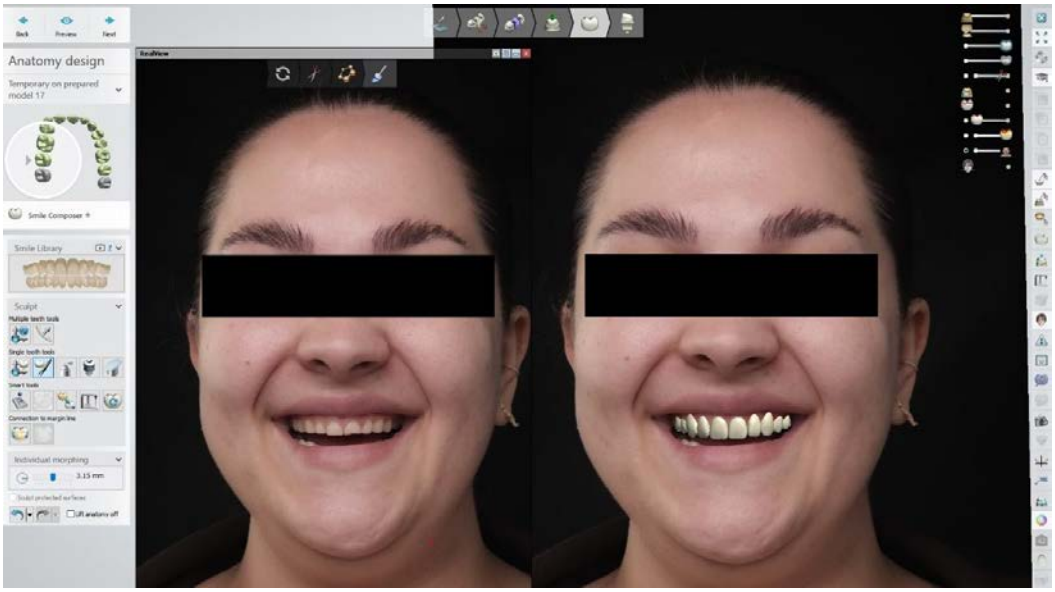

Figure 3. 3D printed model of the proposed final restorations

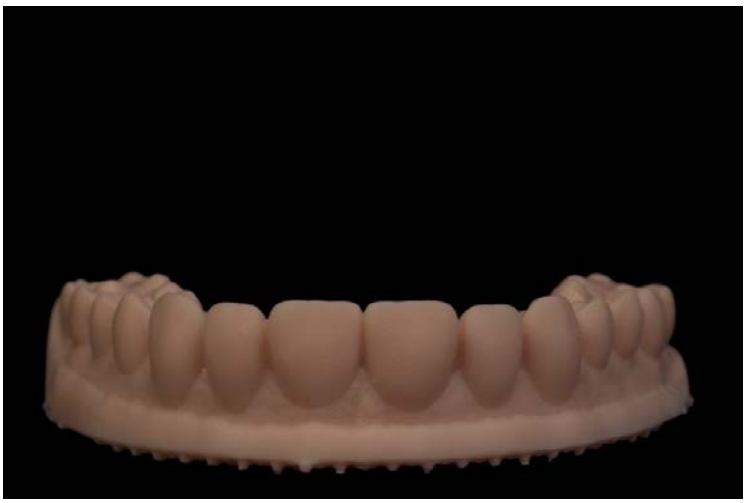

Figure 4a. Mockup (extraoral)

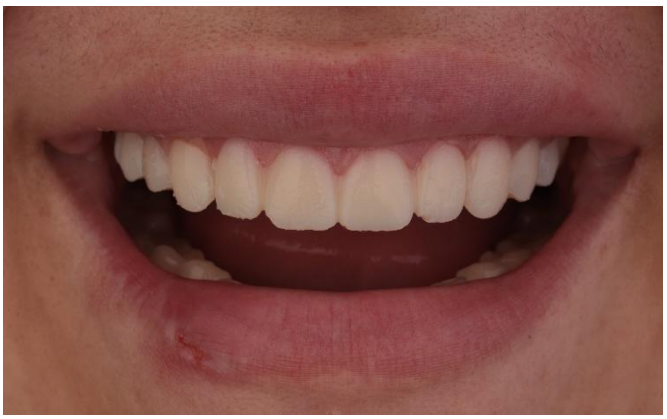

Figure 4a. Mockup (intraoral)

The diagnostic mock-up provides the patient with a preliminary visual preview of the future restoration.

Additional diagnostics were performed using cone-beam computed tomography (CBCT) to assess all dental surfaces as well as the soft and hard tissues. The CBCT scans were imported into the Planmeca Romexis software (Helsinki, Finland), where the STL model was aligned. Lines defining the smile's zenith curve were then drawn to determine the amount of soft and bone tissue to be removed (Figure 5). The surgical guide was subsequently designed and fabricated using 3D printing (Sprint Ray Inc., USA) in 1 minute thick surgical guide resin (Figure 6).

48 hours before surgical intervention, the patient was instructed to rinse the mouth with 0.12% chlor-hexidine-dicluconate for 1min three times a day and additionally 5 minutes before intervention. Concurrently, the printed surgical guide was submerged in 2% chlorhexidine-digluconate solution.

Subsequently to setting up the surgical field with sterile surgical drapes, local anesthesia - articaine 40 mg/ml; epinephrine 10 µg/ml-was administered by local infiltration at the bottom of the maxillary vestibule and the bases of interdental papillas.

Using a millimeter probe (Carl Martin GmbH, Germany), measuring of the supracrestal tissue attachment vertical dimension, from the free gingival rim to the depth of the bone crest, was done and compared with the delimitation of the surgical guide positioned in the mouth (Figure 7). Afterwards, the internal bevel incision was done using a 15c surgical blade (Swann-Norton, England) (Figure 8 and 9).

After the stent was removed, an intrasulcular incision was done one distal tooth beyond the surgical field perimeter bilaterally, and the full-thickness flap was raised apically beyond the mucogingival junction.

To preserve the biological width, the bone crest was judiciously trimmed by the same amount as the gingivectomy using a fine-grain, flat-top, tapered 014 diamond bur (Komet Dental, Germany) in a slow-speed handpiece with copious saline irrigation. The newly positioned osseous rim was rounded using ball ball-shaped fine-grain 023 diamond bur (Komet Dental, Germany) and the same bur was used to give juxtaradicular bone a physiological contour. Exposed radicular surfaces were polished using multifluted surgical-length football-shaped No. 14 carbide polisher (Komet Dental, Germany).

The surgical field was rinsed with copious amounts of saline and 1% povidone-iodine solution, flap repositioned and sutured with vertically mattress sutures using 5-0 polydioxanone (Ethicon, USA).

Patient released and advised for edema and pain control using coldpacks and 600 mg ibuprofen three times a day for consecutive 48 hours and instructed for 14-day TID regimen of 0,12% chlorhexidine-digluconate mouth rinses (until suture removal).

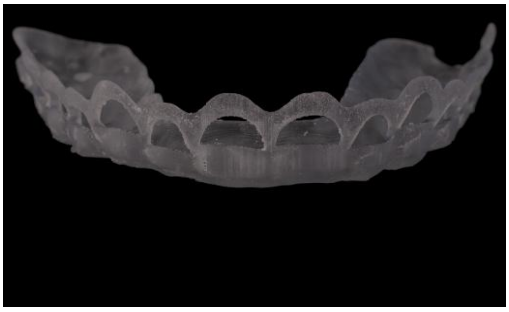

Figure 5. Design of surgical guide

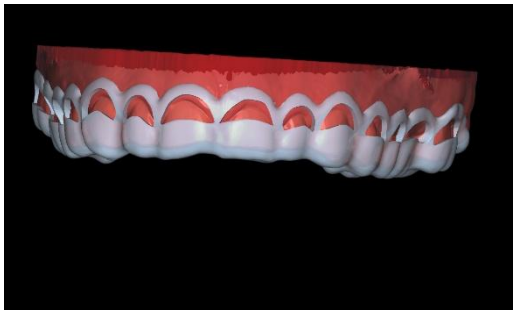

Figure 6. Printet surgical guide

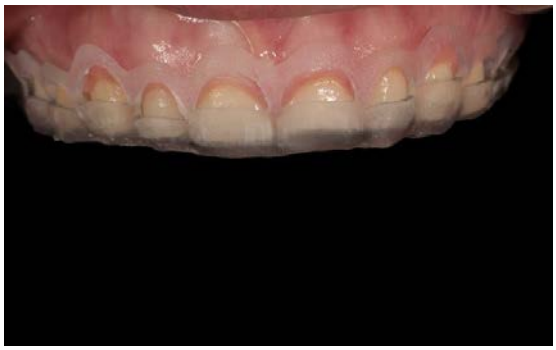

Figure 7. Surgical guide in the mouth

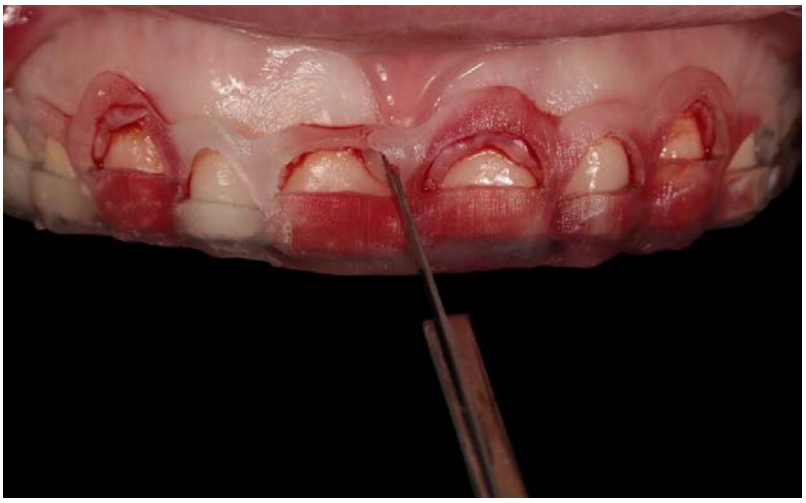

Figure 8. Gingival collar removal

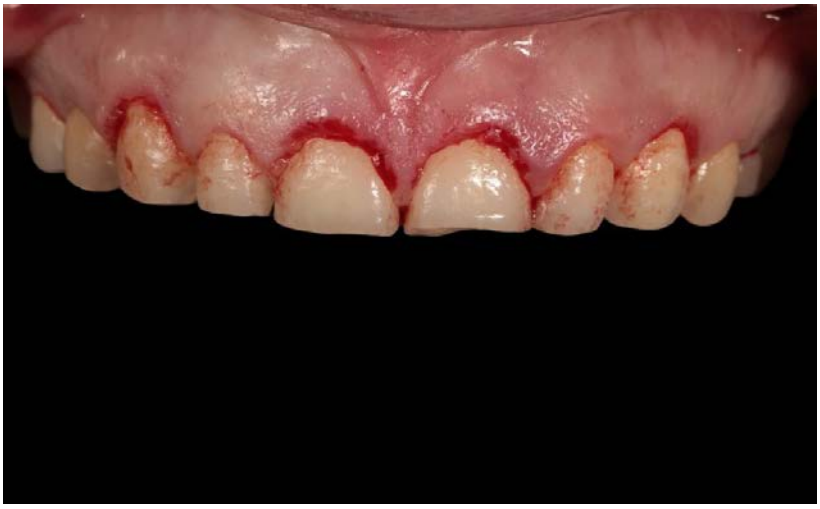

Figure 9. Gingival collar removed

Discussion

A “gummy smile” presents both aesthetic and functional challenges. Accurate diagnosis of the underlying etiology is essential to formulate an appropriate treatment plan, whether the cause is hyperactive upper lip musculature, short clinical crowns, altered passive eruption, or gingival hypertrophy (15, 16). In the present case, a digital workflow was employed for the planning and execution of conventional scalpel-based gingivoplasty using a CAD/CAM-generated surgical guide. This approach underscores the strong interdependence between periodontology and aesthetic dentistry, particularly in patients with excessive gingival display.

Digital planning enables precise determination of the resection line and evaluation of the supracrestal tissue attachment using CBCT analysis, thus preserving the biological width and minimizing the risk of postoperative complications (17, 18). A pre-approved reverse planning protocol, combined with a diagnostic mock-up, provided the patient with a clear visualization of the expected outcome, enhancing confidence in the treatment (19).

Although piezoelectric devices offer selective and safe access to hard tissues, a traditional scalpel technique was utilized in this case. When performed by an experienced clinician and combined with contemporary digital planning, conventional surgery continues to offer a high degree of control and predictable aesthetic outcomes (20).

The use of CAD/CAM technology allows for the fabrication of customized surgical guides that define the exact amount of gingival tissue to be removed, thereby improving both the predictability and safety of the procedure (21, 22). Digital guides significantly contribute to surgical efficiency by reducing operative time, minimizing postoperative discomfort, enhancing tissue healing

(23), and facilitating improved communication among the dentist, surgeon, and dental laboratory (24).

Clinical crown lengthening remains an effective method for correcting a gummy smile, reducing gingival exposure relative to the upper lip while preserving natural interproximal relationships (25, 26). Among various therapeutic options, periodontal plastic surgery, particularly gingivoplasty, remains the gold standard in addressing this aesthetic concern (19, 27).

Modern periodontology operates within a digital environment. Technological tools such as CBCT imaging, intraoral and facial scanning, stereolithography, and surgical guides substantially improve the quality of treatment planning and procedural predictability (18). While clinical probing has traditionally been used to locate the cementoenamel junction, contemporary protocols incorporate the merging of CBCT scans with intraoral scans and the use of retractors, resulting in a highly precise three-dimensional visualization with minimal invasiveness (28).

In conclusion, this case demonstrates that conventional surgery, when supported by digital technologies, can yield excellent aesthetic outcomes with high precision and patient satisfaction (29).

