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CONTENTS

M E D I C I N S K I P R E G L E D
ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
Novi Sad *Vase Stajića 9* *Srbija*

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EDITORIAL
UVODNIK

T-CELL RECEPTOR CONSTANT BETA CHAIN ISOFORMS – NEW PARADIGM IN THE DIAGNOSIS OF T-CELL NEOPLASMS

IZOFORME KONSTANTE BETA LANCA T-ĆELIJSKOG RECEPTORA – NOVA PARADIGMA U DIJAGNOZI T-ĆELIJSKIH NEOPLAZMI

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The adaptive immune system has evolved to recognize as many foreign antigens as possible [1]. The diversity of antigen recognition abilities by B and T-cells is achieved through combinatorial diversity and "imprecision" diversity. The former is the result of functional antigen-recognizing receptors (immunoglobulin receptor (Ig) and T-cell receptor (TCR)) being modular molecules. There are many variable segments with high sequence variability (V-regions of heavy and light chains of immunoglobulin receptor and alpha-through-delta chains of TCR) and few constant segments (C-regions, in translated protein close to/embedded in the membrane of B and T-lymphocytes). RAG-mediated DNA recombination during the development of B and T-cells in the bone marrow and thymus, respectively, combines variable and constant regions (as well as diversity-D and junction-J segments) into recombined DNA ready for transcription and translation of the message into a functional surface receptor protein [2, 3].

B and T-cell clones are cells with identical recombined antigen receptor. Clones may transiently emerge in reactive conditions such as infections or autoimmune processes. However, the term "clone" is most often used to describe abnormal neoplastic proliferations of cells with identical antigen receptor, presumed to originate from a single cell. In clinical laboratories, testing for B and T-cell clonality is routinely performed for diagnosing lymphoproliferative disorders. While new methods of antigen receptor sequencing by NGS offer the most direct evidence of clonality, most clinical laboratories use PCR method, where polyclonal populations exhibit a vaguely Gaussian distribution of PCR product lengths, while (mono)clonal population is represented by a single prominent PCR product peak [4, 5].

Immunophenotyping has been long used as a surrogate for clonality in B-cell neoplasms, enabled by mutually exclusive use of kappa and lambda light chains in B-cells, and availability of antibodies that recognize constant regions of kappa and lambda chains. Normal polyclonal B-cells are a mixture of kappa and lambda-expressing cells, usually in a 2:1 ratio as kappa locus rearranges first during the B-cell development. In contrast, B-cell clones are typically restricted to one of the two light chains.

Immunophenotyping of T-cells to prove clonality has been significantly more difficult. Flow cytometry immunophenotyping of V-beta chain usage is labor-intensive and complicated for analysis, thus rarely implemented in clinical practice [6]. Instead, phenotypic T-cell aberrancies, such as the loss of pan-T-cell antigens (CD2, CD5, CD7) or markedly skewed CD4:CD8 ratios, have been used as soft indicators of potential clonality. This all changed in 2017 when a British group published a paper describing characterization and use of an antibody directed toward constant region-1 (CR-1) of the TCR-beta chain, one of the two mutually exclusive isoforms (TRBC-1 and TRBC-2) [7]. Anti-TRBC-1 antibody was originally produced and partially characterized in early 1990s [8], and was re-discovered in an attempt to limit CAR-T therapy to only a subset of patient's T-cells, ameliorating the immune suppression following such treatment. The diagnostic community quickly realized the importance of this discovery and followed with a massive effort to implement this antibody in indirect T-cell clonality testing [9–16]. Normal T-cells are a mixture of mutually ex-

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clusive TRBC-1 and TRBC-2 expressing cells, akin to kappa and lambda expression in normal B-cells. Even with a single anti-TRBC-1 antibody, it is possible to easily determine whether a certain T-cell population is likely clonal (if flow cytometry identifies the expression of TRBC1 present in less than 15% or more than 85% of the gated population) [15].

The inclusion of TRBC-1 (and, more recently, TRBC-2) [13] antibody in routine T-cell flow cytometry analysis enables rapid, accurate and cost-effective detection and quantification of T-cell clones, and it has dramatically changed the flow cytometry practice in the past several years. Of note, the increased sensitivity of this type of testing also results in detection of spurious small T-cell clones which may arise in reactive conditions (T-cell clones of undetermined significance – TCUS) [15], emphasizing the importance of clinical correlation, as is recommended for any laboratory test.

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ORIGINAL STUDIES
ORIGINALNI NAUČNI RADOVI

IDENTIFYING OF PATIENTS WITH ADVANCED OVARIAN CANCER EXPERIENCING THE HIGHEST BENEFIT FROM BEVACIZUMAB IN THE FIRST-LINE SETTING ON THE BASIS OF KELIM SCORE

IDENTIFIKACIJA PACIJENATA SA UZNAPREDOVALIM KARCINOMOM JAJNIKA KOJI IMAJU NAJVEĆU KORIST OD PRIMENE BEVACIZUMABA U PRVOJ LINIJI NA OSNOVU PRIMENE KELIM SKORA

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Abstract

Introduction. Ovarian cancer represents the deadliest gynecological malignancy. The aim of this study is to validate the predictive value of Cancer antigen-125 Elimination rate constant K score regarding progression-free survival among patients with Stage IIIc-IV high-grade serous ovarian cancer according to the International Federation of Gynecology and Obstetrics, treated with bevacizumab. Additionally, the study aims to determine the significance of a score in the assessment of progression-free survival in relation to operation status and disease stage. **Material and Methods.** The retrospective study was conducted at the Oncology Institute of Vojvodina and included 56 patients treated for high-grade serous ovarian cancer. The treatment protocol consisted of chemotherapy with paclitaxel and carboplatin, followed by bevacizumab maintenance therapy. The Cancer Antigen-125 Elimination Rate Constant K score was calculated using three Cancer Antigen 125 tumor marker values. Based on this calculation, the score was dichotomized into favorabe (≥1) and unfavorable (<1) groups. **Results.** The analysis of the score's impact on progression-free survival revealed that patients with a score ≥1 had longer mean survival time compared to a score <1 (19.4 vs 17.0 months). This difference was not statistically significant (p=0.549). The operation status in relation to a score was the only statistically significant factor with progression-free survival benefit (p=0.002). **Conclusion.** In our study, Cancer antigen-125 Elimination rate constant K score did not emerge as a statistically significant prognostic factor for identifying patients most likely to benefit from bevacizumab. The operative status showed significant predictive value.

Key words: Ovarian Neoplasms; Bevacizumab; CA-125 Antigen; Poly(ADP-ribose) Polymerase Inhibitors; Treatment Outcome; Prognosis

Sažetak

Uvod. Karcinom jajnika predstavlja najletalniji ginekološki malignitet. Cilj rada je potvrditi prediktivnu vrednost *Cancer antigen-125 Elimination rate constant* K skora u odnosu na preživljavanje bez progresije kod pacijentkinja sa seroznim karcinomom jajnika visokog stepena, stadijuma IIIC-IV prema Internacionalnoj federaciji za ginekologiju i akušerstvo, koje su lečene primenom bevacizumaba; utvrditi značaj ovog skora u proceni preživljavanja bez progresije u odnosu na operacioni status i stadijum bolesti. **Materijal i metode.** Istraživanje predstavlja retrospektivnu studiju koja je sprovedena u Institutu za onkologiju Vojvodine. Studijom je obuhvaćeno 56 pacijentkinja koje su lečene od seroznog karcinoma jajnika visokog stepena primenom hemioterapije na bazi paklitaksela i karboplatine i biološkom terapijom održavanja bevacizumabom. Pacijentkinjama je na osnovu tri vrednosti tumorskog markera karcinom antigena 125 izračunat *Cancer antigen-125 Elimination rate constant* K skor koji je tumačen kao povoljan (≥ 1) ili nepovoljan (< 1). **Rezultati.** Ispitivanjem uticaja *Cancer antigen-125 Elimination rate constant* K skora na preživljavanje bez progresije utvrđeno je duže prosečno preživljavanje kod pacijentkinja sa skorom ≥ 1 u odnosu na skor < 1 (19,4 vs. 17 meseci), međutim ono nije bilo statistički značajno (p = 0,549). Operacioni status u odnosu na *Cancer antigen-125 Elimination rate constant* K skor pokazan je kao jedini statistički značajan faktor koji doprinosi preživljavanju bez progresije bolesti (p = 0,002), dok stadijum bolesti nije pokazao statističku značajnost. **Zaključak.** U našem istraživanju *Cancer antigen-125 Elimination rate constant* K skor nije pokazan kao značajan prognostički faktor u odabiru pacijentkinja koje bi imale najveću korist od primene bevacizumaba; operacioni status u odnosu na *Cancer antigen-125 Elimination rate constant* K skor jeste.

Ključne reči: tumori jajnika; bevacizumab; CA-125 antigen; poli adenozin-difosfat riboza polimeraza inhibitori; ishod lečenja; prognoza

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Abbreviations

PARP	– poly adenosine diphosphate-ribose polymerase
KELIM score	– Cancer Antigen-125 Elimination Rate Constant K
PFS	– progression free survival
FIGO	– The International Federation of Gynecology and Obstetrics
CA-125	– Cancer Antigen-125
VEGF	– vascular endothelial growth factor
VEGFR-1,2	– vascular endothelial growth factor receptor 1,2
BRCA gene	– Breast Cancer gene
GCIG	– Gynecologic Cancer Intergroup
OS	– overall survival

Introduction

Ovarian carcinomas rank as the eighth most common malignancy and the eighth leading cause of death among women worldwide, with a 5-year survival rate of 46% [1]. It is the most lethal gynecological malignancy and the second most common in developed countries after endometrial cancer. In Serbia, however, it ranks third, following endometrial and cervical cancers. Approximately 820 new cases are reported annually in Serbia, where it remains the leading cause of death among gynecological malignancies and ranks sixth in overall statistics [1, 2].