Conclusion

The integration of digital planning with conventional surgical techniques facilitates the achievement of high precision and favorable aesthetic outcomes in “gummy smile” correction, while enhancing procedural efficiency, accuracy, and safety. This approach underscores that digital technology serves as a valuable adjunct to, rather than a replacement for, traditional methodologies, augmenting clinical control and increasing patient confidence. The synergy between advanced technological tools and clinical expertise is essential for optimizing treatment success in contemporary periodontal and aesthetic dentistry.

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References

1. Bhola M, Thomas BS, Bhandari S. Management of gummy smile with botulinum toxin type-A: A case report. J Indian Soc Periodontol 2015;19(4):446–9.
2. Rosenblatt A, Simon Z. Lip repositioning for reduction of excessive gingival display: A clinical report. Int J Periodontics Restorative Dent 2006;26(5):433–7. [PubMed]
3. Peck S, Peck L, Kataja M. The gingival smile line: Clinical characteristics and control. J Esthet Restor Dent 1992;4(1):3–10.
4. Moraes SL, Pellizzer EP, de Moraes M, Neto RT, Santiago-Júnior JF, Bonachela WC. Digital planning and guided periodontal surgery for the correction of gummy smile: A clinical case report. Clin Adv Periodontics 2022;12(1):23–30.
5. Cervino G, Fiorillo L, Arzukanyan AV, Spagnuolo G, Cicciu M. Dental restorative digital workflow: digital smile design from aesthetic to function. Dent J (Basel) 2019;7(2):30. [CrossRef][PubMed]
6. Ting-Shu S, Jian S. Intraoral digital impression technique: a review. J Prosthodont 2015;24:313–21. [CrossRef][PubMed]
7. Santos FR, Kamarowski SF, Lopez CAV, Storrer CLM, Neto AT, Deliberador TM. The use of the digital smile design concept as an auxiliary tool in periodontal plastic surgery. Dent Res J (Isfahan) 2017;14:158–61. [CrossRef][PubMed]
8. Zanardi PR, Zanardi LR, Stegun RC, Sesma N, Costa BN, Cruz Laganá D. The use of the digital smile design concept as an auxiliary tool in aesthetic rehabilitation: A case report. Open Dent J 2016;10:28–34. [CrossRef][PubMed]
9. Coachman C, Paravina RD. Digitally enhanced esthetic dentistry—from treatment planning to quality control. J Esthet Restor Dent 2016;28:3–4. [CrossRef][PubMed]
10. Lee EA, Evian CI. Clinical considerations for the restorative dentist in the management of the periodontally involved dentition. Compend Contin Educ Dent 2010;31(4):258–64.
11. Speer A, Honegger D, Stavropoulos A. Biologic width and its relevance in periodontology and restorative dentistry. Periodontol 2000 2020;84(1):125–38.
12. Lee SH, Kim JW, Choi BH. Conventional gingivectomy versus digitally guided surgery: Comparison of outcomes. Int J Periodontics Restorative Dent 2017;37(3):385–91.
13. Mezzadri J, Silva K, Moraes GF, Manfron APT. Guided periodontal surgery: Digital workflow for correction of a gingival smile. J Surg Surg Res 2021;7(2):131–8. [CrossRef]
14. Ribeiro FV, da Silva CO, Sallum EA. Digital technologies in esthetic periodontal plastic surgery: A review and case series. J Esthet Restor Dent 2022;34(5):825–34.
15. Chu SJ, Karabin S, Mistry S. Short tooth syndrome: diagnosis, etiology, and treatment management. J Calif Dent Assoc 2009;37(3):143–52. [CrossRef][PubMed]
16. Garber DA, Salama MA. The aesthetic smile: diagnosis and treatment. Periodontol 2000 1996;11(1):18–28. [CrossRef][PubMed]
17. Ribeiro FV, Duarte PM, de Mendonca AC, Santos VR, Bastos MF, Figueiredo LC. Esthetic crown lengthening with or without flap elevation: A randomized clinical study. J Periodontol 2020;91(3):341–9.
18. Coachman C, Calamita MA, Sesma N, Coachman FG. Digital smile design: A tool for treatment planning and communication in esthetic dentistry. Quintessence Int 2017;48(1):9–20.
19. Evangelista IM, Candido LR, Araújo JJS, Scriboni AB. Major considerations of gingivoplasty: a concise systematic review. MedNEXT J Med Health Sci 2024;5(S4). [CrossRef]
20. Kassab MM, Cohen RE. The etiology and prevalence of gingival recession. J Am Dent Assoc 2003;134(2):220–5. [CrossRef][PubMed]
21. Wang X, Zhang Y, Li W. CAD/CAM surgical guides in aesthetic periodontal therapy: clinical applications and outcomes. J Clin Periodontol 2020;47(4):509–17. [CrossRef][PubMed]
22. Kim H, Lee JH, Cho YS. CAD/CAM surgical guides in periodontal plastic surgery: A new approach. Int J Periodontics Restorative Dent 2019;39(1):e13–8.
23. Martinez F, Petrović M. Advances in digital workflows for periodontal surgery: Clinical applications and benefits. Periodontol Today 2021;45(4):22–9.
24. Thompson JM, Suarez MA, Ferraz R. The influence of digital surgical guides in esthetic periodontal surgery. J Esthet Restor Dent 2018;30(5):410–6.
25. Ramesh A, Vellayappan R, Ravi S, Gurumoorthy K. Esthetic lip repositioning: a cosmetic approach for correction of gummy smile—a case series. J Indian Soc

Periodontol 2019;23(3):290–4. [CrossRef][PubMed]
26. Diaspro A, Cavallini M, Piersini P, Sito G. Gummy smile treatment: proposal for a novel corrective technique and a review of the literature. Aesthet Surg J 2018;38:1330–8. [CrossRef][PubMed]
27. Silva KC, Mezzadri J, Manfron APT. Digital planning and diode laser in guided surgery for gingival smile correction: A case report. J Res Dent 2021;9(2):35–40.
28. Patel N, Kaner D, Ghoneima A. Three-dimensional evaluation of the cemento-enamel junction using cone beam computed tomography: implications for periodontal diagnosis. Clin Oral Investig 2014;18(4):1067–72.
29. Jivraj S, Chee W, Corrado P. Digital dentistry: A review of the literature. Br Dent J 2021;230(9):561–8.

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DIGITALNI PROTOKOL U KOREKCIJI PREVIŠE IZRAŽENIH DESNI

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Previše izražene desni prilikom osmehivanja, pojava poznata kao *gummy smile*, može negativno uticati na estetski izgled i samopouzdanje pacijenata. Digitalni tok rada u korekciji ovog stanja uključuje primenu tehnologija poput intraoralnog skeniranja, CBCT (engl. *cone beam computed tomography* – CBCT) dijagnostike i CAD/CAM (engl. *Computer-Aided Design/Computer-Aided Manufacturing* – CAD/CAM) sistema za izradu hirurških vodiča. U ovom radu je predstavljen klinički slučaj u kojem je korišćen digitalni protokol za planiranje i izvođenje gingivoplastike uz pomoć hirurškog vodiča. Dobijeni rezultati su pokazali visok stepen preciznosti, minimalnu invazivnost i predvidivu estetsku korekciju. Primena digitalnih tehnologija poboljšava komunikaciju sa pacijentima, smanjuje vreme trajanja i povećava sigurnost u izvođenju zahvata, čineći ga efikasnim u estetskoj stomatologiji.

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***Ključne reči**: digitalno planiranje, computer-aided design / computer-aided manufacturing, previse izražene desni, gingivoplastika, hirurški vodič, estetika*

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EFFECTS OF PROLONGED EXPOSURE TO CIGARETTE SMOKE ON PHENOTYPE AND FUNCTION OF INNATE IMMUNE CELLS IN THE LUNGS

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Cigarette smoke contains various toxic compounds that can alter the phenotype and function of immune cells within the lungs. To investigate how extended exposure to cigarette smoke affects innate immune cells (including neutrophils, macrophages, dendritic cells (DCs), natural killer (NK) cells, and natural killer T (NKT) cells), we exposed BALB/c mice to cigarette smoke for 4 or 8 weeks, with control animals being exposed to air. The cigarette smoke was generated using an LM1 Borgwaldt smoking machine. Flow cytometry analysis and intracellular staining of lung-infiltrated immune cells revealed that prolonged exposure to cigarette smoke notably increased the number of inflammatory cluster of differentiation (CD14) receptor-expressing neutrophils, tumor necrosis factor-α (TNF-a)-producing NK and NKT cells, and inflammatory cluster of differentiation (CD40)-expressing macrophages. Additionally, significantly higher numbers of interleukin-12 (IL-12) and interleukin-23 (IL-23) producing DCs were observed in the lungs of mice exposed to cigarette smoke for 8 weeks compared to those exposed to air. Moreover, a greater number of TNF-α, IL-12, and IL-23-producing macrophages, along with TNF-α-producing neutrophils, NK, and NKT cells, were found in the lungs of mice exposed to cigarette smoke for 8 weeks compared to those exposed for 4 weeks. In conclusion, prolonged cigarette smoke exposure promotes an inflammatory phenotype in lung-infiltrating innate immune cells, contributing to the progression and worsening of inflammatory lung diseases.

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Key words: *cigarette smoke, lungs, macrophages, neutrophils, dendritic cells*

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Introduction

Cigarette smoke is composed of numerous harmful substances that severely impact the respiratory system and play a major role in the onset and advancement of lung-related diseases (1–5). Formaldehyde, acrolein, benzene, and heavy metals cause oxidative stress-induced damage of alveolar epithelial cells, resulting in structural changes in the lungs (1, 2). Continuous and long-term exposure to these harmful components can lead to the destruction of alveolar walls, resulting in the development of emphysema (3, 4). Additionally, alarmins released from injured alveolar epithelial cells can recruit fibroblasts and trigger excessive collagen deposition, leading to the development of pulmonary fibrosis (4). Moreover, as highly carcinogenic compounds, polycyclic aromatic hydrocarbons from cigarette smoke can cause mutations in lung parenchymal cells, paving the way for the development of lung cancer (5).

Cigarette smoke significantly affects the microbiome of the respiratory tract, leading to the development of dysbiosis, an imbalance in the microbial communities within the lungs (6, 7). Long-term exposure to the harmful chemicals of cigarette smoke creates a hostile environment for beneficial bacteria while promoting the growth of pathogenic microorganisms (6, 7). This dysbiotic state can impair the lungs' ability to defend against microbial pathogens and may induce a detrimental immune response in the lungs (6, 7). The inflammatory response triggered by the altered microbiome could create a vicious cycle, in which the inflammation promotes the persistence of harmful microbes, exacerbating lung damage and contributing to the development and progression of chronic pulmonary diseases (8).

Importantly, harmful components of cigarette smoke may disrupt the normal homeostasis of the immune system in the lungs, enabling the development of immune cell-driven inflammatory diseases (9). An altered expression

of adhesion molecules (selectins and integrins) was observed on the endothelial cells that were continuously exposed to cigarette smoke (10). Accordingly, prolonged exposure to cigarette smoke affects the migration of circulating immune cells in the lungs, impairing their capacity to elicit an appropriate anti-microbial immune response (9). Additionally, harmful components of cigarette smoke could trigger a potent, detrimental immune response in the lungs by inducing the massive release of pro-inflammatory cytokines from lung tissue-resident innate immune cells (natural killer (NK), natural killer T (NKT) cells, macrophages, and dendritic cells (DCs)), setting off a cycle of chronic inflammation (11). Continuously activated innate immune cells orchestrate detrimental inflammatory response by promoting activation of adaptive immune cells, T and B lymphocytes (11, 12). Persistent activation of immune cells within the lungs over an extended period leads to significant tissue injury and structural changes in the airways, ultimately contributing to the emergence of chronic inflammatory lung conditions such as asthma and chronic obstructive pulmonary disease (COPD) (9, 13).

In order to better understand how prolonged exposure to cigarette smoke affects lung neutrophils, macrophages, DCs, NK, and NKT cells, we herewith used a well-established murine model of lung inflammation induced by cigarette smoke (3, 11) to examine the variations in phenotype and cytokine production of these innate immune cells following one and two months of cigarette smoke exposure.

Materials and Methods

Experimental Animals

This study involved BALB/c mice aged six to eight weeks. The animals were kept under specific-pathogen-free conditions in a climate-regulated facility, featuring a 12-hour alternating light/dark cycle, at the Animal Facility of the Faculty of Medical Sciences, University of Kragujevac, Serbia. They were provided unlimited access to clean water and a standard diet formulated for laboratory rodents. All procedures involving the animals complied with ethical standards and were approved by the Animal Ethics Committee of the same institution, ensuring the welfare of the animals throughout the study.

Design of the Study

The mice were randomly assigned to three groups: a control group exposed only to ambient air, and two experimental groups subjected to cigarette smoke for either 4 weeks (Group 1) or 8 weeks (Group 2). Cigarette smoke was produced using Marlboro Red (MR) cigarettes and delivered via an LM1 Borgwaldt smoking apparatus. Exposure followed the Health Canada Intense (HCI) puffing protocol, which involves drawing nine puffs per cigarette with each puff lasting 2 seconds and delivering 55 mL of smoke in a bell-shaped flow profile (27.5 mL/s), taken every 30 seconds with blocked filter vents, down to a butt length of 36 ± 0.5 mm. Before use, MR cigarettes were conditioned for a minimum of 48 hours at 60 ± 3% relative humidity and 22 ± 1°C. Smoke was administered in an environment maintained at 60 ± 5% relative humidity and 22 ± 2°C, as per ISO 3402:1999 standards (11). The animals were euthanized after completing the exposure protocol, specifically 24 hours following their final exposure to cigarette smoke.

Isolation of Immune Cells From the Mice Lungs

Initially, the mice lungs underwent enzymatic digestion. Once removed, the lungs were placed in a petri dish, rinsed with phosphate-buffered saline (PBS), and finely minced using a scalpel or razor blade. The resulting tissue fragments were transferred into 15 mL conical tubes containing a digestion mixture composed of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 0.5 mg/mL collagenase IV and 1 mg/mL DNase. The samples were incubated at 37°C in a water bath for 45 minutes with continuous agitation at 80 rpm. After digestion, the cell suspensions were first passed through 100 µm nylon mesh filters into 50 mL tubes containing DMEM enriched with 10% fetal bovine serum (FBS), followed by centrifugation. The resulting cell pellets were then separated from the supernatant and treated with 5 mL of lysis buffer for 5 minutes to remove red blood cells. A subsequent centrifugation step was performed, after which the cells were resuspended in fresh medium and prepared for intracellular staining and flow cytometric evaluation (11).

Flow Cytometry Analysis and Intracellular Staining of Immune Cells

Immune cells extracted from the lungs of mice exposed to either ambient air or cigarette smoke were assessed for a range of surface and intracellular markers using flow cytometry (11). To reduce potential cell loss or damage resulting from tissue dissociation processes, viable cells were selectively isolated using the MACS® Dead Cell Removal Kit (Miltenyi Biotec, Bergisch Gladbach, Germany; Cat. No. 130-090-101). For this process, single-cell suspensions were incubated with 100 µL of Dead Cell Removal MicroBeads per 10 million cells at room temperature for 15 minutes. The mixture was then passed through MS columns pre-filled with MACS Binding Buffer, allowing live cells to pass through while dead cells were retained.

To prevent nonspecific interactions with Fc receptors, the isolated live cells were pre-treated with anti-mouse cluster of diferentation (CD) 16 and 32 (CD16/CD32) (BD Fc Block), using 1 µg per

million cells in 100 µL of staining buffer composed of Dulbecco's PBS (DPBS) without calcium or magnesium, 1% heat-inactivated fetal bovine serum (FBS), and 0.09% sodium azide. The incubation was carried out for 15 minutes at 4°C. After blocking, the cells were washed and stained with fluorescently labeled antibodies.

A total of 1 × 10^6 cells were incubated for 30 minutes in the dark at 4°C with antibodies targeting CD45, CD3, CD4, CD8, F4/80, CD11c, Ly6G/C, CD49, CD14, CD40, and TLR-2. These antibodies were conjugated with various fluorophores, including FITC, PE, PerCP, and APC (BD Biosciences, San Jose, CA, USA). The staining process concluded with two washing steps using staining buffer, followed by centrifugation to pellet the cells for subsequent analysis.

For intracellular marker detection, cells were activated using 50 ng/mL phorbol 12-myristate 13-acetate (PMA) and 500 ng/mL ionomycin for a duration of 5 hours. After this stimulation period, GolgiStop (BD Biosciences, San Jose, CA, USA; Cat. No. 554715) was introduced to inhibit protein transport. Cells were subsequently fixed and permeabilized using the BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit (Cat. No. 554714), with incubation at 4°C for 20 minutes. Following fixation, cells were rinsed twice with BD Perm/Wash™ buffer and centrifuged.

The permeabilized cells were then resuspended in 50 µL of BD Perm/Wash™ buffer containing pre-titrated fluorochrome-conjugated antibodies targeting inducible nitric oxide synthase (iNOS), and pyrin damen-conteining protein 3 (NLRP3), tumor necrosis factor-α (TNF-α), interleukin-12 (IL−12), and interleukin-23 (IL−23). These antibodies, specific for mouse antigens, were labeled with FITC, PE, PerCP, or APC fluorophores (BD Biosciences, San Jose, CA, USA). Staining was carried out at 4°C in the dark for 30 minutes. Afterward, cells underwent two additional washes with Perm/Wash™ buffer and were finally resuspended in staining buffer for flow cytometric analysis. Data collection was conducted using a BD FACSCalibur system, and results analyzed using Flowing software (Turku Bioscience Centre).

Statistical Analysis

All statistical evaluations were performed using SPSS software version 21.0 for Windows (SPSS, Inc., Chicago, IL, USA). To determine variations in group means, a one-way analysis of variance (ANOVA) was employed. In cases where ANOVA revealed statistically significant differences, *post hoc* comparisons between specific groups were conducted using two-tailed Student's t-tests, applying the Tukey method to adjust for multiple comparisons. Results are expressed as mean values ± standard error of the mean (SEM). A p-value less than 0.05 was regarded as indicating statistical significance, and all tests were two-tailed.

Results

Extended Cigarette Smoke Exposure Promotes Accumulation of Inflammatory Neutrophils, Natural Killer and Natural Killer T Cells in Lung Tissue

Flow cytometric evaluation of immune cells isolated from the lungs demonstrated that chronic exposure to cigarette smoke markedly elevated the levels of inflammatory neutrophils, natural killer (NK) cells, and natural killer T (NKT) cells in the lungs of treated mice (Figure 1). A substantial increase in $CD14^+CD45^+Ly6G^+$ neutrophil populations was observed in the lungs of mice exposed to smoke for 4 weeks (Figure 1A; $p < 0.01$) and even more so after 8 weeks of exposure (Figure 1A; $p < 0.001$), in comparison to the air-exposed control group. In addition, cigarette smoke inhalation for 8 weeks significantly enhanced the presence of neutrophils expressing TLR-2 ($Ly6G^+CD45^+$) in the lung tissue (Figure 1B; $p < 0.01$).

Further intracellular staining revealed that ongoing smoke exposure drove these neutrophils toward a more pro-inflammatory profile (Figure 1C-D). Mice exposed to cigarette smoke for 8 weeks exhibited a markedly higher number of inducible nitric oxide synthase (iNOS)-positive neutrophils (Figure 1C; $p < 0.001$), as well as TNF-α-secreting $CD45^+Ly6G^+$ neutrophils (Figure 1D; $p < 0.01$), compared to those exposed for only 4 weeks.

Similarly, prolonged exposure to cigarette smoke resulted in an increased presence of inflammatory TNF-α-producing CD49b+ NK cells (Figure 1E) and CD49b+CD3+ NKT cells (Figure 1F) in the lungs of mice. Significantly higher numbers of TNF-α-producing NK and NKT cells were detected in the lung tissue of mice exposed to cigarette smoke for both 4 weeks (Figure 1E-F; $p < 0.01$) and 8 weeks (Figure 1E-F; $p < 0.05$), compared to the control group exposed only to air. Notably, extended cigarette smoke exposure notably increased TNF-α production by both NK and NKT cells in the lungs, with the 8-week exposure group showing a significantly higher proportion of TNF-α-producing CD49b+ and CD49b+CD3+ cells when compared to the 4-week exposure group (Figure 1E-F; $p < 0.05$).

Flow cytometric analysis of immune cells in the lungs revealed that BALB/c mice exposed to cigarette smoke for 8 weeks had the highest levels of CD45+Ly6G/C+ neutrophils expressing CD14 (A), TLR-2 (B), iNOS (C), and TNF-α (D), as well as the highest numbers of TNF-α-producing NK (E) and NKT cells (F), compared to mice exposed to cigarette smoke for 4 weeks or air-exposed control mice. Data are shown as Mean ± SEM; n = 6 mice per group; *p < 0.05, **p < 0.01, *** p < 0.001.

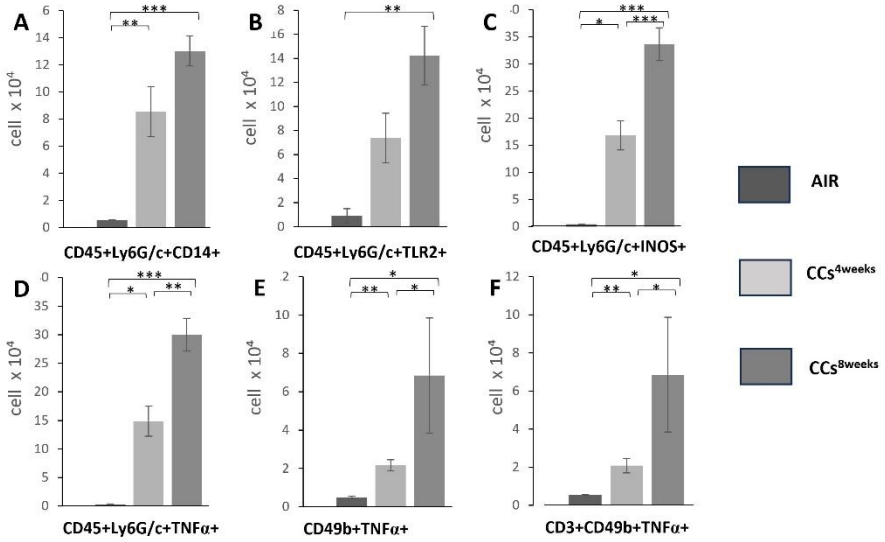

Figure 1. The impact of extended cigarette smoke exposure on the presence of neutrophils, NK, and NKT cells in the lungs of mice

Chronic Cigarette Smoke Exposure Led to the Development of an Inflammatory Profile in Lung Macrophages

Analysis of lung cell populations revealed that extended exposure to cigarette smoke significantly increased the number of inflammatory macrophages (Figure 2). The presence of NLRP3 inflammasome-activated F4/80+ macrophages was notably higher in mice exposed to smoke for 4 weeks (Figure 2A; $p < 0.05$) and 8 weeks (Figure 2A; $p < 0.001$), compared to the control group exposed to air. Additionally, after 8 weeks of smoke exposure, there was a marked increase in the number of F4/80+ macrophages expressing iNOS (Figure 2B; $p < 0.001$), CD40 (Figure 2C; $p < 0.05$), and CD14 (Figure 2D; $p < 0.05$) in the lungs of the experimental animals.

Intracellular staining further demonstrated that prolonged exposure to cigarette smoke induced the production of pro-inflammatory cytokines such as IL-12, IL-23, and TNF-α in lung macrophages (Figure 2D-F). Significantly elevated levels of IL-12 (Figure 2D; $p < 0.001$), IL-23 (Figure 2E; $p < 0.001$), and TNF-α-producing macrophages (Figure 2F; $p < 0.05$) were detected in the lungs of mice exposed to cigarette smoke for 4 weeks, compared to those exposed to air. This effect was more pronounced in animals exposed to smoke for 8 weeks, with substantially higher numbers of IL-12 (Figure 2D; $p < 0.001$), IL-23 (Figure 2E; $p < 0.001$), and TNF-α-producing F4/80+ macrophages (Figure 2F; $p < 0.05$) observed in the 8-week exposure group compared to the 4-week exposure group.

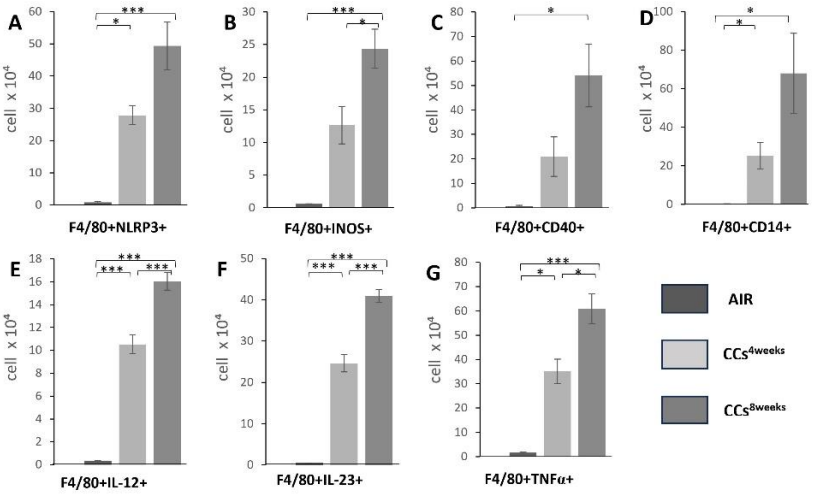

Figure 2. The lasting impact of cigarette smoke on the phenotype and functionality of lung macrophages

A notable increase in the total number of F4/80+ macrophages expressing NLRP3 inflammasome (A), iNOS (B), CD40 (C), CD14 (D), and producing IL-12 (E), IL-23 (F), and TNF-α (G) was observed in BALB/c mice exposed to cigarette smoke for 4 or 8 weeks, compared to the air-exposed control group. Data are presented as Mean ± SEM; n = 6 mice per group; *p < 0.05, **p < 0.01, *** p < 0.001.

Chronic Cigarette Smoke Exposure Increased the Number of Inflammatory DCs in the Lungs of Mice

Similar to the effects seen in macrophages, sustained exposure to cigarette smoke promoted the development of an inflammatory phenotype in dendritic cells (DCs) in the lungs (Figure 3). A significant rise in the numbers of IL-12 and IL-23 producing CD11c+ DCs (Figure 3A-B; p < 0.05) was observed in the lungs of mice exposed to smoke for 8 weeks, compared to the air-exposed control group. Additionally, the total count of NLRP3+CD11c+ dendritic alls (DCs) was significantly higher in the lungs of mice exposed to cigarette smoke for both 4 weeks and 8 weeks (Figure 3C; p < 0.001), compared to the control group.

The substantial increase in inflammatory DCs in the lungs coincided with a significant rise in the number of inflammatory T cells infiltrating the lungs (Figure 3D–E). Notably, the number of TNF-α-producing helper CD4+ T cells was significantly higher in mice exposed to cigarette smoke for 4 weeks (Figure 3D; p < 0.01) and 8 weeks (Figure 3D; p < 0.001). Similarly, a marked increase in TNF-α-producing cytotoxic CD8+ T cells was observed in the lungs of mice exposed to cigarette smoke for both 4 weeks (Figure 3E; p < 0.05) and 8 weeks (Figure 3E; p < 0.001).