In terms of histological characteristics, ovarian cancers are divided into four major groups: epithelial tumors, germ cell tumors, stromal tumors of the genital cord, and sarcomas. The most common histological subtype is epithelial adenocarcinoma, which accounts for 95% of cases, with high-grade serous carcinomas comprising 70-80% of all ovarian cancers. Due to the lack of symptoms in earlier stages and the impossibility of screening, 80% of cases are diagnosed in advanced stages of the disease (International Federation of Gynecology and Obstetrics (FIGO) III-IV) [2–4].

Metastases typically occur on the omentum and peritoneum, while lymphogenous and hematogenous spread occurs in only 2-3% of cases. Once the disease spreads in the abdomen, often presenting as ascites, it progresses to the chest, causing malignant pleural effusions. The cytokine vascular endothelial growth factor (VEGF) plays a pivotal role in the development of ascites and malignant pleural effusions by promoting fluid extravasation from vascular spaces [2]. VEGF also contributes to tumor growth and metastasis, exacerbating the prognosis of ovarian cancer [5]. It exerts its effects by binding to two tyrosine kinase receptors on the surface of endothelial cells of tumor blood vessels: vascular endothelial growth factor receptor 1 (VEGFR1) and 2 (VEGFR2) [6]. Bevacizumab, a humanized monoclonal antibody, selectively binds to circulating VEGF, preventing its receptor binding. By reducing tumor microvascular angiogenesis, bevacizumab restricts the oxygen and nutrient supply to the tumor, decreases interstitial pressure, and enhances vascular permeability, thereby improving the flow and effect of chemotherapeutic agents in the tumor [7]. Despite its proven efficacy in prolonging progression-free survival (PFS), bevacizumab is associated with significant adverse effects, including gastrointestinal perforation, hypertension, proteinuria, thromboembolism, bleeding, and impaired wound healing, which can limit its use [6, 7]. Therefore, identifying patients who benefit most from bevacizumab is crucial.

First-line treatment of ovarian cancer includes primary or interval surgery and platinum- and taxane-based chemotherapy, which may be combined with maintenance therapies such as bevacizumab or poly adenosine diphosphate-ribose polymerase (PARP) inhibitors, where applicable [8]. In Serbia, bevacizumab is approved alongside standard chemotherapy with carboplatin and paclitaxel for patients with epithelial ovarian carcinomas (FIGO stage IIIC: suboptimally operated or inoperable cases, and FIGO stage IV), fallopian tube carcinomas, and primary peritoneal carcinomas. Eligible patients must be in good general condition (performance status 0-1), without significant comorbidities or intestinal infiltration. PARP inhibitors, such as olaparib, are indicated as maintenance therapy for newly diagnosed advanced (FIGO stages III-IV) serous/endometrioid epithelial ovarian, fallopian tube, or primary peritoneal cancers with high-grade tumors and Breast Cancer (BRCA) gene 1 or 2 mutations (germinal or somatic) that achieve complete or partial response after completing the first-line platinum-based chemotherapy [9].

Bevacizumab has shown greater activity of in patients with platinum-resistant recurrent ovarian cancer [10]. This highlights the importance of evaluating the role of intrinsic tumor chemosensitivity in determining the effectiveness of bevacizumab in first-line therapy. The Cancer Antigen-125 Elimination Rate Constant K (KELIM) is a score calculated based on at least three measurements of the Cancer Antigen-125 (CA-125) tumor marker during the first 100 days of chemotherapy. It reflects the clearance rate of CA-125, with higher values indicating faster elimination of CA-125, and greater chemosensitivity to the applied therapy [11]. CA-125 is primarily associated with ovarian cancer but may be elevated in other conditions. According to the Gynecologic Cancer Intergroup (GCIG) criteria, CA-125 is used to monitor treatment response in recurrent disease or to define progression after first-line chemotherapy. Assessing the prognostic value of CA-125 over time for recurrence poses statistical challenge, as its trajectory can be summarized in various ways (e.g., KELIM after three months, or CA-125 values or

decreases at three or six months), and its prognostic role has been analyzed in many studies. Measurement error and biological variation further complicate its use as a biomarker, prompting some researchers to advocate for modeling techniques to better estimate its evolution. The KELIM score, based on longitudinal CA-125 measurements and incorporating pharmacokinetic and pharmacodynamic parameters, has gained interest in multiple contexts, including recurrent disease and neoadjuvant or initial treatments. The prognostic role of the KELIM score after 100 days (approximately three months) in adjuvant and neoadjuvant treatments had been tested in several studies [8].

Material and Methods

Research Goals

The objectives of this research are:

1. To validate the predictive value of the KELIM score for PFS period in patients with high-grade serous ovarian cancer, FIGO stages IIIC-IV, treated with bevacizumab.

2. To assess the significance of the KELIM score in evaluating PFS in relation to surgical status and disease stage.

This retrospective study was conducted at the Oncology Institute of Vojvodina in Sremska Kamenica, utilizing data from the Oncology Institute's BirPis21 database. The study included patients treated for high-grade serous ovarian cancer, FIGO stage IIIC-IV, between January 2019 and December 2021. These patients received a chemotherapy regimen of paclitaxel/carboplatin administered every 21 days in six cycles, followed by biological maintenance therapy with bevacizumab. Out of 91 patients, 56 had documented values of at least three theca-125 tumor marker measurements within the first 100 days of chemotherapy, required for KELIM score calculation. CA-125 levels were measured in local laboratories. Patient follow-up continued until December 2023 to determine the time in months post-chemotherapy until disease progression occurred. Ethical approval was obtained from the Ethics Committee of the Oncology Institute of Vojvodina in Sremska Kamenica.

Data collected from the BirPis21 information system included: patient's year of birth, FIGO stage, operative status, dates of chemotherapy cycles, date of bevacizumab initiation, CA-125 values and corresponding dates, progression-free survival in months following the last chemotherapy cycle.

Calculation of the KELIM score

KELIM scores were computed using a validated online tool (Biomarker Kinetics) [12]. Three CA-125 values obtained during the first 100 days of chemotherapy, as along with the dates of chemotherapy cycles, were imputed into the calculator. The results were classified as either favorable (KELIM ≥ 1) or unfavorable (KELIM < 1).

Statistical data processing

Statistical analyses were performed using SPSS Statistics version 25.0 software package. Statistically significant differences were observed between suboptimally operated FIGO stage IIIC and non-operated FIGO stage IIIC-IV patients regarding KELIM scores and the benefit of bevacizumab for PFS. Kaplan-Meier analysis and Cox regression were employed to examine correlations between the studied parameters. Statistically significant difference was defined as $p<0.05$ (p – level of statistical significance).

Results

A total of 91 patients with high-grade serous ovarian cancer (FIGO stage IIIC-IV) were included in this study. Of these, 32 were excluded due to insufficient CA-125 data (fewer than three documented values within the first 100 days of chemotherapy, required to calculate the KELIM score), and three patients were excluded due to the endometrioid subtype of epithelial ovarian cancer. All patients were treated with a standard chemotherapy protocol comprising paclitaxel and carboplatin, followed by maintenance therapy with bevacizumab. Data from the remaining 56 patients were analyzed using univariate and multivariate methods. The average patient age was 62 years (range: 36-79). The average progression-free survival (PFS) from the last chemotherapy cycle was 13.4 months (range: 2-51). Key patient characteristics included FIGO stage, operative status, and KELIM score. Among the participants, 39 (69.6%) had FIGO stage IIIC and 17 (30.4%) had stage IV. Regarding the operative status, 34 (60.7%) underwent surgery, while 22 (39.3%) did not. Based on KELIM scores, 48 patients (85.7%) had an unfavorable score (KELIM < 1), while 8 (14.3%) had a favorable score (KELIM ≥ 1).

Univariate analysis of PFS relative to KELIM scores i summarized in **Table 1.** Patients with KELIM ≥ 1 had a longer PFS compared to those with KELIM<1 (19.4 vs. 17.0). However, the difference was not statistically significant (p=0.549), likely due to the small proportion of patients with KELIM ≥ 1 (14.3%). **Graph 1** illustrates differences between these groups.

The multivariate analysis, detailed in **Table 2**, assessed the effect of operative status and FIGO stage on PFS in patients with an unfavorable KELIM score (KELIM < 1).

Table 1. Analysis for progression-free survival (PFS) in relation to KELIM score

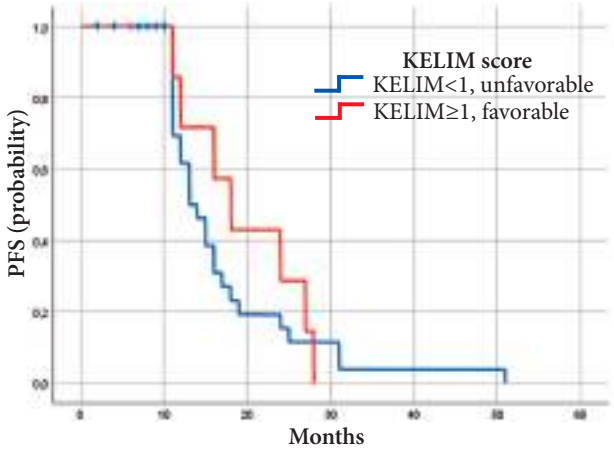
	PFS				
	n	Mean value (months)	95% CI (month)	Median (months)	p
Favorable KELIM≥1	8	19.4	14.3-24.6	18.0	0.549
Unfavorable KELIM<1	48	17.0	13.5-20.5	13.0	

n – number of patients 95%; CI – 95% confidence interval; p – statistical level significance; PFS – progression-free survival

Table 2. Multivariate analysis for progression-free survival (PFS) vs operative status and FIGO disease stage in patients with KELIM<1

		n	Mean value PFS (month)	Estimate	OR	95% CI	p
Operation status for KELIM<1	Unoperated	18	9.8	Ref	Ref	Ref	0.002
	Operated	30	14.4	-1.624	0.20	0.07-0.55	
FIGO stage for KELIM<1	IIIC	35	14.2	Ref	Ref	Ref	0.166
	IV	13	8.6	0.696	2.01	0.75-5.37	