Crucially, prolonged cigarette smoke exposure significantly enhanced TNF-α production in both helper and cytotoxic T lymphocytes, as evidenced by a higher number of TNF-α-producing CD4+ (Figure 3D; p < 0.05) and CD8+ T cells (Figure 3E; p < 0.01) in the lungs of mice exposed to smoke for 8 weeks, compared to those exposed for 4 weeks.

Flow cytometry analysis of immune cells infiltrating the lungs showed that the highest numbers of IL-12 (A) and IL-23 producing (B) NLRP3 inflammasome-activated (C) CD11c+DCs, as well as TNF-α-producing CD4+ T helper (D) and cytotoxic CD8+ T cells (E), were found in the lungs of BALB/c mice exposed to cigarette smoke for 8 weeks, compared to those exposed for 4 weeks or the air-exposed control group. Data are presented as Mean ± SEM; n = 6 mice per group; *p < 0.05, **p < 0.01, *** p < 0.001.

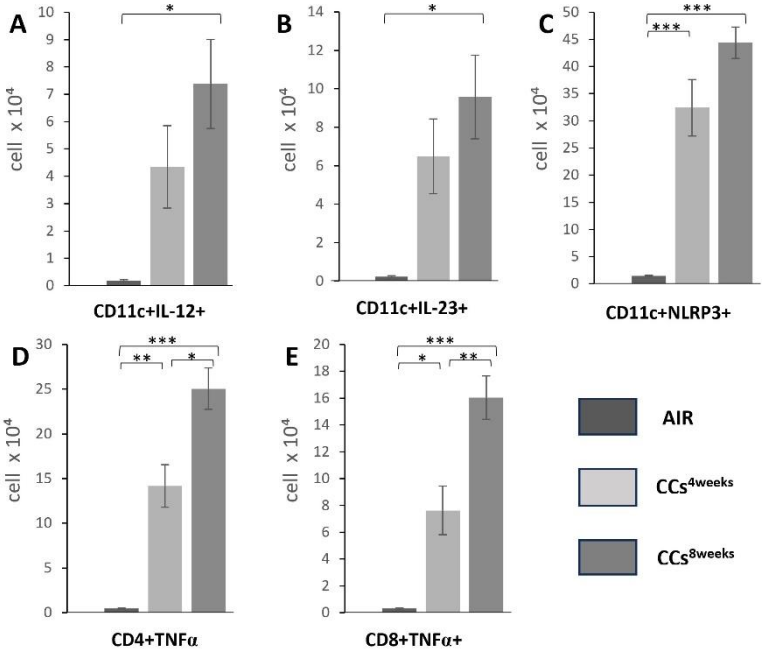

Figure 3. The effect of extended cigarette smoke exposure on the presence of DCs in the lungs

Discussion

Cigarette smoke exposure leads to an increased influx of neutrophils into the lungs, where they become activated and release various pro-inflammatory cytokines and chemokines (14, 15). These smoke-activated neutrophils generate reactive oxygen species (ROS) and proteolytic enzymes, which can damage lung tissue and worsen chronic inflammatory conditions such as COPD and asthma (14). Moreover, cigarette smoke alters neutrophil functions by suppressing apoptosis, thereby prolonging their survival in the lungs and contributing to ongoing inflammation (15). In our study, we observed that prolonged exposure to cigarette smoke enhanced the inflammatory response of neutrophils in the lungs. Specifically, long-term smoke exposure significantly increased the number of CD14 and TLR-2-expressing neutrophils, which play a crucial role in initiating and exacerbating lung inflammation. These receptors facilitate the detection of pathogen-associated molecular patterns, leading to neutrophil activation and a surge in ROS and inflammatory cytokine production, which further damages lung tissue (16). Consistent with these findings, we noted a significant increase in the number of iNOS-expressing and TNF-α-producing neutrophils in the lungs of mice exposed to cigarette smoke for 8 weeks.

Furthermore, our data showed that prolonged exposure to cigarette smoke led to an elevated presence of TNF-α-producing NK and NKT cells in the lungs. NK cells are essential for recognizing and eliminating infected or transformed cells through cytotoxic mechanisms (17), while NKT cells, which share features of both T and NK cells, contribute significantly to the development and progression of lung inflammation (17). Upon activation, these cells release large amounts of TNF-α, which induces the expression of adhesion molecules such as E and P selectins on endothelial cells, thereby promoting the recruitment of circulating leukocytes into the lungs (8, 17). The increase in TNF-α-producing NK and NKT cells due to cigarette smoke exposure may play a critical role in driving tissue damage and worsening inflammatory lung diseases. Chronic inflammation caused by these cells can lead to the destruction of alveolar structures, which is a key factor in the development of emphysema in lungs exposed to cigarette smoke over time (9).

Macrophages play a crucial role in the immune defense of the lungs, participating in phagocytosing pathogens,antigens presentation, and the modulation of inflammatory responses (18). When exposed to cigarette smoke, a variety of toxic chemicals are introduced that can alter their function and characteristics, leading to an imbalance in the macrophage-driven immune response (19). Macrophages exposed to cigarette smoke show reduced phagocytic activity, weakening their ability to eliminate inhaled microbial threats (19). This impairment is partly due to the oxidative stress caused by cigarette smoke, which interferes with the signaling pathways required for macrophage activation and phagocytosis (19, 20). In our study, we found that prolonged exposure to cigarette smoke led to a higher number of macrophages with an activated NLRP3 inflammasome and increased expression of iNOS.

Additionally, cigarette smoke affects the ability of lung macrophages to present antigens effectively (11, 19). Exposure to smoke can alter the expression of co-stimulatory molecules, which hampers their capacity to present antigens to T cells, potentially leading to ineffective T cell activation and contributing to T cell-mediated lung inflammation (8). Our results align with this, showing a significant increase in macrophages expressing the CD14 receptor and the co-stimulatory CD40 molecule in the lungs of mice exposed to cigarette smoke for 8 weeks.

Cigarette smoke significantly impacts professional antigen-presenting cells in the lungs, such as DCs and macrophages, by promoting a pro-inflammatory state marked by the elevated production of cytokines like TNF-α, IL-12, and IL-23 (8, 11, 19). These cytokines contribute to the sustained inflammatory environment in the lungs by driving Th1 and Th17 cell-mediated lung damage (21, 22). In our study, we found that continuous exposure to cigarette smoke substantially increased the number of macrophages producing TNF-α, IL-12, and IL-23, as well as DCs expressing NLRP3 and producing IL-12 and IL-23, thereby likely contributing to the development and progression of Th1 and Th17 cell-induced inflammation in the lungs. Notably, the increased presence of IL-12 and IL-23-producing DCs was associated with a higher count of TNF-α-producing CD4+ and CD8+ T cells in the lungs of mice exposed to cigarette smoke for 8 weeks. These T cells, in a TNF-α-dependent manner, trigger apoptosis in alveolar epithelial cells, resulting in alveolar damage and compromised gas exchange (23, 24). Moreover, TNF-α produced by T cells increases the expression of E and P selectins on lung endothelial cells, facilitating the recruitment of circulating leukocytes into the lungs, which further intensifies the inflammatory response and worsens lung injury (24).

Conclusion

In conclusion, extended exposure to cigarette smoke promoted the development of an inflammatory phenotype in innate immune cells within the lungs, contributing to the progression and worsening of immune cell-driven inflam-matory lung diseases.

References

1. Hikisz P, Jacenik D. The Tobacco Smoke Component, Acrolein, as a Major Culprit in Lung Diseases and Respiratory Cancers: Molecular Mechanisms of Acrolein Cytotoxic Activity. Cells 2023;12(6):879. [CrossRef][PubMed]
2. Szoka P, Lachowicz J, Cwiklińska M, Lukaszewicz A, Rybak A, Baranowska U, et al. Cigarette Smoke-Induced Oxidative Stress and Autophagy in Human Alveolar Epithelial Cell Line (A549 Cells). Adv Exp Med Biol 2019;1176:63-9. [CrossRef][PubMed]
3. Upadhyay P, Wu CW, Pham A, Zeki AA, Royer CM, Kodavanti UP, et al. Animal models and mechanisms of tobacco smoke-induced chronic obstructive pulmonary disease (COPD). J Toxicol Environ Health B Crit Rev 2023;26(5):275-305. [CrossRef][PubMed]
4. Casal A, Suárez-Antelo J, Riveiro V, Ferreiro L, Rodríguez-Núñez N, Toubes ME, et al. Smoking-related interstitial lung disease: A narrative review. Chron Respir Dis 2024;21:14799731241291538. [CrossRef][PubMed]
5. Li Y, Hecht SS. Carcinogenic components of tobacco and tobacco smoke: A 2022 update. Food Chem Toxicol 2022;165:113179. [CrossRef][PubMed]
6. Furnari S, Emma R, Caruso M, Furneri PM, Fuochi V. Evaluating the Risks of Heated Tobacco Products: Toxicological Effects on Two Selected Respiratory Bacteria and Human Lung Cells. Toxics 2025;13(2):70. [CrossRef][PubMed]
7. Fan J, Zhou Y, Meng R, Tang J, Zhu J, Aldrich MC, et al. Cross-talks between gut microbiota and tobacco smoking: a two-sample Mendelian randomization study. BMC Med 2023;21(1):163. [CrossRef][PubMed]
8. Hikichi M, Mizumura K, Maruoka S, Gon Y. Pathogenesis of chronic obstructive pulmonary disease (COPD) induced by cigarette smoke. J Thorac Dis 2019;11(Suppl 17):S2129-S2140. [CrossRef][PubMed]
9. Quan DH, Kwong AJ, Hansbro PM, Britton WJ. No smoke without fire: the impact of cigarette smoking on the immune control of tuberculosis. Eur Respir Rev 2022;31(164):210252. [CrossRef][PubMed]
10. Caruso M, Emma R, Distefano A, Rust S, Poulas K, Giordano A, et al. Comparative assessment of electronic nicotine delivery systems aerosol and cigarette smoke on endothelial cell migration: The Replica Project. Drug Test Anal 2023;15(10):1164-74. [CrossRef][PubMed]
11. Kastratovic N, Markovic V, Harrell CR, Arsenijevic A, Stojanovic MD, Djonov V, et al. Effects of Combustible Cigarettes and Electronic Nicotine Delivery Systems on the Development and Progression of Chronic Lung Inflammation in Mice. Nicotine Tob Res 2024;26(6):704-14. [CrossRef][PubMed]
12. Kastratovic N, Zdravkovic N, Cekerevac I, Sekerus V, Harrell CR, Mladenovic V, et al. Effects of Combustible Cigarettes and Heated Tobacco Products on Systemic Inflammatory Response in Patients with Chronic Inflammatory Diseases. Diseases 2024;12(7):144. [CrossRef][PubMed]
13. Kastratovic N, Cekerevac I, Sekerus V, Markovic V, Arsenijevic A, Volarevic A, et al. Effects of combustible cigarettes and heated tobacco products on immune cell-driven inflammation in chronic obstructive respiratory diseases. Toxicol Sci 2024;200(2):265-76. [CrossRef][PubMed]
14. Strzelak A, Ratajczak A, Adamiec A, Feleszko W. Tobacco Smoke Induces and Alters Immune Responses in the Lung Triggering Inflammation, Allergy, Asthma and Other Lung Diseases: A Mechanistic Review. Int J Environ Res Public Health 2018;15(5):1033. [CrossRef][PubMed]
15. McGrath JJC, Vanderstocken G, Dvorkin-Gheva A, Cass SP, Afkhami S, Fantauzzi MF, et al. Cigarette smoke augments CSF3 expression in neutrophils to compromise alveolar-capillary barrier function during influenza infection. Eur Respir J 2022;60(2):2102049. [CrossRef][PubMed]
16. Riaz B, Sohn S. Neutrophils in Inflammatory Diseases: Unraveling the Impact of Their Derived Molecules and Heterogeneity. Cells 2023;12(22):2621. [CrossRef][PubMed]
17. Bergantini L, Cameli P, d'Alessandro M, Vagaggini C, Refini RM, Landi C, et al. NK and NKT-like cells in granulomatous and fibrotic lung diseases. Clin Exp Med 2019;19(4):487-94. [CrossRef][PubMed]
18. Malainou C, Abdin SM, Lachmann N, Matt U, Herold S. Alveolar macrophages in tissue homeostasis, inflammation, and infection: evolving concepts of therapeutic targeting. J Clin Invest 2023;133(19):e170501. [CrossRef][PubMed]
19. Lugg ST, Scott A, Parekh D, Naidu B, Thickett DR. Cigarette smoke exposure and alveolar macrophages: mechanisms for lung disease. Thorax 2022;77(1):94-101. [CrossRef][PubMed]
20. Cristaldi M, Buscetta M, Cimino M, La Mensa A, Giuffrè MR, Fiore L, et al. Caspase-8 activation by cigarette smoke induces pro-inflammatory cell death of human macrophages exposed to lipopolysaccharide. Cell Death Dis 2023;14(11):773. [CrossRef][PubMed]
21. Riches DWH, Martin TR. Overview of Innate Lung Immunity and Inflammation. Methods Mol Biol 2018;1809:17-30. [CrossRef][PubMed]
22. Misra DP, Agarwal V. Th17.1 lymphocytes: emerging players in the orchestra of immune-mediated inflammatory diseases. Clin Rheumatol 2022;41(8):2297-308. [CrossRef][PubMed]
23. Williams M, Todd I, Fairclough LC. The role of CD8 + T lymphocytes in chronic obstructive pulmonary disease: a systematic review. Inflamm Res 2021;70(1):11-8. [CrossRef][PubMed]
24. Kheradmand F, Zhang Y, Corry DB. Contribution of adaptive immunity to human COPD and experimental models of emphysema. Physiol Rev 2023;103(2):1059-93. [CrossRef][PubMed]