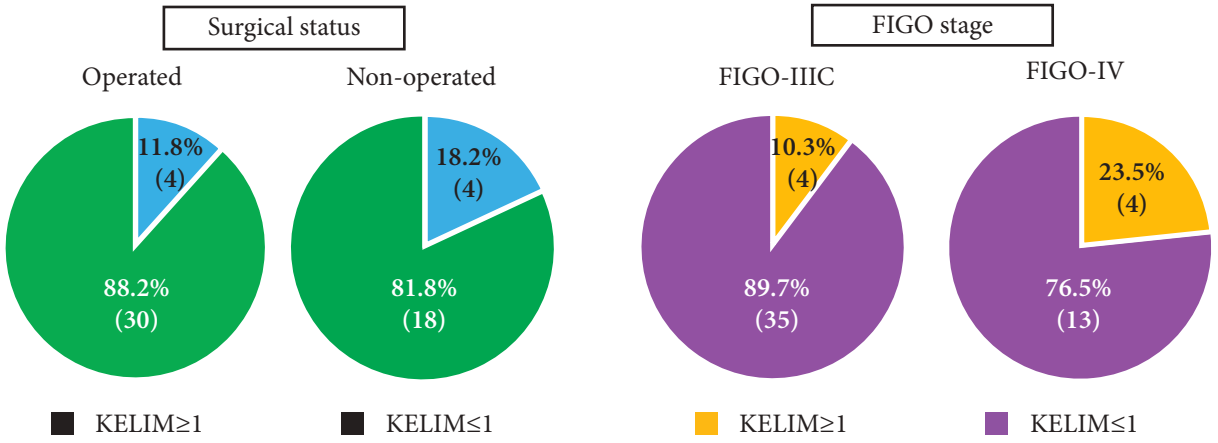
n – number of patients; PFS – progression free survival; Estimate – evaluation; OR – odd ratio; 95% CI – 95% confidence interval; p – level of statistical significance; Ref – reference



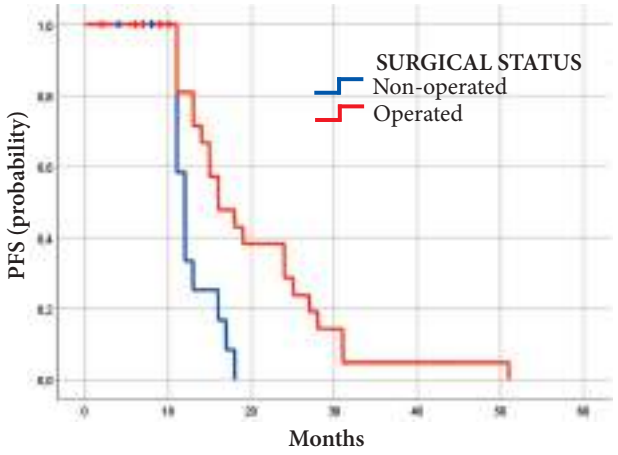
KELIM<1	48	64.6% (31)	10.4% (5)	6.25% (3)	2.1% (1)	2.1% (1)	0
KELIM≥1	8	87.5% (7)	37.5% (3)	0	0	0	0

Graph 1. PFS curves according to KELIM score

Among the 56 patients, 34 (60.7%) underwent surgery, of whom 4 (11.8%) had a favorable KELIM score (KELIM ≥ 1) and 30 (88.2%) had an unfavorable score (KELIM < 1). Among the 22 (39.3%) non-operated patients, 4 (18.2%) had a favorable score (KELIM ≥ 1), while 18 (81.8%) had an unfavorable score (KELIM <1) (**Graph 2**). Comparisons were not made in the KELIM ≥1 subgroup due to the small portion of patients. In the KELIM < 1 subgroup, surgery was associated with significantly longer average PFS compared to non-operated patients (14.4 vs. 9.8 months) (p=0.002). Operated patients had an 80% lower likelihood of disease progression (OR=0.20). **Graph 3** illustrates PFS comparisons between operated and non-operated patients with an unfavorable KELIM score. Regarding FIGO stage, 39 patients (69.6%) were stage IIIC, and 17 (30.4%) were stage IV. In the stage IIIC group, 4 patients (10.3%) had KELIM ≥ 1, and 35 (89.7%) had KELIM <1. In the stage IV group, 4 patients (23.5%) had KELIM ≥1, while 13 (76.5%) had KELIM < 1 **(Graph 2)**. Due to the small portion of patients with KELIM ≥1, comparisons were made in the KELIM <1 group only. Among the patients with KELIM < 1, those with stage IIIC had longer average PFS than stage IV (14.2 vs. 8.6). However, this difference was not statistically significant. A high odds ratio (OR=2.01) indicated a two-fold increased risk of disease progression in stage IV disease, but the wide

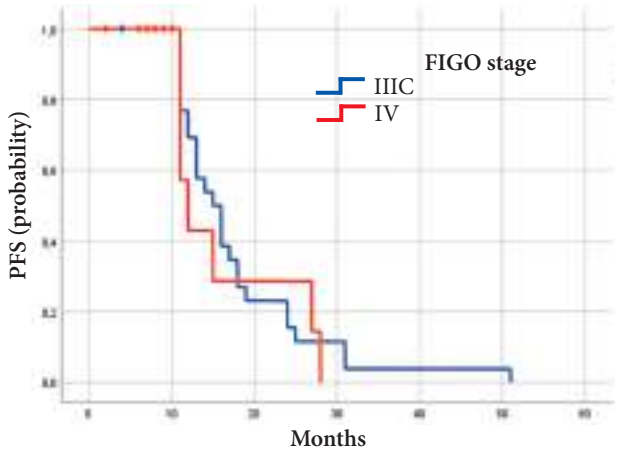


Graph 2. Patient distribution based on KELIM score in relation to surgical status and FIGO stage



Non-operated with KELIM<1	18	50% (9)	0	0	0	0	0
Operated with KELIM<1	30	56.7% (17)	16.7% (5)	10% (3)	3.3% (1)	3.3% (1)	0

Graph 3. PFS curves of patients with KELIM < 1 according to surgical status



IIIC with KELIM<1	32	62.9% (22)	14.3% (5)	8.6% (3)	2.9% (1)	2.9% (1)	0
IV with KELIM<1	13	30.8% (4)	0	0	0	0	0

Graph 4. PFS curves of patients with KELIM < 1 according to FIGO stage

confidence interval (95% CI: 0.75-5.37) suggests further validation is needed. **Graph 4** compares PFS across FIGO stages in the KELIM < 1 subgroup.

Discussion

Substantial efforts have been made to identify reproducible markers predicting bevacizumab efficacy in first-line therapy in advanced ovarian cancer [13, 14]. The KELIM score has recently emerged as a promising prognostic and predictive factor, identifying patients likely to benefit most from bevacizumab.

In our study, the KELIM score was not proven to be a statistically significant predictor of response to bevacizumab in the first-line therapy, based on PFS (p=0.549). Nonetheless, graphical representation suggested improved PFS in patients with KELIM ≥ 1. Multivariate analysis demonstrated statistically significantly longer PFS in operated patients compared to non-operated patients within the KELIM < 1 group (p=0.002), whereas FIGO stage did not prove to be a statistically significantly influence PFS in this population.

In 2022, You et al. [15] published a paper examining the relationship between intrinsic tumor chemosensitivity and the benefits of bevacizumab as the first-line treatment. This retrospective confirmatory analysis of the GOG-0218 trial, an international, multicenter, double-blind, placebo-controlled phase III clinical study, involved 1662 patients. The study demonstrated a statistically significant improvement in PFS OS with bevacizumab compared to placebo in patients with an unfavorable KELIM score (KELIM < 1). Conversely, no such benefit was observed in patients with a favorable KELIM score (KELIM ≥ 1). The most pronounced benefit was seen in patients with KELIM <1 and high-risk disease, specifically those with suboptimal surgical outcomes in FIGO stage IIIC and those in FIGO stage IV. Unlike their study, which compared bevacizumab and placebo groups, all patients in our study received bevacizumab. We observed that patients with KELIM ≥1 had longer PFS, as did those with KELIM <1 who underwent surgical treatment.

In 2020, You et al. [16] presented findings from the CHIVA study, a phase II clinical trial involving 134 patients treated with neoadjuvant chemotherapy, interval surgical treatment, and nintedanib or placebo. This study validated the independent predictive value of the KELIM score for tumor response rate, likelihood of complete surgical resection after neoadjuvant chemotherapy, risk of platinum-resistant relapse, PFS, and OS. Similar results were reported by Van Wagensveld et al. [17] in 2021. Their study, which analyzed 1,582 patients receiving neoadjuvant chemotherapy, identified the KELIM score as the most significant predictive factor for tumor response rate, complete surgical resection, PFS, and OS. Piedimonte et al. [18] conducted a 2022 study involving 217 patients with platinum-resistant tumor treated with neoadjuvant chemotherapy. They found that KELIM ≥1 was associated with statistically significantly longer PFS (p < 0.001) and OS (p = 0.014) compared to KELIM <1. Similarly, in 2023, Liontos et al. [19] evaluated the predictive value of the KELIM score on PFS and OS in real-world clinical settings among 99 patients receiving neoadjuvant chemotherapy. Their result confirmed a statistically significant correlation between the KELIM score and both PFS and OS. Moreover, they demonstrated adding

bevacizumab after neoadjuvant chemotherapy increased the statistical significance in regard to PFS and OS only in patients with an unfavorable KELIM score.

Our study is limited by the small sample size. Tumor marker CA-125 levels were measured in local laboratories, potentially introducing variability in the KELIM score due to non-standardized immunoassays. However, this variability is likely minimized by the scoring method, which utilizes multiple CA-125 measurements over time. Additionally, some patients were excluded from the final analysis due to insufficient CA-125 data, as the findings were self-reported by female patients. Another limitation is the small proportion of patients with a positive KELIM score (14.3%), which reduced statistical power and precluded their inclusion in the final multivariate analysis. Furthermore, surgeries were performed by different surgeons at various institutions, potentially affecting the quality of surgical resection and its influence on PFS in the operated group. Finally, since the patients were not prospectively monitored, OS data are unavailable, limiting this study to PFS evaluation.