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UTICAJ PRODUŽENOG IZLAGANJA DUVANSKOM DIMU NA FENOTIP I FUNKCIJU ĆELIJA URODJENE IMUNOSTI U PLUĆIMA

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Duvanski dim sadrži različite štetne supstance koje mogu menjati fenotip i funkciju imunskih ćelija u plućima. Da bismo bolje razumeli kako produženo izlaganje duvanskom dimu utiče na ćelije urođene imunosti (neutrofile, makrofage, dendritske ćelije, prirodne ćelije ubice (engl. *natural killer* – NK) i prirodne ćelije ubice T (engl. *natural killer T* – NKT) u plućima, obavili smo istraživanje na BALB/c miševima, koji su bili izloženi duvanskom dimu četiri ili osam nedelja. Duvanski dim je generisan pomoću *LM1 Borgwaldt* mašine. Miševi iz kontrolne grupe bili su izloženi vazduhu. Korišćenjem protočne citometrije i intracelularnim bojenjem imunskih ćelija infiltriranih u plućima uočeno je da produženo izlaganje duvanskom dimu značajno povećava prisustvo inflamacijskih neutrofila koji eksprimiraju CD14 (engl. *cluster of differentiation 14* – CD14) receptor, NK i NKT ćelija koje produkuju faktor nekroze tumora-α (engl. *tumor necrosis factor* – TNF-α), kao i makrofaga koji eksprimiraju CD40 (engl. *cluster of differentiation 40* – CD40) receptor. Takođe, značajno veći broj denditritskih ćelija koje produkuju interleukin-12 (IL-12) i interleukin-23 (IL-23) primećen je u plućima miševa koji su bili izloženi duvanskom dimu osam nedelja nego u plućima životinja koje su bile izložene vazduhu. Pored toga, značajno veći broj makrofaga koji proizvode TNF-α, IL-12, IL-23, kao i značajno veći broj neutrofila i NK i NKT ćelija koje proizvode TNF-α primećen je u plućima miševa koji su bili izloženi duvanskom dimu osam nedelja nego u plućima životinja koje su bile izložene duvanskom dimu četiri nedelje. Dakle, može se zaključiti da produženo izlaganje duvanskom dimu pospešuje stvaranje inflamacijskog fenotipa u urođenim imunskim ćelijama i da tako podstiče progresiju i pogoršanje inflamacijskih oboljenja pluća.

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***Ključne reči**: duvanski dim, pluća, makrofagi, neutrofili, dendritske ćelije*

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UNDERSTANDING THE RISK FACTORS ASSOCIATED WITH VIOLENT BEHAVIOR IN SCHIZOPHRENIA

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This review examines the risk factors associated with violent behavior in individuals with schizophrenia, highlighting public perceptions and empirical evidence in this area. While schizophrenia affects approximately 1% of the population, research indicates that 5% of homicide offenders have been diagnosed with the disorder. Public stereotypes link mental illness with aggression, yet studies show only a modest association between schizophrenia and violent behavior. Proposed risk factors include younger age, male gender, unemployment, economic disadvantage, childhood abuse, and poor anger management. Substance misuse, particularly among individuals with schizophrenia, significantly increases the risk of violence, while the duration of untreated psychosis is also correlated with a higher likelihood of violent behavior. Individuals experiencing first-episode psychosis face al elevated risks of violence prior to receiving treatment. Research indicates that symptoms suchos hallucinations and delusions correlate with aggressive behavior, with personality disorders, particularly antisocial personality disorder, identified as significant predictors. It is important to note that while individuals with schizophrenia typically demonstrate lower rates of violence, the existence of comorbid conditions or acute psychotic symptoms can markedly increase the associated risk. Early identification and appropriate treatment can help reduce these risks and improve community safety.

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Key words: *violence, schizophrenia, risk factors*

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Introduction

Aggressiveness is an inherent instinct in humans, as in other living organisms. This behavior is essential for the survival of the individual, which in turn contributes to the species' continued existence from an evolutionary perspective (1). Violence represents an extreme manifestation of aggression aimed at inflicting significant physical harm (2). While the prevalence of schizophrenia is generally estimated at around 1% of the population, a study involving 1,594 homicide offenders found that 5% had been diagnosed with schizophrenia (3).

Public perceptions of individuals with psychosis and other severe mental illnesses continue to be disproportionately damaging (4). The general population frequently associates mental illness, especially psychotic disorders, with aggression, violent behavior, and a perceived threat to safety (5). Despite negative public perceptions and exaggerated beliefs regarding violence in individuals with psychosis and other severe mental illnesses, empirical studies, meta-analyses, and literature reviews indicate a consensus that there is a modest but consistent increase in the risk of aggression and violence among individuals with psychosis (6–8). A meta-analysis of 204 studies, encompassing 166 independent datasets, revealed that psychosis was associated with a 49–68% increase in the likelihood of violent behavior (9).

Several recent systematic reviews and meta-analyses have been conducted in this area. A notable limitation of these studies is the overly broad terminology, particularly in relation to violence and diagnostic categories. Violence encompasses a wide range of behaviors, from verbal threats and aggression to homicide (10). There may be a difference in the psychological characteristics associated with milder aggression and more serious acts of hostility. Studies have shown contradictory findings since the term "violence" has not been defined precisely and

operationally, making it difficult to draw firm conclusions on the factors linked with violence.

The objective of this review was to assess the empirical evidence that supports the relationship between the risk factors associated with severe violence in individuals with schizophrenia and to identify them.

Risk Factors

Several factors were found to predict violent behavior during the follow-up period, including the severity of prior criminal activity, the frequency of previous arrests, younger age, male gender, unemployment, living in an economically disadvantaged neighborhood, and a history of childhood abuse. Other predictors included having a father with a history of substance abuse or who left the family before the child reached the age of 15, poor anger management, violent fantasies, a history of head injuries or loss of consciousness, and involuntary psychiatric hospitalization (11).

Substances Misuse

Contrary to expectations, a diagnosis of schizophrenia was associated with a reduced likelihood of post-hospital violence. In contrast, personality disorders, adjustment disorders, and substance use disorders were associated with an increased risk. Additionally, individuals with schizophrenia showed lower rates of post-hospital violence compared to those with bipolar disorder or depression. These findings have been widely interpreted (11).

Research has shown that substance misuse among people with schizophrenia is associated with the most serious forms of violence. A meta-analysis focusing on violence during the first episode of psychosis (FEP) found that substance use was associated with violence of varying intensity, with an odds ratio (OR) of 2.33. After the first psychotic episode, this increased to 2.74 (8). The OR for pre-hospital drug or alcohol abuse in psychotic patients who committed homicide after discharge was 4.3 in one study, compared to patients who did not engage in violent crime. It was discovered that 73% of psychotic individuals accused of murder reported engaging in substance misuse, even though only 35% reported being under the influence at the time of the crime (12, 13). In addition, a comprehensive analysis that used meta-regression analysis to identify risks of violence found that, when focusing on studies that measured serious violence, the OR for substance misuse was 2.2. Although some argue that substance use is the leading cause of violence in people with schizophrenia and that specific psychotic factors have an almost negligible influence, data show that patients with schizophrenia without comorbid substance abuse also have an increased risk of violent behavior (14). Furthermore, a study conducted in Japan found no association between substance misuse and violence (15). The most reliable evidence of the link between schizophrenia and violence is substance misuse. Although substance use can explain a significant portion of the variance in violence when comparing patients with violent schizophrenia those who do not, it does not account for all aspects of that behavior. When evaluating independent psychological characteristics that indicate a higher risk of violence, drug misuse is less important because it is more commonly seen as a co-occurring illness within the disease.

Duration of Illness and Treatment

Recent research shows that people who experience a first episode of psychosis are at greater risk of committing serious violent acts compared to those who experience later episodes. A review of violence associated with first-episode psychosis found that approximately one-third of patients exhibit some violent behavior before first receiving treatment, while approximately 16% engage in acts of more serious violence (8, 13). A meta-analysis that analyzed homicide rates in first-episode psychosis cases found that 38.5% of homicides occurred during this critical period (16). Regarding the duration of untreated psychosis (DUP), there is evidence to suggest that longer duration directly increases the likelihood of serious violence (13, 15, 17). Compliance with treatment also plays a significant role in this context. Research indicates that coercive treatment is strongly associated with violent behavior that occurs after initiation of therapy. Non-adherence to prescribed treatment is also linked to serious acts of violence. These findings emphasize the importance of early intervention and effective treatment strategies to reduce the risk of violence during the first episode of psychosis. Addressing the duration of untreated psychosis (DUP) is essential, as prolonged untreated psychosis may increase the likelihood of violent outcomes (8, 12).

About one-third of people with first episode of psychosis commit minor violent acts, putting them at a significantly higher risk of violence overall and especially before they get treatment. Research indicates that the highest risk for homicide occurs in first-episode patients prior to receiving their initial psychotic treatment, with 38.5% of all psychosis-related homicides taking place during this period (18). The objective of one study was to examine whether individuals with first-episode psychosis are more prone to committing violent crimes compared to the general population. They analyzed the annual crime rates recorded for a specific cohort between 2009 and 2016. They compared these with the crime rates of the general population in the same region over the same period. The analysis revealed no significant differences in the types of violent acts committed by people with FEP. However, the most common type of violence observed was intrafamily violence. This finding is consistent with prior research, which indicates that

violence is primarily directed at family members and typically occurs within the home (19). A significant portion of individuals with first-episode psychosis commit violent acts before seeking treatment, including some who engage in more severe violence, resulting in harm to others. However, instances of severe violence leading to serious or permanent injury to the victim are relatively rare in this population (8).

Abuse in Childhood

Research that focused on patients with early-onset psychosis found that individuals who were victims of childhood abuse were more likely to display violent behavior later in life. A meta-analysis examining violence in psychosis included studies on both physical and sexual childhood abuse. The results demonstrated is a moderate association between violence and reported cases of physical abuse (20). One study provided strong evidence that various forms of childhood adversity were linked, to different extents, with a higher likelihood of developing psychotic disorders in adulthood. Additionally, exposure to multiple adversities was associated with a gradual increase in the risk of psychosis. In particular, extreme hardship, including aggressiveness, threats, and physical violence, may significantly increase the risk. Also, the timing of specific events during adolescence may be particulary important (e.g., sexual abuse, bullying) (21).

Clinical characteristics

Hallucinations and Delusions

Research has shown a link between auditory hallucinations and violent behavior, with findings indicating that people who experience such hallucinations are more prone to violence. Also, evidence suggests that there is a significant association between hallucinatory behavior and an increased risk of serious violence. In a study with a sample of 88 psychotic patients who had committed murder, auditory hallucinations that resulted in persecutory delusions were recognized as the most prevalent symptom. Furthermore, another study indicated that 81% of individuals responsible for serious acts of violence reported having experienced auditory hallucinations (13, 22, 23).

Research has identified a significant relationship between serious violence and grandiosity. A study examining patients with psychotic disorders involved in severe violent incidents found that a substantial majority reported experiencing delusions during their offenses (22, 24). When comparing those engaged in serious violence with a non-violent subgroup over two years, a strong correlation emerged between severe violence and delusions that suggested threats. These included beliefs of being monitored, persecutory delusions, and notions of conspiracy. It is significant to recognize that the anger associated with these delusions appears to be crucial for understanding the relationship between psychosis and violence (25). Participants also showed a high link between excessive violence and paranoia. Paranoid ideation correlated with several forms of violence. In addition, studies investigating the association between paranoia and aggression in psychotic individuals have concluded that there is mixed support for the existence of a link between paranoia and aggression in individuals with schizophrenia. However, when the quality of the studies was considered, those of higher quality tended to show a positive correlation between the two factors (26, 27).

Personality Disorder and Impulsivity

The incidence of violence in individuals with psychosis is often lower compared to other mental health disorders, including personality and substance use disorders. Antisocial personality disorder has been recognized as a significant clinical factor associated with aggressive violence in individuals with comorbid psychosis (28, 29). Research has shown that past suicidal threats and attempts increased the likelihood of violent behavior in male schizophrenia patients by 3.8%, compared to a 2.8% increase in those without a history of suicidal behavior. In female patients, past suicidal threats and attempts raised the odds ratio for violence to 9.4%, compared to a 4.4-fold increase in the non-suicidal group. These findings suggest that both suicidal behavior and violence are linked to a broader tendency toward impulsivity, with severe impulsivity, mainly when expressed as intense aggression toward oneself, potentially serving as an indicator of the risk for violence toward others (30).

Some studies suggest that impulsivity contributes to reactive violence, crime, and antisocial behavior (31–34). Consequently, schizophrenia is consistently associated with an elevated risk of violent behavior compared with the general population and some other psychiatric disorders, across countries and definitions of violence. In schizophrenia, conceptual models of violence propose that the psychopathological symptoms of the disorder (such as hallucinations and delusions) are the cause of the violent behavior in patients. According to a recent literature analysis, violence that is precipitated by a mental illness, such as schizophrenia, is classified as medical. However, aggression that is motivated by delusions or hallucinations can also be classified as impulsive, premeditated, or compulsive (23, 35). In patients with severe violence, weak impulse control, as measured by Positive and Negative Syndrom Scale the (PANSS), was significantly increased (36). One review only identified four studies that investigated the correlation between impulsivity and violence in individuals with psychosis. The

evidence of a link between impulsivity and violence in psychotic patients is equivocal (37). In individuals diagnosed with psychosis, a strong association between weak impulse control and the risk of violence was determined by a meta-analysis (24). Overall, the evidence demonstrates that impulsivity might be an important factor in the violent and aggressive behavior of people with schizophrenia, both forensically and clinically.

Conclusion

Patients diagnosed with schizophrenia exhibit low rates of violent behavior, both in terms of minor and severe violence. However, the presence of comorbid conditions such as dual pathology, substance abuse, or positive symptoms at the time of an aggressive act—categorized as hostility, impulsivity, persecutory delusions, and plausible hallucinations—interpunkcija significantly associated with aggressive behavior. Aggressive behavior in newly admitted acute psychiatric inpatients with untreated or insufficiently treated psychosis is mainly driven by psychotic symptoms and disrupted impulse regulation. Many patients are incarcerated in forensic psychiatric institutions, jails, or prisons, where substantial obstacles exist in accessing appropriate mental health care.

References

1. Nielssen O, Large M. Homicides in psychiatric hospitals: Absence of evidence or evidence of absence? Crim Behav Ment Health 2022;32(1):60–6. [CrossRef][PubMed]
2. Allen JJ, Anderson CA. Aggression and Violence: Definitions and Distinctions. In: Sturmey P. The Wiley Handbook of Violence and Aggression. Hoboken (NJ): John Wiley & Sons, Ltd.; 2017. p. 1–14. [CrossRef]
3. Shaw J, Hunt IM, Flynn S, Meehan J, Robinson J, Bickley H, et al. Rates of mental disorder in people convicted of homicide. National clinical survey. Br J Psychiatry 2006;188:143–7. [CrossRef][PubMed]
4. Pescosolido BA, Manago B, Monahan J. Evolving Public Views On The Likelihood Of Violence From People With Mental Illness: Stigma And Its Consequences. Health Aff (Millwood) 2019;38(10):1735–43. [CrossRef][PubMed]
5. Varshney M, Mahapatra A, Krishnan V, Gupta R, Deb KS. Violence and mental illness: what is the true story? J Epidemiol Community Health 2016;70(3):223–5. [CrossRef][PubMed]
6. Edlinger M, Rauch AS, Kemmler G, Yalcin-Siedentopf N, Wolfgang Fleischhacker W, Hofer A. Risk of violence of inpatients with severe mental illness--do patients with schizophrenia pose harm to others? Psychiatry Res 2014;219(3):450–6. [CrossRef][PubMed]
7. Iozzino L, Ferrari C, Large M, Nielssen O, De Girolamo G. Prevalence and Risk Factors of Violence by Psychiatric Acute Inpatients: A Systematic Review and Meta-Analysis. PLoS One 2015;10(6):e0128536. [CrossRef][PubMed]
8. Large MM, Nielssen O. Violence in first-episode psychosis: a systematic review and meta-analysis. Schizophr Res 2011;125(2–3):209–20. [CrossRef][PubMed]
9. Douglas KS, Guy LS, Hart SD. Psychosis as a risk factor for violence to others: a meta-analysis. Psychol Bull 2009;135(5):679–706. [CrossRef][PubMed]
10. Harris AWF, Large MM, Redoblado-Hodge A, Nielssen O, Anderson J, Brennan J. Clinical and cognitive associations with aggression in the first episode of psychosis. Aust N Z J Psychiatry 2010;44(1):85–93. [CrossRef][PubMed]
11. Silverstein SM, Del Pozzo J, Roché M, Boyle D, Miskimen T. Schizophrenia and violence: realities and recommendations. Rev Crime Psychol 2020;181–202. [CrossRef]
12. Fazel S, Buxrud P, Ruchkin V, Grann M. Homicide in discharged patients with schizophrenia and other psychoses: a national case-control study. Schizophr Res 2010;123(2–3):263–9. [CrossRef][PubMed]
13. Nielssen OB, Westmore BD, Large MMB, Hayes RA. Homicide during psychotic illness in New South Wales between 1993 and 2002. Med J Aust 2007;186(6):301–4. [CrossRef][PubMed]

14. Rund BR. A review of factors associated with severe violence in schizophrenia. Nord J Psychiatry 2018;72(8):561–71. [CrossRef][PubMed]
15. Imai A, Hayashi N, Shiina A, Sakikawa N, Igarashi Y. Factors associated with violence among Japanese patients with schizophrenia prior to psychiatric emergency hospitalization: a case-controlled study. Schizophr Res 2014;160(1–3):27–32. [CrossRef][PubMed]
16. Nielssen O, Large M. Rates of homicide during the first episode of psychosis and after treatment: a systematic review and meta-analysis. Schizophr Bull 2010;36(4):702–12. [CrossRef][PubMed]
17. Humphreys MS, Johnstone EC, MacMillan JF, Taylor PJ. Dangerous behaviour preceding first admissions for schizophrenia. Br J Psychiatry 1992;161:501–5. [CrossRef][PubMed]
18. Nielssen OB, Malhi GS, McGorry PD, Large MM. Overview of violence to self and others during the first episode of psychosis. J Clin Psychiatry 2012;73(5):e580-7. [CrossRef][PubMed]
19. Esbec E, Echeburúa E. Violencia y esquizofrenia: un análisis clínico-forense. Anu Psicol Jurídica 2016;26(1):70–9. [CrossRef]
20. Spidel A, Lecomte T, Greaves C, Sahlstrom K, Yuille JC. Early psychosis and aggression: predictors and prevalence of violent behaviour amongst individuals with early onset psychosis. Int J Law Psychiatry 2010;33(3):171–6. [CrossRef][PubMed]
21. Morgan C, Gayer-Anderson C, Beards S, Hubbard K, Mondelli V, Di Forti M, et al. Threat, hostility and violence in childhood and later psychotic disorder: population-based case–control study. Br J Psychiatry 2020;217(4):575-82. [CrossRef][PubMed]
22. Yee NYL, Large MM, Kemp RI, Nielssen OB. Severe non-lethal violence during psychotic illness. Aust N Z J Psychiatry 2011;45(6):466–72. [CrossRef][PubMed]
23. Swanson JW, Swartz MS, Van Dorn RA, Elbogen EB, Wagner HR, Rosenheck RA, et al. A national study of violent behavior in persons with schizophrenia. Arch Gen Psychiatry 2006;63(5):490–9. [CrossRef][PubMed]
24. Witt K, van Dorn R, Fazel S. Risk factors for violence in psychosis: systematic review and meta-regression analysis of 110 studies. PLoS One 2013;8(2):e55942. [CrossRef][PubMed]
25. Coid JW, Ullrich S, Kallis C, Keers R, Barker D, Cowden F, et al. The relationship between delusions and violence: findings from the East London first episode psychosis study. JAMA Psychiatry 2013;70(5):465–71. [CrossRef][PubMed]
26. Ullrich S, Keers R, Coid JW. Delusions, anger, and serious violence: new findings from the MacArthur Violence Risk Assessment Study. Schizophr Bull 2014;40(5):1174–81. [CrossRef][PubMed]
27. Darrell-Berry H, Berry K, Bucci S. The relationship between paranoia and aggression in psychosis: A systematic review. Schizophr Res 2016;172(1–3):169–76. [CrossRef][PubMed]
28. Rampling J, Furtado V, Winsper C, Marwaha S, Lucca G, Livanou M, et al. Non-pharmacological interventions for reducing aggression and violence in serious mental illness: A systematic review and narrative synthesis. Eur Psychiatry 2016;34:17–28. [CrossRef][PubMed]
29. Lamsma J, Harte JM. Violence in psychosis: Conceptualizing its causal relationship with risk factors. Aggress Violent Behav 2015;24(5):75–82. [CrossRef]
30. Witt K, Hawton K, Fazel S. The relationship between suicide and violence in schizophrenia: analysis of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) dataset. Schizophr Res 2014;154(1–3):61–7. [CrossRef][PubMed]
31. Brown CS, Kent TA, Bryant SG, Gevedon RM, Campbell JL, Felthous AR, et al. Blood platelet uptake of serotonin in episodic aggression. Psychiatry Res 1989;27(1):5–12. [CrossRef][PubMed]
32. Dolan M Fullam R. Behavioural and psychometric measures of impulsivity in a personality disordered population. The Journal of Forensic Psychiatry & Psychology 2004;15(3):426–50. [CrossRef]
33. Hynan DJ, Grush JE. Effects of impulsivity, depression, provocation, and time on aggressive behavior. J Res Pers 1986;20(2):158–71. [CrossRef]
34. Krakowski M. Schizophrenia with aggressive and violent behaviors. Psychiatr Ann 2005;35(1):45–9. [CrossRef]
35. Joyal CC, Dubreucq J-L, Gendron C, Millaud F. Major Mental Disorders and Violence: A Critical Update. Curr Psychiatry Rev 2007;3(1):33–50. [CrossRef]
36. Verma S, Poon LY, Subramaniam M, Chong SA. Aggression in Asian Patients with First-Episode Psychosis. Int J Soc Psychiatry 2005;51(4):365-71. [CrossRef][PubMed]
37. Bjørkly S. A systematic review of the relationship between impulsivity and violence in persons with psychosis: Evidence or spin cycle? Aggress Violent Behav 2013;18(6):753–60. [CrossRef]

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RAZUMEVANJE FAKTORA RIZIKA POVEZANIH SA NASILNIM PONAŠANJEM U SHIZOFRENIJI

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U ovom radu se ispituju faktori rizika povezani sa nasilnim ponašanjem osoba koje boluju od shizofrenije. Shizofrenija pogađa otprilike 1% populacije, a istraživanja pokazuju da je kod 5% počinilaca ubistava dijagnostikovana upravo ova bolest. Premda stereotipi povezuju mentalne bolesti sa agresijom, studije ukazuju na to da postoji samo neznatna povezanost između shizofrenije i nasilnog ponašanja. Predloženi faktori rizika uključuju mlađi uzrast, muški pol, nezaposlenost, nepovoljnu ekonomsku situaciju, zlostavljanje u detinjstvu i lošu kontrolu impulsa. Zloupotreba supstanci, posebno među osobama koje boluju od shizofrenije, značajno povećava rizik od ispoljavanja nasilnog ponašanja. Što se duže psihoza ne leči, veća je verovatnoća da će doći do pojave nasilja. Kod osoba sa prvom psihotičnom epizodom postoji povećan rizik od ispoljavanja nasilja, naročito pre početka tretmana. Istraživanja pokazuju da su simptomi poput halucinacija i deluzija u korelaciji sa agresivnim ponašanjem, sa poremećajima ličnosti, posebno sa antisocijalnim poremećajem ličnosti, koji su identifikovani kao značajni prediktori. Važno je istaći da je kod osoba koje boluju od shizofrenije stopa ispoljavanja nasilnog ponašanja niža, ali i da postojanje komorbiditeta ili akutnih psihotičnih simptoma može značajno povećati rizik da će doći do pojave nasilja. Rana identifikacija pomenutih faktora rizika i odgovarajući tretman mogu pomoći da se osigura bezbednost i zajednice.

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***Ključne reči**: nasilje, shizofrenija, faktori rizika*

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EFFECT OF GESTATIONAL AGE, BODY WEIGHT AND SERUM CREATININE CONCENTRATION ON SERUM VANCOMYCIN CONCENTRATION IN THE TREATMENT OF NEONATAL SEPSIS: A SINGLE-CENTER PILOT STUDY

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Neonatal sepsis is a serious medical condition characterized by systemic infection in infants younger than 28 days. Therapy for late neonatal sepsis is a combination of vancomycin and meropenem. The aim of this single-center pilot study is to examine the influence of newborn body weight, gestational age, and creatinine values on the serum concentration of vancomycin in the treatment of neonatal sepsis. The study included 13 patients admitted to the Pediatrics Clinic University Clinical Center Niš, with a diagnosis of neonatal sepsis in the period from June to October 2024. The value of serum vancomycin between 10 and 15 μg/ml was recorded in 38.5% of subjects, which is preferred therapeutic range. Due to the small sample size, the effect of gestational age, body weight, and serum creatinine concentration on serum vancomycin concentration weren't found to be statistically significant. However, descriptive statistics show that a significant difference can be found between these groups, suggesting a potential effect worth further investigation.

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Key words: *neonatal sepsis, vancomycin, therapeutic drug monitoring*

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Introduction

Neonatal sepsis is a serious medical condition characterized by systemic infection in infants younger than 28 days. Various factors can influence the onset of neonatal sepsis, such as gestational age and birth weight. In relation to the time of appearance of the first symptoms, neonatal sepsis can be divided into early and late. Early neonatal sepsis occurs in the first 72 hours after birth, and the most common causative agents are bacteria that colonize the birth canal. Late neonatal sepsis occurs after 72 hours of birth, and procedures performed in the intensive care unit, such as central venous and umbilical catheters, thoracic puncture, and the use of parenteral therapy, are cited as a risk. The risk group includes infants with low birth weight, as well as infants who are on parenteral nutrition or mechanical ventilation. Due to long hospital stays and despite a number of preventive measures, late neonatal sepsis is a problem in intensive care units worldwide (1).

Infectious agents that lead to sepsis can be different. In neonatal sepsis, these are mainly bacteria, which can be divided into gram-positive and gram-negative. The most common gram-positive causative agents are *Staphylococcus aureus, Enterococcus, and Streptococcus pneumoniae*, while characteristic gram-negative causative agents are *Klebsiella pneumoniae, Enterobacter, Salmonella, E. coli*. *Staphylococcus aureus* is mostly sampled from purulent skin infections, while gram-negative bacilli such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Serratia* are mostly isolated from infants with tracheal intubation and mechanical ventilation (2, 3).

Treatment of neonatal sepsis must be started as early as possible. It is recommended to begin empiric therapy before blood culture

results. The choice of antibiotic depends on whether it is early or late neonatal sepsis.

The causative agents of early neonatal sepsis are mainly group B beta-hemolytic streptococci, as well as gram-negative bacteria, such as *E. coli*. Accordingly, antibiotic therapy is started with a combination of ampicillin and aminoglycosides (e.g., gentamicin, amikacin). A combination of aminoglycosides and third-generation cephalosporins can also be used in therapy. In case of suspicion of meningitis, it is necessary to double the doses of ampicillin or penicillin G and include a cephalosporin.