Given that the KELIM score is a relatively new prognostic tool, primarily studied in controlled clinical trials with homogeneous patient populations, further research is required to validate its applicability in real-world clinical settings. Further studies should include diverse patient populations and explore its use across various therapeutic regimens, including bevacizumab and/or PARP inhibitors. These treatments, especially among of patients with BRCA 1/2 mutations, have shown a positive impact on survival and are increasingly used in the first-line therapy [20, 21].

Conclusion

Although our study did not find a statistically significant correlation between the Cancer Antigen-125 Elimination Rate Constant K score and progression-free survival in bevacizumab-treated patients, the findings has one significant limitation that is reflected in the small sample of patients, primarily those with a favorable the Cancer Antigen-125 Elimination Rate Constant K score. Certainly, the Cancer Antigen-125 Elimination Rate Constant K score calculator remains an accessible prognostic tool, and its utility in identifying patients who would benefit most from bevacizumab in the first-line therapy warrants further investigation. The patient operative status, on the other hand, proved to be the most significant factor contributing to survival, which is in line with the literature.

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BOTULINUM TOXIN-A THERAPY IN PEDIATRIC POPULATION – SINGLE-CENTER EXPERIENCE

TERAPIJA BOTULIN TOKSINOM A U PEDIJATRIJSKOJ POPULACIJI – ISKUSTVO JEDNE KLINIKE

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Abstract

Introduction. This study aims to evaluate the efficacy and safety of Botulinum toxin-A therapy in pediatric population with lower urinary tract symptoms. **Material and Methods**. A retrospective review was conducted on our initial clinical experience in treating lower urinary tract symptoms with Botulinum toxin-A at the Institute for Child and Youth Health Care of Vojvodina. Given the diversity of symptoms and the heterogeneity of underlying pathological conditions, each patient and their treatment outcomes were individually analyzed. **Results**. Our clinical experience revealed that 80% of the patients demonstrated significant improvement in symptoms following Botulinum toxin-A injections. Specifically, of the 17 patients treated, 15 reported symptom relief, with 7 achieving complete regression of lower urinary tract symptoms. Only two patients with developmental delays exhibited partial improvement, with persistent lower urinary tract symptoms. **Conclusion**. Botulinum toxin-A injections have shown promising efficacy in managing refractory bladder dysfunction in the pediatric population. The majority of patients experienced symptom regression, with many achieving complete remission. The treatment protocol was well-tolerated, with no adverse effects observed. However, the variability in treatment responses, particularly in patients with developmental delays, underscores the need for individualized treatment planning. Larger cohort studies with extended follow-up periods are needed to validate the long-term efficacy and safety of Botulinum toxin-A therapy in pediatric population. Among available therapeutic options, Botulinum toxin-A plays a significant role in improving the quality of life for children with lower urinary tract symptoms.

Keywords: Botulinum Toxins, Type A; Pediatrics; Child; Lower Urinary Tract Symptoms; Urinary Bladder, Overactive; Urinary Bladder Diseases; Treatment Outcome

Sažetak

Uvod. Cilj rada je procena efikasnosti i sigurnosti terapije botulin toksinom A kod pedijatrijske populacije, a u sklopu lečenja simptoma donjeg urinarnog trakta. **Materijal i metode.** Sprovedena je retrospektivna studija koja predstavlja pregled inicijalnog kliničkog iskustva u lečenju tegoba donjeg urinarnog trakta botulin toksinom A u pedijatrijskoj populaciji na Institutu za zdravstvenu zaštitu dece i omladine Vojvodine. Zbog velike raznovrsnosti tegoba i heterogenosti patoloških stanja, pacijenti i rezultati njihovih lečenja razmatrani su pojedinačno. **Rezultati.** Naše kliničko iskustvo pokazalo je da je 80 procenata pacijenata imalo značajno poboljšanje simptoma nakon injekcija botulin toksina A. Konkretno, 15 od 17 pacijenata prijavilo je smanjenje tegoba, a kod sedam dolazi do potpune regresije tegoba. Kod samo dva pacijenta, koji su praćeni zbog kašnjenja u razvoju, postignuto je delimično poboljšanje, uz perzistiranje simptoma donjeg urinarnog trakta. **Zaključak.** Upotreba injekcija botulin toksina A u pedijatrijskoj populaciji sa refraktornom disfunkcijom bešike, pokazala je obećavajuće rezultate. Kod većine pacijenata verifikovano je regrediranje tegoba, dok je značajan broj pacijenata bez ikakvih simptoma nakon tretmana. Pacijenti su dobro tolerisali terapijski protokol i nisu registrovani neželjeni efekti. Varijabilnost u samom odgovoru na terapiju, posebno kod pacijenata sa kašnjenjem u razvoju, ističe potrebu za individualizovanim planom lečenja. Kako bi se potvrdila dugoročna efikasnost i sigurnost primene botulin toksina A u pedijatrijskoj populaciji, potrebne su studije koje bi obuhvatile veći broj pacijenata i duži period praćenja. Opravdano, terapija botulin toksinom A ima značajno mesto u lečenju i unapređivanju kvaliteta života pacijenata sa simptomima u donjem urinarnom traktu.

Ključne reči: botulin toksin-A; pedijatrija; dete; simptomi donjeg urinarnog trakta; hiperaktivna bešika; bolesti mokraćne bešike; ishod lečenja

Introduction

Lower urinary tract symptoms (LUTS) encompass a wide range of issues stemming from various disorders involving the central or peripheral nervous systems. General classification of causes refers to any anatomic, neurogenic, or non-neurogenic conditions [1, 2]. Regardless of etiology, treatment aims to address two primary goals: optimizing bladder storage and ensuring effective bladder emptying. These measures are essential for preventing permanent damage to both the upper and lower urinary tract. In older children, the primary therapeutic challenge is achieving social continence and fostering independence in bladder man-

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Abbreviations

LUTS – lower urinary tract symptoms
ICCS – International Children's Continence Society
OAB – overactive bladder
IU – international unit

agement, which should persist into adulthood. Diagnosing bladder disorders in children typically involves physical examination, urinalysis and urine culture, biochemical tests, abdominal ultrasound, and urodynamic investigations. Additional tests such as cystography, renal scans, intravenous pyelography, and computed tomography may also be employed to assess the extent and severity of the condition. Early evaluation and appropriate treatment are crucial for minimizing or preventing damage to the upper urinary tract and the bladder, and maximizing safe, socially acceptable continence. Management strategies focus on reducing bladder pressures, increasing capacity and compliance. The International Children's Continence Society (ICCS) recommends urotherapy as the initial treatment for all types of LUTS. This non-invasive, non-pharmacological approach emphasizes bladder re-education or rehabilitation to optimize filling and voiding functions. Success rates for urotherapy range from 40 to 90 percent [3, 4]. This treatment protocol combines education and demystification, behavioral modification guidance, lifestyle adjustments regarding fluid intake, and tracking of voiding frequencies, volumes, and incontinence episodes, along with constant support and encouragement for both children and their guardians [2, 5]. Standard urotherapy is a valuable cost-free option as it is a purely behavioral intervention with no side effects that can sometimes lead to spontaneous resolution of the symptoms [2, 6]. When conservative measurements fail to provide sufficient results, pharmacological treatments are indicated. Antimuscarinic agents, particularly oxybutynin, are the first-line pharmacologic options for pediatric patients. Oxybutynin, accepted and validated for use in children for over 20 years, is the only antimuscarinic medication approved by the U.S. Food and Drug Administration for managing overactive bladder symptoms in children aged five and older. Unlike other anticholinergics, oxybutynin is a non-selective and, short-acting drug, and its dosage can be tailored to periods of peak symptoms [3]. Newer generation of antimuscarinics, including propiverine, tolterodine, and solifenacin, have recently gained approval for use in pediatric patients, although they are still less commonly prescribed than oxybutynin. While all antimuscarinics are considered safe and well-tolerated, certain side effects such as dry mouth, dizziness, headache, and constipation, occur in 3% to 23% percent of cases, sometimes prompting discontinuation [2, 3]. Additional pharmacological options include mirabegron and imipramine. Mirabegron is an innovative drug, recognized as the first ß3-adrenergic receptor agonist available for treating overactive bladder (OAB). While proven effective and safe in adults, its efficacy in pediatric patients remains under investigation. In severe OAB cases, combination therapy involving anticholinergic and β2-adrenergic receptor agonists offers a potent alternative for pediatric patients unresponsive to single-agent anticholinergic therapy [3]. Recent pediatric literature highlights Botulinum toxin-A as a treatment option for bladder disorders and lower urinary tract symptoms. The substance is effective in managing high bladder pressures and incontinence caused by both uninhibited bladder contractions and sphincter dysfunction. As evidence supporting its safety and efficacy grows, pediatric urologists increasingly adopt Botulinum toxin-A as a preferred long-term treatment of children with both non-neurogenic and neurogenic bladder disorders.

Material and Methods

We reviewed our initial clinical experience with pediatric patients treated with Botulinum toxin-A at the Institute for Child and Youth Health Care of Vojvodina. The patients included were those with neurogenic bladder, overactive bladder with daytime incontinence, and patients with non-monosymptomatic nocturnal enuresis. The inclusion criteria for the procedure were the absence of a clinical response to any of the treatment protocols such as standard urotherapy, biofeedback, medicaments (such as antimuscarinics and/or B3 agonists), clean intermittent catheterization, etc. For patients with neurogenic bladder, the indication for Botox instillation besides the positive findings on urodynamic testing was incontinence between catheterizations. For patients with therapy-resistant enuresis, inclusion required that the patient had been on a combined treatment regimen for at least 3 months, in addition to the diagnostic workup. The exclusion criteria were any contraindications to Botulinum toxin-A. All patients underwent a comprehensive diagnostic evaluation before the intervention, including mandatory urodynamic testing. Both pre and post-intervention data were collected through medical history. The data included symptoms of the lower urinary tract, such

as urgency, frequency, and urinary incontinence, as well as drug therapy, uroflowmetry, and urodynamic studies. Due to a great diversity of underlying conditions, all patients and their outcomes were presented individually.