Staphylococcus aureus is the main cause of late neonatal sepsis. In case of suspicion of a hospital strain of staphylococcus, vancomycin is included in the therapy as a backup antibiotic. In addition to staphylococci, the cause of neonatal sepsis can also be *Pseudomonas aeruginosa*, in which case the treatment involves a combination of an antipseudomonal cephalosporin with an aminoglycoside or carbapenems. In anaerobic infections, metronidazole or clindamycin is included in the therapy (4–6).

After obtaining the results of the antibiogram, it is necessary to correct the therapy depending on the causative agent. The length of therapy depends on the clinical picture and microbiological findings. In addition to causal treatment, treatment of neonatal sepsis requires symptomatic therapy.

The Aim

Our research aims to examine the influence of newborn body weight, gestational age, and creatinine values on the serum concentration of vancomycin in the treatment of neonatal sepsis, as well as whether the monitoring of these parameters is sufficient to determine the dose necessary for treatment.

Materials and Methods

The research included newborns admitted to the University Clinical Center Niš, at the Clinic of Pediatrics, in the period from June to October 2024 with a diagnosis of neonatal sepsis, who received vancomycin as part of the therapy. Patients received vancomycin intravenously at a dose of 15 mg/kg every 8 hours. If patients were premature infants up to 29th weeks of gestation, they received vancomycin every 12 hours.

The study was conducted in premature infants up to the 32nd gestational week, premature infants between the 33rd and 36th gestational weeks, and term infants of both sexes with a diagnosis of neonatal sepsis. One blood sample was collected from each subject. 1 ml of blood was taken from the subjects during the period when blood is sampled for routine biochemical analyses after reaching the equilibrium state of vancomycin in the body (30 to 60 minutes before the fourth dose of vancomycin), at the Clinic of Pediatrics of the University Clinical Center Niš. A portion of the sampled blood of 0.5 ml, used to determine the serum concentration of vancomycin, was centrifuged for 10 minutes at 3000 revolutions per minute, after which the separated serum was transferred to tubes for analysis. The remaining sampled blood of 0.5 ml was used to determine routine biochemical analyses.

Determining the serum concentration of vancomycin is a routine method for which the AU680 device, Beckman Coulter, was used in the Center for Medical and Clinical Biochemistry, of University Clinical Center Niš.

The study was conducted in compliance with the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the University Clinical Center Niš, Serbia (EK 8604).

Results were statistically evaluated using Microsoft Excel.

Results

The study included 13 patients admitted to the Clinic of Pediatrics, University Clinical Center Niš, with a diagnosis of neonatal sepsis. To collect demographic data, medication data, and microbiological and biochemical analyses results, medical records were reviewed.

Of the 13 patients admitted to the Pediatrics Department, 6 patients were male (46%), while 7 patients were female (54%). In terms of gestational age, patients were divided into three groups: infants born before 32 weeks of gestation, infants born between 33 and 36 weeks of gestation, and infants born at term. Newborns born at term had significantly greater average body weight, compared to the first two groups of newborns ($p < 0.001$) (Table 1)

The majority of patients were admitted during the 1st day of age (40%) with a diagnosis of early neonatal sepsis, with initial antibiotic therapy of ampicillin and amikacin or ampicillin and gentamicin. If the initial therapy proved ineffective or after blood culture results arrived, the initial treatment would be replaced by a therapeutic combination of Vancomycin and meropenem. The majority of patients admitted during the first day of age were infants born before the 32nd week of gestation (50%). There was a moderate positive correlation between gestational age group and the age of admission ($\rho = 0.563$, $p < 0.01$).

Table 1. Average body weight of subjects (g)

Newborns born before 32 weeks of gestation	1605 ± 267	F = 41.703 p < 0.001
Newborns born between 32 and 36 weeks of gestation	2069 ± 247	
Newborns born at term	3461 ± 581	

Vancomycin therapy lasted for 7 days (52%) for the majority of subjects, while a smaller group (16%) was treated for just 5 days; all subjects were newborns born at term. In newborns born before the 32nd gestational week, as well as in newborns born between the 32nd and 36th gestational week, vancomycin therapy lasted for 7 days in the largest number of subjects. No statistically significant association was found between gestational age and the duration of vancomycin treatment.

Blood cultures were performed 29 times, 15 of which were sterile (52%), which may be due to several different factors. One possible explanation is that in newborns, especially preterm infants, the number of bacteria present in the blood may have been insufficient to be detected by standard techniques, while another possible explanation is that due to the need to minimize blood loss in newborns, the blood sample taken was insufficient to determine sensitivity. The highest number of blood cultures was *Staphylococcus epidermidis* (17%) and *E. coli* (10%). The presence of *Staphylococcus spp, Staphylococcus haemolyticus, Enterococcus faecium, and Klebsiellae pneumoniae* was also recorded. (Table 2).

Vancomycin was used in combination with meropenem in the treatment of late neonatal sepsis (85%). The most common type of additional therapy included rehydration (36%), symptomatic therapy (28%), systemic antimycotics (24%), and substitution with human albumin (20%) (Table 3.)

Table 2. Blood culture

Staphylococcus epidermidis	5
Staphylococcus sp.	1
Sterilo	15
E. coli ESBL+	3
Staphylococcus haemolyticus	1
Enterococcus faecium	1
Klebsiellae pneumoniae	2
Candida	1

Table 3. Additional drugs used in therapy

Rehydration	9
Symptomatic therapy	7
Systematic antimycotic	6
Phototherapy	6
Substitution with human albumins	5
Vitamin K	4
Caffeine citrate	4
Surfactant	3
Fresh frozen plasma	2
Phenobarbitone	2
Substitution of filtered erythrocytes	2

Vancomycin serum concentration

The value of vancomycin serum concentration was expressed in μg/ml (as the concentration was measured). Guidelines for vancomycin serum concentrations in adults recommend a reference range above 10 μg/ml, except for Methicilin-resistant *Staphylococcus Aureus* (MRSA) pneumonia, endocarditis, or bone infections in infants, while reference values between 15 and 20 μg/ml are advised. It is necessary to note that the reference values were determined based on research carried out on adults, without a study that would determine the corresponding reference values in the neonatal population (7). Some studies suggest that in newborns, the reference values should be between 10 and 15 μg/ml, while others suggest an even smaller range between 10 and 12 μg/ml to avoid potential nephrotoxicity (8, 9).

The highest value of serum vancomycin determined in our research was 46.8 μg/ml, and was recorded in a newborn born at 36 gestational weeks, with a body weight of 2090 g, while the lowest value was 1.9 μg/ml and was measured in newborns born at term, with a body weight of with a mass of 3600 g.

Out of the total number of subjects, only one had a concentration lower than 10 μg/ml, which is also the lowest recorded dose. An equal number of respondents had values between 20 and 30 μg/ml (23%), as well as over 30 μg/ml (23%), while the largest number of respondents had values between 10 and 20 μg/ml (46.2%). The value of serum vancomycin between 10 and 15 μg/ml was recorded in 38.5% of subjects, while the values of vancomycin in serum between 15 and 20 μg/ml were recorded in 7.7% of subjects (Figure 1).

In infants born before 32 weeks of gestation, the mean value of vancomycin concentration in the blood was 24.6 ± 15.23 μg/ml, with a minimum value of 12.2 μg/ml, and a maximum of 43.9 μg/ml. In infants born between 32 and 36 weeks of gestation, the mean value was similar at 27.08 ± 15.03 μg/ml, with the lowest measured value in this group at 11 μg/ml, and the highest at 46.8 μg/ml. For newborns born at term, the average value of serum vancomycin concentration was 17.76 ± 13.06 μg/ml, with a minimum values of 1.9 μg/ml, while the highest was 37.9 μg/ml (Table 4). The differences in vancomycin concentration between the three gestational age groups were not statistically significant.

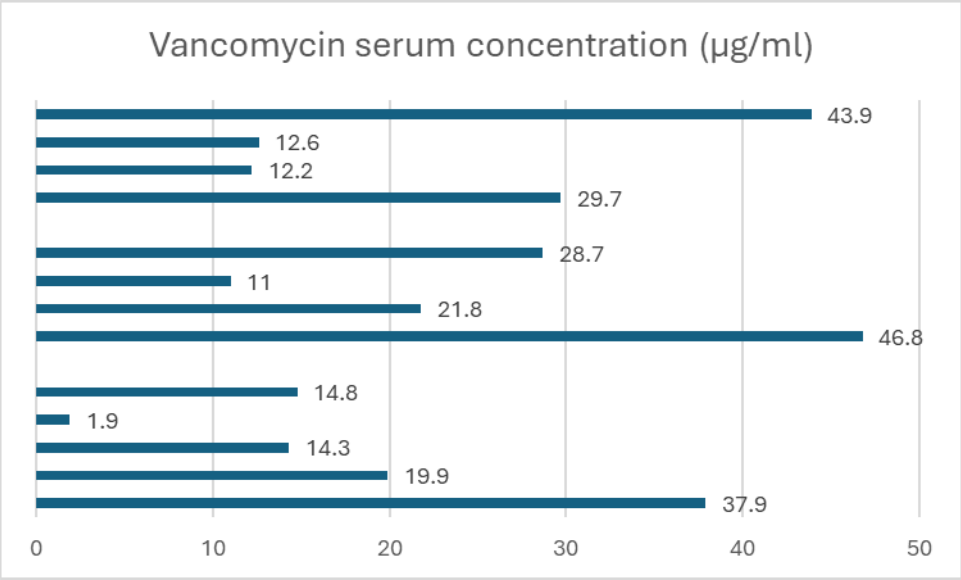

Figure 1. Vancomycin serum concentration (μg/ml)

Table 4. Effect of gestational week on serum vancomycin concentration

Weeks of gestation	average	min	max	F=0.518 p=0.611
Newborns born before 32 weeks of gestation	24.60±15.23	12.2	43.9	
Newborns born between 33 and 36 weeks of gestation	27.08±15.03	11	46.8	
Newborns born at term	17.76±13.06	1.9	37.9	

In newborns whose birth weight was less than 2000 g, the average value of vancomycin concentration was 23.02 ± 13.29 µg/ml, while in newborns whose birth weight was between 2000 and 3000 it was 31.60 ± 12.96 µg/ml, and in newborns whose birth weight was over 3000g, the average concentration of vancomycin is 10.33 ± 7.31 µg/ml, which is the closest to the reference values of vancomycin in serum (Table 5.). These differences were not found to be significant. Although there are no statistically significant differences, it was observed that as the body weight increases over 3000g, the concentrations of vancomycin in the serum decrease, while in the other groups of subjects (birth weight less than 2000g and birth weight between 2000g and 3000g), the results were well above the reference values.

In subjects whose measured values of serum creatinine were less than 60 µmol/l, the average value of vancomycin in serum was 17.24 ± 13.26 µg/ml, which is the smallest deviation from the reference values. With a serum creatinine concentration between 60 and 90 µmol/l, there is also an increased level of vancomycin in the serum, namely the mean value is 22.62 ± 13.56 µg/ml, the lowest value is 11 µg/ml, while the highest measured value is 46.8 µg/ml. In the case when serum creatinine values exceed 90 µmol/l, the value of vancomycin in serum also increases, with an average value of 36.80 ± 10.04 µg/ml, where the minimum value was 29.7 µg/ml, and the maximum value was 43.9 µg/ml (Table 6). These differences were not found to be statistically significant. However, there are noticeable differences between different values of creatinine concentration, indicating that higher creatinine levels lead to increased vancomycin serum concentration.

Table 5. Effect of body weight on vancomycin concentration

Body weight	average	min	max	F = 2.599 P = 0.123
< 2000	23.02 ± 13.29	11	43.9	
2000–3000	31.60 ± 12.96	19.9	46.8	
> 3000	10.33 ± 7.31	1.9	14.8	

Table 6. Effect of serum creatinine concentration on serum vancomycin concentration

serum creatinine concentration (µmol/L)	average	min	max	F = 1.587 P = 0.252
< 60	17.24 ± 13.26	1.9	37.9	
60–90	22.62 ± 13.56	11	46.8	
> 90	36.80 ± 10.04	29.7	43.9	

Discussion

Therapeutic drug monitoring entails adjusting medication dosage based on the serum concentration of the drug in blood in order to achieve the optimal effect with minimal toxicity. The most accurate method for monitoring the efficacy and safety of vancomycin is the determination of the trough concentration of the drug in the serum. The determination of the lowest drug concentration is made immediately before administration of a new dose.

Determining the concentration allows for informed decisions to be made in order to maintain the drug concentration within therapeutic limits, which enables us to optimize therapy while minimizing toxic effects. Therapeutic monitoring is extremely important, especially in newborns due to the very pharmacokinetic specificity of this population, which leaves little room for error (10, 11).

Our reserch showed in subjects whose birth weight was over 3000 g, the smallest deviations from the reference values were recorded, which may be the result of several factors. The first is the development of the kidneys, because a birth weight of over 3000 g is characteristic for full-term newborns. As another factor, we have to take into account that in newborns with a lower birth weight, there is a higher proportion of body fluid (80–85%) and a lower proportion of fat compared to newborns with a higher birth weight. In low-weight infants, the combination of a high proportion of body fluid and a much smaller proportion of body

fat leads to an uneven distribution of vancomycin, i.e., higher concentrations in the blood. On the other hand, as newborns with a higher birth weight have a higher percentage of fat in relation to body fluid, the distribution of the drug will be more even, thus a lower concentration of vancomycin in the serum is recorded (12, 13).

Elevated concentrations of serum creatinine are commonly indicative of reduced renal function. When renal function is reduced, the efficiency of glomerular filtration is also reduced, causing an increase of serum creatinine levels. Vancomycin is also primarily eliminated by glomerular filtration. The reduced ability to excrete vancomycin can lead to an increase in vancomycin serum level. Concentration of serum creatinine can be a valuable marker for determining appropriate dosing of vancomycin to avoid potential toxicity (14–16).

Research has shown that when the value of the lowest concentration of vancomycin is greater than 15 μg/ml, there is an increase in the incidence of nephrotoxicity by 2.67 times. The increased incidence of vancomycin-induced nephrotoxicity also leads to prolonged duration of therapy. A retrospective observational study conducted in 10 US medical centers showed that in patients treated with vancomycin for 7 days, approximately 20% of subjects developed vancomycin-induced nephrotoxicity (17).

Meta-analysis done by Tsutsuura et al. showed that acute kidney injury (AKI) incidence rates reportedly increase with trough concentrations ≥ 15 μg/mL and further increase for trough concentrations ≥ 20 μg/ml. They believe that vancomycin trough concentrations should be kept below 20 μg/mL at all times and minimized wherever possible (18).

Longer duration of vancomycin can also lead to reduced vancomycin-associated AKI (19). Also, some drugs can affect the serum concentration of vancomycin, for example, ibuprofen treatment may increase the risk of overly high trough levels (20).

Research conducted in Europe showed that in intensive care units, the combination of ampicillin and gentamicin is most often prescribed as therapy for early neonatal sepsis, while vancomycin, gentamicin, cefotaxime, and meropenem are most often prescribed for late neonatal sepsis (21).

Certain studies recommend that the dosing of vancomycin in newborns should be guided by body weight, gestational age, and serum creatinine concentration. Due to the high degree of variability of pharmacokinetics in infants, it is considered that the concentration of serum vancomycin should be between 10 and 15 μg/ml (22) to achieve its antibacterial effect in the treatment of gram-positive bacteria .

Research, such as a retrospective study conducted in Taiwan, shows that only half of the examined infants receiving vancomycin as part of empiric therapy for late neonatal sepsis achieved the target serum concentration of 10 to 20 μg/ml. Risk factors for elevated serum levels of vancomycin include elevated serum creatinine levels, as well as therapeutic use of ibuprofen (23).

These results are confirmed by a retrospective study conducted in China, which shows that the percentage of subjects who achieved the target serum concentration was 45% with the empirical initial regimen, while in the case of dose adjustment, that percentage was 55.2%. These results indicate that the optimal serum concentration of vancomycin cannot be achieved based on empirical dose adjustment alone (24).

Limitations

The main limitation of this study is the small sample size. However, to the best of our knowledge, this is the first time that this type of study is conducted in neonatal patients in Serbia. Another limitation of this study includes that the detailed Minimum inhibitorycon centration of vancomycin were not available in the analysis of the effectiveness of vancomycin target trough concentrations.

Conclusion

Sepsis represents a complex clinical syndrome with potential life-threatening organ dysfunction. A big challenge in therapy is the use of antibiotics in neonates with sepsis. Especially the use of antibiotics such as vancomycin, in which, due to physiologically reduced function of organs in newborns and pathophysiological changes caused by sepsis, potentiation of nephro- and ototoxicity can occur. Therapeutic monitoring of vancomycin is critically important, especially in newborns, due to the very pharmacokinetic specificity of this population.

Due to the small sample size, the effects of gestational age, body weight, and serum creatinine concentration on serum vancomycin concentration in newborns were not found to be statistically significant. However, descriptive statistics show that significant differences can be found in these groups, especially in the group examining effects of serum creatinine on serum vancomycin concentration, which shows that higher creatinine levels lead to increased serum vancomycin concentrations. Additionally, only in the group of subjects whose body weight was over 3000 g was the serum concentration found to be within the reference values for newborns, suggesting a potential effect worthy of further investigation.

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References

1. De Rose DU, Ronchetti MP, Martini L, Rechichi J, Iannetta M, Dotta A, et al. Diagnosis and management of neonatal bacterial sepsis: current challenges and future perspectives. Trop Med Infect Dis 2024;9(9):199. [CrossRef][PubMed]
2. Celik IH, Hanna M, Canpolat FE, Pammi M. Diagnosis of neonatal sepsis: the past, present and future. Pediatr Res 2022;91(2):337-50. [CrossRef][PubMed]
3. Yadav P, Yadav SK. Progress in Diagnosis and Treatment of Neonatal Sepsis: A Review Article. JNMA J Nepal Med Assoc 2022;60(247):318-24. [CrossRef][PubMed]
4. Molloy EJ, Bearer CF. Paediatric and neonatal sepsis and inflammation. Pediatr Res 2022;91(2):267-9. [CrossRef][PubMed]
5. Procianoy RS, Silveira RC. The challenges of neonatal sepsis management. J Pediatr (Rio J) 2020;96(Suppl 1):80-6. [CrossRef][PubMed]
6. Darlow CA, da Costa RMA, Ellis S, Franceshi F, Sharland M, Piddock, L, et al. Potential Antibiotics for the Treatment of Neonatal Sepsis Caused by Multidrug-Resistant Bacteria. Paediatr Drugs 2021;23(5):465-84. [CrossRef][PubMed]
7. Mejías-Trueba M, Alonso-Moreno M, Gutierrez-Valencia A, Herrera-Hidalgo L, Guisado-Gil AB, Jimenez-Parrilla F, et al. Association between Vancomycin Pharmacokinetic Parameters and Clinical and Microbiological Efficacy in a Cohort of Neonatal Patients. Antimicrob Agents Chemother 2022;66(11):e0110922. [CrossRef][PubMed]
8. University of North Carolina Medical Center. Vancomycin dosing and monitoring guide: neonatal and pediatric. Chapel Hill (NC): UNC Medical Center; 2023.
9. Alrahahleh D, Xu S, Luig M, Kim HY, Alffenaar JW. Dosing of vancomycin and target attainment in neonates: a systematic review. Int J Antimicrob Agents 2022;59(2):106515. [CrossRef][PubMed]
10. Kim SM, Lee HS, Hwang NY, Kim K, Park HD, Lee SY. Individualized Vancomycin Dosing with Therapeutic Drug Monitoring and Pharmacokinetic Consultation Service: A Large-Scale Retrospective Observational Study. Drug Des Devel Ther 2021;15:423-40. [CrossRef][PubMed]
11. Lee SM, Yang S, Kang S, Chang MJ. Population pharmacokinetics and dose optimization of vancomycin in neonates. Sci Rep 2021;11(1):6168. [CrossRef][PubMed]
12. Alsultan A, Al Munjem MF, Atiq KM, Aljehani ZK, Al Muqati H, Almohaizeie A, et al. Population pharmacokinetics of vancomycin in very low birth weight neonates. Front Pediatr 2023;11:1093171. [CrossRef][PubMed]
13. Leroux S, van den Anker JN, Smits A, Pfister M, Allegaert K. Maturational changes in vancomycin protein binding affect vancomycin dosing in neonates. Br J Clin Pharmacol 2019;85(5):865-7. [CrossRef][PubMed]
14. Cao L, Li Z, Zhang P, Yong S. Relationship between Vancomycin Trough Serum Concentrations and Clinical Outcomes in Children: a Systematic Review and Meta-Analysis. Antimicrob Agents Chemother 2022;66(8):e0013822. [CrossRef][PubMed]
15. Altowayan WM, Mobark MA, Alharbi A, Alduhami AA, Rabbani SI. The influence of vancomycin on renal functions, the predictors and associated factors for nephrotoxicity. PLoS One 2023;18(4): e0284223. [CrossRef][PubMed]
16. Bruniera FR, Ferreira FM, Saviolli LRM, Bacci MR, Feder D, da Luz Goncalves Pedreira M, et al. The use of vancomycin with its therapeutic and adverse effects: a review. Eur Rev Med Pharmacol Sci 2015;19(4):694-700. [PubMed]
17. Al-Maqbali JS, Shukri ZA, Sabahi NA, Al-Riyami I, Al Alawi AM. Vancomycin Therapeutic Drug Monitoring (TDM) and Its Association with Clinical Outcomes: A Retrospective Cohort. J Infect Public Health 2022;15(5):589-93. [CrossRef][PubMed]
18. Tsutsuura M, Moriyama H, Kojima N, Mizukami Y, Tashiro S, Osa S, et al. The monitoring of vancomycin: a systematic review and meta-analyses of area under the concentration-time curve-guided dosing and trough-guided dosing. BMC Infect Dis 2021;21(1):153. [CrossRef][PubMed]
19. Murphy ME, Tang Girdwood S, Goldman JL, Scheetz MH, Downes KJ. Precision dosing of vancomycin: in defence of AUC-guided therapy in children. J Antimicrob Chemother 2021;76(10):2494-7. [CrossRef][PubMed]
20. Cristea S, Allegaert K, Falcao AC, Falcao F, Silva R, Smits A, et al. Larger dose reductions of vancomycin required in neonates with patent ductus arteriosus receiving indomethacin versus ibuprofen. Antimicrob Agents Chemoter 2019;63:e00853-19. [CrossRef][PubMed]
21. Garrido F, Allegaert K, Arribas C, Villamor E, Raffaeli G, Paniagua M, et al. Variations in Antibiotic Use and Sepsis Management in Neonatal Intensive Care Units: A European Survey. Antibiotics (Basel) 2021;10(9):1046. [CrossRef][PubMed]
22. Pham JT. Challenges of Vancomycin Dosing and Therapeutic Monitoring in Neonates. J Pediatr Pharmacol Ther 2020;25(6):476-84. [CrossRef][PubMed]
23. Lee TY, Hung YL, Shen CM, Kao CL, Hsieh WS. Reappraisal of therapeutic vancomycin trough concentrations with empirical dosing in neonatal infections. Pediatr Neonatol 2023;64(2):176-82. [CrossRef][PubMed]
24. Weng XH, Zhu CQ, Duan LF, Li L, Yang ZM, Wang SM, et al. Vancomycin in neonatal sepsis: predictive performance of a Chinese neonatal population pharmacokinetic model and clinical efficacy evaluation. Eur J Hosp Pharm 2022;29(2):101-8. [CrossRef][PubMed]

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UTICAJ GESTACIJSKE STAROSTI, TELESNE MASE I KONCENTRACIJE KREATININA U SERUMU NA KONCENTRACIJU VANKOMICINA U SERUMU PRILIKOM LEČENJA NEONATALNE SEPSE: PILOT STUDIJA JEDNOG CENTRA

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Neonatalna sepsa je ozbiljno zdravstveno stanje, koje karakteriše sistemska infekcija kod novorođenčadi mlađe od dvadeset osam dana. Terapija kasne neonatalne sepse zasnovana je na kombinaciji vankomicina i meropenema. Cilj ove pilot studije bio je da se ispita uticaj telesne težine novorođenčeta, gestacijske starosti i vrednosti kreatinina na koncentraciju vankomicina u serumu prilikom lečenja neonatalne sepse. Istraživanje je obuhvatilo trinaest pacijenata koji su od juna do oktobra 2024. godine primljeni na Kliniku za pedijatriju Univerzitetskog kliničkog centra Niš sa dijagnozom neonatalne sepse. Poželjna koncentracija vankomicina u serumu, koja iznosi između 10 µg/ml i 15 µg/ml, zabeležena je kod 38,5% ispitanika. Budući da uzorak uzet u obzir nije velik, može se reći da uticaj gestacijske starosti, telesne težine i vrednosti kreatinemije na koncentraciju vankomicina u serumu nije statistički značajan. Međutim, deskriptivna statistika pokazuje da se može uočiti značajna razlika između ovih grupa, tako da je poželjno obaviti dalja istraživanja.

Acta Medica Medianae 2025; 64(4):159–166.

***Ključne reči**: neonatalna sepsa, vankomicin, terapijsko praćenje lekova*

CHANGES OF MYOCARDIAL GLYCOGEN CONTENT IN RATS ADMINISTERED WITH MODERATE DOSES OF FURFURAL (Vol 53, No. 1, pages 5–9, 2014)

The editorial board of the journal Acta Medica Medianae

Abstract

This erratum is issued because there was an oversight and the name of one of the co-authors was misspelled. Co-authors herself, Sanja Mladenović, noticed this mistake. Mistake was simple spelling error that occured during the aplication process in which first author misspelled the name of her colleague. We are very grateful to Sanja Mladenović that she noticed this mistake and we are very sorry that this happened.

In the article *Changes Of Myocardial Glycogen Content in Rats Administered with Moderate Doses of Furfural* authored by: Dragana Veličković and co-authors Branislava Miličić i Tanja Mladenović published in the journal Acta medica Medianae Vol. 53 (No. 1), pages 5–9, 2014, the name of one of the co-authors was wrongly spelled. Name of the co-author is not Tanja Mladenović, but Sanja Mladenović and should be evidenced as such.

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Acta Medica Medianae 2025; 64(4):167–168.

PROMENE SADRŽAJA GLIKOGENA U MIOKARDU PACOVA KOD PRIMENE UMERENIH DOZA FURFURALA (Vol 53, broj 1, str. 5–9, 2014)

Uređivački odbor časopisa Acta Medica Medianae

Apstrakt

Izdajemo ovaj erratum zbog greške koja je nastala u procesu tehničkog uređivanja časopisa koja se odnosi na ime koautorke rada. Naime, zbog greške u kucanju pogrešno je navedeno ime koautorke. Grešku je primetila sama koautorka rada, Sanja Mladenović, na čemu smo mu joj zahvalni i ovom prilikom izražavamo žaljenje zbog propusta.

U radu *Promene sadržaja glikogena u miokardu pacova kod primene umerenih doza furfurala* autora: Dragane Veličković i koautora Branislave Miličić i Tanje Mladenović objavljenog u časopisu Acta medica Medianae Vol. 53 (No. 1), 2014. godine, strane 5–9, pogrešno je zavedeno ime koautorke rada. U radu kao ime koautorke stoji Tanja Mladenović, dok je ime koautorke Sanja Mladenović.

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Acta Medica Medianae 2025; 64(4):167–168.

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