Results

In this retrospective clinical study, we reviewed the medical records of 17 pediatric patients treated with Botulinum toxin-A (Botox) at the Institute for Child and Youth Health Care of Vojvodina, Department of Surgery. The cohort included 3 patients with overactive bladder (OAB), 7 with neurogenic bladder, 5 with non-monosymptomatic nocturnal enuresis, and 2 with other underlying conditions. All procedures were performed under general anesthesia, with intravenous antibiotic prophylaxis administered preoperatively. The intervention involved rigid cystoscopy and the intradetrusor injection of Botox, diluted in 10 ml of 0.9% saline. Twenty injections, each approximately 0.5 ml, were distributed evenly through the detrusor muscle, avoiding the trigone area, using a Deflux needle. The dosing was determined based on clinical experience rather than specific symptomatology or urodynamic parameters. A total of 14 patients received 100 international units (IU) of Botox, while 3 older patients (aged over 15 years) received 200 IU. Post-intervention catheterization was maintained for 48 hours for all patients. To maximize the bladder volume and facilitate continence, all patients received urotherapist-guided bladder training following the procedure.

Among the 7 patients with neurogenic bladder, 4 had myelomeningocele, 1 had a spinal cord injury, 1 had undergone pelvic neuroblastoma surgery, and 1 had for a history of sacral teratoma surgery. Of the 3 patients with OAB, 2 achieved complete symptom resolution after a single treatment. The remaining patient, a female now over 18 years old, continues to experience symptoms. Among the 5 patients with therapy-resistant enuresis, 3 became symptom-free after one treatment, while 2 with developmental delays continued to experience symptoms; one received a single treatment, and the other received two treatments. Additionally, two patients were not classified within any category due to their underlying conditions.

The first, diagnosed with urinary incontinence, exhibited bladder dysfunction confirmed through urodynamic studies. Extensive evaluations by neurosurgeons, neurologists, and gastroenterologists revealed no significant dysfunctions. After radiography and MRI of the lumbosacral region, the treatment regimen started involving Macrogolaxan and other supportive therapies. The patient underwent an initial Botulinum toxin-A treatment (100 IU) coupled with biofeedback therapy, followed by a second Botox intervention, which reinforced the positive outcomes of the first intervention, resulting in a marked reduction in incontinence.

The second patient initially presented with urinary retention and hematuria. An abdominal ultrasound identified a bladder wall lesion protruding into the lumen. Cystoscopy with biopsy revealed a lesion at the entrance of the bladder, with histopathological analysis confirming the diagnosis of Rhabdomyosarcoma. Surgical treatment was performed at a specialized center abroad, with administration of neoadjuvant therapy followed by partial resection of the bladder, and brachytherapy targeting the bladder and prostate. The completion of postoperative chemotherapy finalized the primary treatment course. After completing the tumor treatment, the patient continued to experience discomfort such as recurrent episodes of itching and increased urinary frequency, sometimes associated with incontinence. To manage these persistent symptoms, the patient underwent cystoscopy with instillation of Botulinum toxin-A into the bladder wall (100 IU), and was prescribed Solifenacin 5 mg once a day.

Both of these two additional cases highlight the potential efficacy and safety of Botulinum toxin-A in managing refractory LUTS, emphasizing the need for individualized treatment plans in addressing challenging bladder dysfunctions in pediatric patients.

Discussion

Lower urinary tract symptoms affect individuals across all age groups, with two notable incidence peaks, one in childhood and another in adulthood (ages over 40). The overall prevalence of LUTS is estimated to range between 25% and 45%, with 20% to 30% of children experiencing dysfunctional voiding and related symptoms, such as incontinence and enuresis [5, 7, 8]. The causes are typically classified into three main groups: 1) anatomic, 2) neurogenic, and 3) non-neurogenic. Anatomic and neurogenic causes, including posterior urethral valves and spinal dysraphisms, require immediate recognition and management, which extends beyond the scope of this paper. Conversely, non-neurogenic causes, including OAB, voiding postponement, and dysfunctional voiding, stem from a complex interaction of behavioral, genetic, and functional factors [5]. Dysfunctional voiding, also re-

ferred to as "non-neurogenic bladder" or "Hinman-Allen Syndrome", was initially described by Hinman and Baumann in 1973. They observed a group of children who, despite having no signs of neurologic disease, exhibited abnormal non-relaxation of the external sphincter during voiding. Although various patterns of dysfunctional voiding have been identified, they all share the common feature of sphincter non-relaxation during the voiding process [6]. LUTS can manifest as isolated symptoms or may be associated with and/or worsened by any accompanying disorder that affects the nervous system, such as myelomeningoceles, Parkinson's disease, or stroke [2]. In children, LUTS can also be a symptom of various non-neurological voiding disorders. The most common of these include dysfunctional voiding, detrusor overactivity disorder, and giggle incontinence [2–4]. Daytime urinary incontinence, affecting 7% to 10% of children aged 5 to 13, and nocturnal enuresis, defined as sleep-associated incontinence, are particularly prevalent. Monosymptomatic nocturnal enuresis refers to bedwetting without other LUTS, whereas non-monosymptomatic nocturnal enuresis involves bedwetting accompanied by daytime symptoms of the lower urinary tract. Three primary causes of nocturnal enuresis include nocturnal polyuria, detrusor overactivity, and an increased arousal threshold during sleep [3]. As the child matures, voiding gradually comes under voluntary control. The first awareness of bladder function typically occurs around the age of 1 to 2 years, when toilet training is often initiated. Given that each child develops at own pace, it is expected that the adult-like voiding pattern will be established by ages 3 to 5. Consequently, any form of lower urinary tract dysfunction is considered physiological in children up to 5 years old. Beyond this age, any symptoms such as recurrent urinary tract infections (UTIs), incontinence, frequency, hesitancy, and holding maneuvers, should be carefully evaluated [2]. Child bladder capacity is estimated by two formulas: for children younger than 2, the formula is 7 x weight in kg, and for older ones (ages in years + 1) x 7. As bladder capacity increases and the cortical pathways regulating detrusor and sphincter function continue to develop, there is a corresponding decrease in voiding frequency. This frequency typically reduces from around 24 times per day at birth to about 4 to 6 times daily by the age of 4 [2, 5].

Diagnostic tools include thorough medical history, physical examinations, bladder diaries, questionnaires, urinalysis, post-void residual volume measurement, flowmetry, and ultrasound [3, 9]. The primary objective of these initial assessments is to identify the specific subtype of LUTS, evaluate the frequency and severity of the symptoms, assess their impact on the patient's quality of life, and determine the appropriate level of treatment required [7]. A detailed voiding and medical history explores voiding frequency, sensation of urgency, hesitancy, feelings of complete or incomplete emptying, daytime and/or night-time incontinence, and the quality of the urinary stream. This helps physicians determine patterns and triggers associated with a particular subtype of disorder. After the detailed anamnesis, a physical examination focuses on anatomic abnormalities and other possible contributing causes [1]. While, the majority of physical examinations of the abdomen, lower back, and genitalia are regular, the examination can also identify a distended bladder, full rectal ampulla, signs of an occult spinal dysraphism, or other malformations of the external genitalia (phimosis, meatal stenosis, or labial fusion). Bladder diaries are typically recorded on paper charts or through various smart device applications, tracking both voided volumes and liquid intake. Most physicians who specialize in voiding disorders emphasize the importance of using incontinence questionnaires. Over the past few decades, several voiding and elimination questionnaires have been developed specifically for children with voiding disorders, with the Dysfunctional Voiding Symptom Score being the most widely used [3]. Once a comprehensive voiding history is gathered, further diagnosis steps are taken. Initially, urine studies, including urinalysis and urine culture, are conducted to rule out urinary tract infections as a potential cause of the symptoms. The post-void residual calculation is also useful if the child is suspected for overflow incontinence. It can be measured using either ultrasound or intermittent bladder catheterization after the patient has emptied their bladder fully to determine the amount of urine left in the bladder. Volumes greater than 200 ml are indicative of overflow incontinence, while a residual volume of less than 50 ml effectively rules out overflow incontinence as a contributing factor to a patient's symptoms [1]. Transabdominal ultrasound is a valuable tool for visualizing the bladder, assessing bladder wall thickness, determining whether the bladder neck is open or closed, and measuring the diameter of the rectum [2, 3]. Uroflowmetry, as a next step in the diagnostic protocol, provides a graphical recording of the urinary stream, offering insights into voided volume, flow time, initial stream velocity, maximal flow rate, and the flow pattern.

Cystometry and urodynamics are also beneficial diagnostic tools [3]. According to the ICCS, a conservative approach should be prioritized in treatment strategies. If necessary, pharmacological treatment may be added but always combined with other treatment modalities [3, 7]. As mentioned above, Botulinum toxin injections are one of the options for children with idiopathic detrusor overactivity, refractory to non-invasive procedures [10]. Botulinum toxin is a potent neurotoxin produced by the facultative anaerobic bacteria Clostridium botulinum, discovered and used for medicinal purposes in 1897 by Van Ermegam [11]. For over 30 years, Botulinum toxin-A has been utilized in treating lower urinary tract dysfunction, and it is widely used to restore urinary continence and enhance quality of life. Its effectiveness stems from its ability to inhibit the calcium-mediated release of acetylcholine vesicles at the presynaptic neuromuscular junction in peripheral nerve endings, leading to temporary flaccid muscle paralysis [12]. The mechanism involves inhibiting the abnormal release of neurotransmitters like acetylcholine, adenosine triphosphate, and substance P, as well as the abnormal expression of transient receptor potential cation channel subfamily V member 1 (TRPV1) and the purinergic receptor (P2X3). These neurotransmitters play a role in bladder sensation and inflammation, influencing detrusor muscle contraction [13]. Subureteral toxin injection causes relaxation of striated/smooth muscles and helps control local inflammation. This action increases bladder capacity, reduces intravesical pressure, and leads to an overall improvement in continence. Currently, there are seven serotypes of Botulinum toxin-A (A, B, C1, D, E, F, G), all of which are derivatives of the toxin produced by Clostridium botulinum. Among these, Botulinum toxin-A is the most commonly used for medicinal purposes in humans. The three commercially available formulations of the toxin are Botox, Dysport, and Myobloc [11]. Botulinum toxin is applied intravesically under general anesthesia, with rigid cystoscopy and injections equally distributed into the detrusor muscle. When comparing treatment outcomes across various doses of Botox, it was observed that 100 international units (IU) achieved a 73.3 percent success rate, with fewer adverse events occurring at this dose compared to higher ones. Based on these findings, along with results from other clinical studies, 100 IU of Botulinum toxin-A has become a standard dose that delivers satisfactory outcomes. A double-blind, placebo-controlled, randomized, dose-ranging trial that tested doses of 50, 100, 150, and 200 IU of Botulinum toxin-A found that the 100 IU dose was particularly effective [13]. The higher dose of BTX-A showed a favorable response in the short term but outcomes in the long term were similar between the two groups [12]. The lethal dose of Botulinum toxin for humans is approximately 3000 IU (39 IU per kilogram of body weight), and must be administered intravascularly to achieve lethality [11]. The effects of Botulinum toxin injections typically last 6 to 9 months, which is significantly longer than if the toxin is injected into skeletal muscle. On the other hand, therapeutic effects were found to last longer with repeated Botox injections compared to a single injection. Administering Botox injections every 6 months extended the duration of therapeutic benefits. It is recommended that the interval between retreatments should not be shorter than 3 months, with a preferred interval of 6 to 9 months. This period is recommended to prevent the development of residual circulating antibodies that could diminish the efficacy of subsequent Botox injections [13]. One of the primary drawbacks of this procedure in pediatric patients is the necessity for general anesthesia [14]. Aside this, there are relatively few reported side effects [15]. The most common reasons for discontinuing Botox treatment include inadequate efficacy and issues related to the need for clean intermittent catheterization. Importantly, there have been no reports of serious systemic adverse events, like respiratory depression or generalized muscle weakness, following Botox injection. The most frequently observed local adverse events include gross hematuria, a significant post-void residual volume exceeding 150 mL, difficulty urinating, and urinary tract infections [13, 12]. The primary disadvantage of Botox treatment is its transient effect, typically lasting no more than 6 months, necessitating repeated injections. This requirement for ongoing re-injections can be particularly challenging for patients transitioning to adult care centers [16].

Conclusion

Intravesical Botulinum toxin type A injections have emerged as a promising therapeutic option for pediatric patients. Accordingly, our results demonstrate that intravesical Botulinum toxin-A therapy is a safe and effective treatment option for pediatric patients with neurogenic, non-neurogenic, and refractory overactive bladder. The majority of the patients included in our study showed significant symptom improvement, including reduced urinary incontinence and enhanced

bladder compliance. While generally well-tolerated, individual responses may vary, particularly in patients with other underlying conditions. Further studies with larger cohorts and long-term follow-ups are essential to optimize the dosing strategies and understand the long-term benefits and potential risks associated with repeated injections. Nevertheless, Botulinum toxin-A represents a valuable addition to the therapeutic options available in pediatric urology, improving the quality of life for children with challenging bladder dysfunctions and lower urinary tract symptoms.

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DIFFERENCES IN HEMODYNAMIC PARAMETERS IN GENERAL AND SPINAL ANESTHESIA DURING LOWER LIMB ORTHOPEDIC SURGERIES

RAZLIKE U HEMODINAMIČKIM PARAMETRIMA KOD OPŠTE I SPINALNE ANESTEZIJE TOKOM ORTOPEDSKIH OPERACIJA DONJIH EKSTREMITETA

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Abstract

Introduction. Hemodynamic instability is a frequent adverse effect during anesthesia and one of the significant parameters in determining the type of anesthesia. This study examined the incidence of hypotension and bradycardia during general and spinal anesthesia in hip and knee arthroplasty, alongside potential causes. We hypothesized that hypotension occurs more frequently with general anesthesia, while bradycardia is more prevalent with spinal anesthesia. **Material and Methods.** This retrospective study included patients who underwent total hip or knee alloarthroplasty. Data regarding blood pressure, heart rate, long-term antihypertensive therapy, and comorbidities were extracted from medical records. Patients were divided into two groups based on the type of anesthesia administered: general or spinal anesthesia. **Results.** Among 371 patients, 105 experienced bradycardia, and 308 developed hypotension based on systolic blood pressure measurements. Hypotension was significantly more common in patients receiving general anesthesia, with the lowest mean arterial blood pressure observed in 41 patients (59.4%). However, there was no significant difference in the incidence of bradycardia between spinal and general anesthesia. Similarly, no significant differences were identified in the incidence of hypotension and bradycardia between standard or unilateral spinal anesthesia. Chronic essential hypertension was associated with occurrence higher incidence of bradycardia (86.7%). Additionally, antihypertensive therapy was linked to increased occurrences of both hypotension (134 patients, 43.5%) and bradycardia (74 patients, 70.5%). **Discussion/Conclusion.** Intraoperative hypotension is more prevalent in patients undergoing general anesthesia. However, no significant difference in the occurrence of bradycardia was observed between general and spinal anesthesia. Essential hypertension and long-term antihypertensive therapy are potential risk factors for bradycardia and hypotension during anesthesia.

Key words: Hemodynamic Monitoring; Anesthesia, General; Anesthesia, Spinal; Hypotension; Bradycardia; Risk Factors

Sažetak

Uvod. Hemodinamička nestabilnost je čest neželjeni događaj tokom anestezije. Kako je ovo jedan od značajnih parametara pri izboru vrste anestezije, istraživali smo učestalost hipotenzije i bradikardije kod opšte i spinalne anestezije tokom aloartroplastike kuka i kolena kao i njihove potencijalne uzroke. Pretpostavili smo da je hipotenzija češća u opštoj, a bradikardija u spinalnoj anesteziji. **Materijal i metode.** Istraživanje je sprovedeno kao retrospektivna studija koja je obuhvatila pacijente kojima je izvršena aloartroplastika kuka ili kolena. Iz protokola za anesteziju su preuzeti podaci o vrednostima krvnog pritiska i pulsa, podaci o hroničnoj antihipertenzivnoj terapiji i komorbiditetima. Pacijenti su podeljeni u dve grupe prema vrsti anestezije (opšta i spinalna). **Rezultati.** Od ukupno 371 pacijenta, kod 105 se javila bradikardija, dok je kod 308 došlo do hipotenzije. Pacijenti koji su primili opštu anesteziju imali su značajno češće prisutnu hipotenziju prema vrednostima najnižeg zabeleženog srednjeg arterijskog pritiska (41; 59,4%). Nije pronađena statistički značajna razlika u pojavi bradikardije kod pacijenata u opštoj i spinalnoj anesteziji. Nije pronađena statistički značajna razlika u pojavi hipotenzije i bradikardije kod pacijenata koji su primili standardnu ili unilateralnu spinalnu anesteziju. Hronična esencijalna hipertenzija povezana je sa češćom pojavom bradikardije kod pacijenata (86,7%). Hronična antihipertenzivna terapija je povezana sa češćom pojavom hipotenzije (134, 43,5%) i bradikardije (74, 70,5%) kod pacijenata u toku anestezije. **Diskusija/Zaključak.** Hipotenzija se češće javlja kod pacijenata u opštoj anesteziji. Ne postoji statistički značajna razlika u pojavi bradikardije tokom opšte i spinalne anestezije. Značajni faktori rizika za pojavu hipotenzije i bradikardije tokom anestezije su hronična esencijalna hipertenzija i antihipertenzivna terapija.

Ključne reči: hemodinamski monitoring; opšta anestezija; spinalna anestezija; hipotenzija; bradikardija; faktori rizika

Introduction

Orthopedic surgeries can be performed under general anesthesia, neuraxial block (spinal anesthesia), or peripheral or central blocks. The choice of anesthesia is determined by anesthesiologists, considering factors such as the patient's general condition, age and previous anesthetic complications. The potential advantages and risks, including absolute and relative contraindications, are also taken into consideration [1].

Hemodynamic instability is a common adverse event during both general and spinal anesthesia [2].

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Abbreviations

ASA – American Society of Anesthesiologists
ACE – angiotensin-converting enzyme
ARB – angiotensin II receptor blocker
SBP – systolic blood pressure
MAP – mean arterial pressure
CCB – calcium chanel blocker

Both types of anesthesia suppress the sympathetic nervous system. If the patient is on antihypertensive therapy such as blockers of the renin-angiotensin-aldosterone system blockers (which also reduce adrenergic receptor sensitivity), the likelihood of refractory hypotension increases significantly [3].

Guidelines recommend maintaining systolic blood pressure during surgery at ≥90% of pre-anesthesia values, and preventing it from dropping below 80% [4]. The Oxford Handbook of Anesthesia recommends maintaining mean arterial pressure above 65 mmHg or 80% of baseline values during surgery [5].

The aim of this study was to analyze changes in hemodynamic parameters (blood pressure and heart rate) during general and spinal anesthesia in hip and knee alloarthroplasty. Three hypotheses were proposed before starting the research:

1. Hypotension is more frequent during general anesthesia than spinal anesthesia in hip and knee alloarthroplasty
2. Bradycardia is more frequent during spinal anesthesia than general anesthesia in hip and knee alloarthroplasty
3. Hypotension is more frequent in patients on chronic antihypertensive therapy (renin-angiotensin-aldosterone system inhibitors).

Material and Methods

The retrospective study was conducted at the Clinic for Anesthesia, Intensive Care and Pain Therapy, University Clinical Center of Vojvodina, after obtaining the approval of the University Clinical Center of Vojvodina Ethics Committee. The study covered the period from July 2022 to December 2022 and included medical records of 371 patients undergoing primary hip or knee alloarthroplasty.

The following data were extracted from the anesthesia protocols: procedure type, demographic data (sex, age, body mass, height), ASA (American Society of Anesthesiologists) status, comorbidities (essential arterial hypertension and diabetes mellitus), chronic antihypertensive therapy (angiotensin-converting-enzyme (ACE) inhibitors, beta blockers, calcium channel antagonists, diuretics, and angiotensin II receptor blockers (ARBs), type of anesthesia, blood pressure and heart rate. Comorbidities were recorded as they might contribute to blood pressure and heart rate values.

Blood pressure, heart rate, and their lowest recorded values were taken from the anesthesia protocols at the beginning of surgery. Patients were divided into two groups based on the type of anesthesia: 176 patients received general balanced anesthesia, while 195 received spinal anesthesia (57 of whom received unilateral spinal anesthesia). Patients receiving both general and spinal anesthesia were excluded from our study.

Hypotension during anesthesia was defined as a drop in systolic blood pressure >20% from baseline or a drop in mean arterial pressure < 65 mmHg. Like in previous studies, we used both definitions of hypotension to check for any differences. Bradycardia was defined as a drop in heart rate <50 beats per minute.

Descriptive statistics (measures of central tendency (arithmetic mean), variability (standard deviation), and relative numbers (structure indicators)) were used for the analysis of primary data. Predictive analyses included parametric (Student's t-test) and non-parametric tests. Hypotheses were tested at the statistical significance level of ($\alpha = 0.05$. Results are presented in tables and graphs. Data was processed using standard statistical packages (IBM SPSS Statistics 26).

Results

The study included 371 patients (146 males (39.4%), 225 females (60.6%)), aged 39 – 86 years. The median age of the patients is 68 years, and the mean value is 66.81 years. Of the total of 371 patients, 176 patients (47.4%) received balanced general anesthesia, and 195 patients (52.6%) received regional anesthesia (138 (70.8%) spinal, 57 (29.2%) unilateral spinal).

A statistically significant difference was observed in mean values of systolic blood pressure (SBP) ($t=-3.84$; $p<0.001$) and mean arterial pressure (MAP) ($t=-3.49$; $p<0.001$) between the groups of general and spinal anesthesia. No difference was observed in mean heart rate values in patients receiving different types of anesthesia ($t=1.67$; $p=0.09$). Mean SPB in patients under general anesthesia was 96.48±13.79, compared to 102.22±14.95 in patients under spinal anesthesia. Mean MAP in patients under general anesthesia was 72.97±11.31, compared to 77.24±12.05 in patients under spinal anesthesia.

There was no statistically significant difference in the mean pulse values ($t=0.05$; $p=0.96$). However, a statistically significant difference was observed in the mean values of the lowest SBP ($t=6.24$; $p<0.001$) and the lowest MAP ($t=5.08$; $p<0.001$) in patients who received standard spinal or unilateral spinal anesthesia. Specifically, the mean lowest SBP was 105.98±14.28

Table 1. Incidence of hypotension according to the mean arterial pressure

Type of anesthesia	Hypotension according to the MAP		Total	P
	Non-hypotensive	Hypotensive		
General	135	41	176	0.03
	44.7%	59.4%	47.4%	
Spinal	167	28	195	
	55.3%	40.6%	52.6%	
Total	302	69	371	
	100%	100%	100%	

MAP – mean arterial pressure

for patients under spinal anesthesia and 93.05±12.63 for those under unilateral spinal anesthesia. Similarly, the mean lowest MAP was 79.84±11.49 in the spinal anesthesia group and 70.50±11.19 in the under unilateral spinal anesthesia group.

In our study, 105 patients (28.3%) developed bradycardia (<50/min), while 308 patients (83%) experienced hypotension based on the SBP value recorded at the beginning of operation (a drop below 80% of the baseline value). Furthermore, 280 patients (75.5%) were hypotensive based on the MAP value recorded at the beginning of operation (a drop below 80% of the baseline value), and in 69 patients (18.6%), the MAP value fell below 65 mmHg.

There was no statistically significant difference in the frequency of bradycardia between the general anesthesia and spinal anesthesia groups (χ^2=0.002: p=0.99). Bradycardia occurred in 28.4% of patients under general anesthesia and in 28.2% of patients under spinal anesthesia.

There was no statistically significant difference in the frequency of hypotension based on the lowest recorded SBP between the general and spinal anesthesia group (χ^2=1.831; p=0.21). Based on SBP values, hypotension occurred in 151 patients (85.6%) who received general anesthesia and 157 patients (80.5%) who received spinal anesthesia.

However, hypotension based on the lowest recorded MAP occurred more frequently in the general anesthesia group (41; 59.4%) compared to the group of patients who received one form of regional anesthesia (167; 55.3%) (χ^2=4.88; p=0.03) **(Table 1).** Specifically, the frequency of hypotension based on MAP was 23.3% in the general anesthesia group and 14.4% in the spinal anesthesia group.

There was no statistically significant difference in the presence of bradycardia (χ^2=1.159; p=0.3), hypotension based on SBP (χ^2=1.53; p=0.24), or hypotension based on MAP (χ^2=0, 85; p=0.39) between patients who received spinal anesthesia and those who received unilateral spinal anesthesia.

A significant difference was found between hypertension as a comorbidity in patients with and the ones without bradycardia (χ^2=7.61; p=0.006). There was a higher percentage of hypertensive patients with bradycardia (86.7%) compared to the percentage of hypertensive patients with no bradycardia (73.3%).

It was found that significantly higher percentage of patients with diabetes mellitus did not develop bradycardia (21.1%) compared to the ones with diabetes mellitus who developed bradycardia (8.6%) (χ^2=8.12; p=0.004).

No significant differences were observed in the use of ACE inhibitors among patients with and without bradycardia (χ^2=0.005; p=0.941), or in patients with and without hypotension based on SBP (χ^2=0.25; p=0.619) or MAP (χ^2=0.07; p=0.79).

However, a higher percentage of patients who used calcium channel blockers developed bradycardia during the intervention (39%) compared to those who

Table 2. Incidence of bradycardia according to the use of calcium channel blockers and angiotensin receptor blockers

CCB	Bradycardia		Total	p	ARB	Bradycardia		Total	p
	No	Yes				No	Yes		
No	193	64	257	0.03	No	244	85	329	0.003
	72.6%	61%	69.3%			91.7%	81%	88.7%	
Yes	73	41	114		Yes	22	20	42	
	27.4%	39%	30.7%			8.3%	19%	11.3%	
Total	266	105	371		Total	266	105	371	
	100%	100%	100%			100%	100%	100%	

CCB – calcium channel blockers; ARB – angiotensin receptor blocker

Table 3. Incidence of bradycardia and hypotension according to the use of antihypertensive therapy

Antihypertensives	Bradycardia		Total	Hypotensive SBP		Total	p
	No	Yes		No	Yes		
No	194	31	225	51	174	225	<0.001
	72.9%	29.5%	60.6%	81%	56.5%	60.6%	
Yes	72	74	146	12	134	146	
	27.1%	70.5%	39.4%	19%	43.5%	39.4%	
Total	266	105	371	63	308	371	
	100%	100%	100%	100%	100%	100%	

SBP – systolic blood pressure

did not (27.4%) (χ^2=4.76; p=0.03) **(Table 2).** Similarly, a higher percentage of patients using ARBs developed bradycardia (19%) compared to those who did not (8.3%) (χ^2=8.71; p=0.003) **(Table 2).**

No statistically significant association was found between the use of ARBs and hypotension based on SBP (χ^2=1.87; p=0.17) or MAP (χ^2=1.8; p=0.18).

However, a statistically higher percentage of patients using antihypertensive therapy developed bradycardia (70.5%) compared to those who did not (27.1%) (χ^2= 59.44; p<0.001) **(Table 3).** Similarly, a higher percentage of patients using antihypertensives were hypotensive based on SBP (43.5%) compared to those who were not hypotensive (19%) (χ^2=13.11; p<0.001) **(Table 3).**

Discussion

In this study, 28.3% of patients developed bradycardia, while 83% developed hypotension based on their SBP. The frequency of hypotension during general anesthesia varies widely in the literature (16–93%) [6–9]. In our study, hypotension occurred in 86% of patients under general anesthesia based on SBP and in 23.3% based on MAP. For spinal anesthesia, hypotension occurred in 80.5% of patients based on SBP and 14.36% based on MAP, which is higher than previously reported frequencies of 16-33% [10]. This discrepancy may be attributed to variations in definitions of intraoperative hypotension and the use of different parameters (SBP vs. MAP). Moreover, Südfeld S et al. found that older age may be a potential risk factor for intraoperative hypotension, and the majority of patients included in our research belong to this age group [11].

Our findings support our first hypothesis that hypotension based on MAP occurs more frequently in patients under general anesthesia, consistent with the study by Messina et al. [12].

However, no significant difference in hypotension or bradycardia frequency was observed between patients who received standard spinal anesthesia and those who received unilateral spinal anesthesia, contrary to previous studies suggesting that unilateral blocks, compared to standard spinal anesthesia, can reduce the frequency of hemodynamic instability during orthopedic surgeries [13].

The occurrence of bradycardia was similar between general and spinal anesthesia, with rates of 28.4% and 28.2%, respectively. These results align with earlier studies reporting bradycardia frequencies of 9-74% during spinal anesthesia [14], and 27.3-34.7% during general anesthesia [15, 16]; therefore, our second hypothesis was not confirmed.

The results show that certain comorbidities are associated with the frequency of bradycardia occurrence. Specifically, bradycardia was more common in hypertensive patients (70.5%), likely due to the use of ARBs and calcium channel blockers, which our study proved to contribute significantly to intraoperative bradycardia.

Cardiovascular autonomic neuropathy, characterized by dysregulation of the sympathetic and parasympathetic nervous systems leading to cardiovascular and respiratory disorders, is a common complication in patients with diabetes mellitus. A recently published case report by Lee et al. described a diabetic patient who experienced a significant drop in blood pressure (70/40 mmHg) and a heart rate below 20 beats per minute after induction of anesthesia [17]. The *Oxford Handbook of Anesthesia* identifies autonomic neuropathy in diabetic patients as a risk factor for tachycardia, bradycardia, and postural hypotension [5]. While hemodynamic instability is an expected complication in such patients, our findings revealed an even rarer occurrence of bradycardia. This phenomenon could generally be attributed to a decrease in baroreceptor sensitivity in patients with diabetes mellitus.

A comparison of chronic antihypertensive therapy and intraoperative hemodynamic instability showed a higher incidence of bradycardia in patients on chronic therapy with ARBs and calcium channel blockers, consistent with a Canadian study from 2015 [15]. Our study demonstrated that chronic antihypertensive therapy is a significant factor in the development of hemodynamic instability, including both hypotension and bradycardia.

One study reported a significant incidence of hypotension in patients who received spinal anesthesia while on chronic antihypertensive therapy with calcium channel blockers, attributing this by the vasodilator effect of calcium blockers combined with spinal anesthesia. The same study also noted a higher incidence of bradycardia in patients on chronic beta-blocker therapy [18]. However, our findings differed: bradycardia was associated with the chronic use of calcium channel blockers. Importantly, our study examined the overall statistically significant occurrence of hemodynamic instability in patients on antihypertensive therapy rather than separating patients based on spinal or general anesthesia.

Some studies have observed severe circulatory side effects following anesthesia induction in patients on chronic ACE inhibitors and ARBs [15, 19]. While our study found occurrence higher incidence of bradycardia in patients on ARBs, it did not reveal a significant association between the chronic use of renin-angiotensin-aldosterone system blockers and intraoperative hypotension, failing to confirm our third hypothesis. Given the potential contribution of chronic antihypertensive therapy (ARBs and ACE inhibitors) to intraoperative hypotension, the *Oxford Handbook of Anesthesia* recommends discontinuing these medications 24 hours before surgery to reduce the risk of intraoperative cardiovascular instability [5]. The absence of data on whether the patients refrained from taking these drugs on the day of surgery may have influenced our results.

A case report from 2020 described an elderly patient who underwent elective hip-replacement surgery with spinal anesthesia and subsequently experienced a sudden deterioration in general condition, characterized by tachypnea, decreased oxygen saturation, and neck cyanosis. Noninvasive monitoring revealed hemodynamic instability followed by hypotension and periodic cardiac arrhythmia manifesting with ventricular extrasystoles [20]. While anesthetic agents and antihypertensives are common causes of hemodynamic instability, it can also be a nonspecific symptom of pulmonary thromboembolism, which is yet another reason to investigate all circumstances surrounding intraoperative hemodynamic instability.

Conclusion

Hypotension and bradycardia are frequent complications during both general and spinal anesthesia. General anesthesia is associated with a higher risk of hypotension, making an important consideration when selecting the type of anesthesia, particularly for patients with high perioperative cardiovascular risk. Intraoperative hypotension and bradycardia occur more frequently in patients on chronic antihypertensive therapy, highlighting the need for careful perioperative management of these drugs. Our results did not show a significant difference in the incidence of bradycardia between general and spinal anesthesia. Based on our findings, spinal anesthesia carries a lower risk of intraoperative hypotension. Given the clinical importance of this issue, further studies are needed to determine the incidence of intraoperative hypotension and bradycardia under different types of anesthesia and to more precisely define all associated risk factors.

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ARE THERE ANY DIFFERENCES IN PAIN THRESHOLDS DURING THE MENSTRUAL CYCLE?

POSTOJE LI RAZLIKE U PRAGU BOLA U RAZLIČITIM FAZAMA MENSTRUALNOG CIKLUSA?

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Abstract

Introduction. Pain perception in women may vary due to hormonal fluctuations across the phases of the menstrual cycle. Emotional status also tends to fluctuate significantly during these phases. This study aimed to investigate differences in pressure pain thresholds between the follicular and luteal phases of the menstrual cycle. An additional objective was to evaluate variations in emotional status. **Material and Methods.** The phases of the menstrual cycle were determined using an online ovulation calculator. Participants in the ovulation phase (n=5) were excluded from further analysis. A total of 95 participants (mean age 27.7±7.8 years) were included in the study. Pressure pain threshold testing was conducted on the extensor carpi radialis longus muscle and the paraspinal musculature of the lumbar region using an algometer equipped with a 1 cm^2 rubber tip. The Depression, Anxiety, Stress Scale was employed to evaluate variations in emotional status. **Results.** No significant differences were observed in pressure pain thresholds in the forearm region (follicular phase (33.55 ± 12.15 N/cm^2) vs. luteal phase (33.55 ± 13.65 N/cm^2), t=0.509; p=0.979) or the lower back region (follicular phase (56.85 ± 19.95 N/cm^2) vs. luteal phase (58.93 ± 21.20 N/cm^2), t=0.982; p=0.619) between the follicular and luteal phases of the menstrual cycle. Similarly, no significant differences were identified in levels of depression (χ^2 = 1016.000; p = 0.392), anxiety (χ^2 = 972.500; p=0.243), or stress levels (t = -1.038; p=0.302) during the menstrual cycle. **Conclusion.** The findings indicate no significant variations in pressure pain thresholds or emotional status across different phases of the menstrual cycle.

Key words: Pain Perception; Pain Threshold; Menstrual Cycle; Emotional Regulation

Sažetak

Uvod. Percepcija bola kod žena može varirati usled hormonskih promena tokom različitih faza menstrualnog ciklusa. Takođe, tokom menstrualnog ciklusa može doći i do značajnih promena emocionalnog statusa. Cilj studije bio je da se utvrdi razlika u pragu bola na pritisak kod žena tokom folikularne i lutealne faze menstrualnog ciklusa. Dodatni cilj bio je da se procene razlike u emocionalnom statusu tokom menstrualnog ciklusa. **Materijal i metode.** Faza menstrualnog ciklusa utvrđena je pomoću onlajn ovulacionog kalkulatora. S obzirom da je samo pet ispitanica bilo u fazi ovulacije, one nisu uključene u dalju analizu. U istraživanje je uključeno 95 ispitanica (prosečne starosti 27,7 ± 7,8 godina). Testiranje praga bola pritiskom vršeno je na *m. extensor carpi radialis longus* i na paraspinalnoj muskulaturi lumbalnog dela kičmenog stuba koristeći algometar sa gumenim vrhom površine 1 cm^2. Za procenu emocionalnog statusa korišćena je Skala depresije, anksioznosti i stresa. **Rezultati.** Nisu zabeležene značajne razlike u pragu bola pritiskom na podlaktici (folikularna faza (33,55 N/cm^2 ± 12,15 N/cm^2) vs. lutealna faza (33,55 N/cm^2 ± 13,65 N/cm^2), t = 0,509; p = 0,979) i u lumbalnoj regiji (folikularna faza (56,85 N/cm^2 ± 19,95 N/cm^2) vs. lutealna faza (58,93 N/cm^2 ± 21,20 N/cm^2), t = 0,982; p = 0,619) među ispitanicama u folikularnoj i lutealnoj fazi menstrualnog ciklusa. Nisu pronađene značajne razlike u depresivnosti (χ^2 = 1016.000; p = 0.392), anksioznosti (χ^2 = 972.500; p = 0,243) i stresu (t = -1.038;p = 0.302) tokom menstrualnog ciklusa. **Zaključak.** Ne postoje značajne razlike u pragu bola na pritisak i emocionalnom statusu žena u različitim fazama menstrualnog ciklusa.

Ključne reči: percepcija bola; prag bola; menstrualni ciklus; emocionalna regulacija

Introduction

Pain is a complex physiological response of the sensory nervous system to actual or potential harmful stimuli [1]. The process of pain perception can be divided into four phases: transduction, transmission, modulation, and perception [2]. During transduction, nociceptors convert noxious stimuli into action potentials. In the transmission phase, nerve impulses travel along myelinated A-delta fibers and unmyelinated C fibers from nociceptors to the posterior horn of the spinal cord. These impulses are then relayed through ascending pathways to higher centers in the central nervous system. Modulation occurs when nociceptive signals are processed and modified in the dorsal horn of the spinal cord. Finally, in the perception phase, pain signals reach the cerebral cortex, resulting in the conscious experience of pain [3].

The menstrual cycle (MC) is a physiological process in women, commencing with menarche (the onset of menstruation) at puberty and continuing until menopause. A typical MC spans 28 to 30 days and

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Abbreviations

MC – menstrual cycle
FP – follicular phase
LP – luteal phase
QST – quantitative sensory testing
PPT – pressure pain threshold
DASS 21 – Depression Anxiety Stress Scale

is characterized by significant hormonal fluctuations [4]. The cycle is divided into three phases: the follicular phase (FP), ovulation, and the luteal phase (LP). The FP begins on the first day of menstruation and lasts approximately 14 days, during which ovarian follicles develop and produce estrogen. Ovulation occurs around day 14, when a mature egg is released and travels through the fallopian tube, ready for fertilization. The LP follows ovulation and lasts about 14 days, during which the ruptured follicle forms the corpus luteum, producing progesterone. In the absence of pregnancy, hormone levels decline, triggering menstruation [5]. Hormonal fluctuations throughout the MC have been linked to changes in emotional status, as suggested by some studies [6].

Pain is both sensory and emotional experience, and its perception varies significantly among individuals [7]. Numerous factors influence pain perception, including spontaneous neural activity, expectations, sleep patterns, emotional stress, and stress levels [8–11]. Gonadal hormones play a pivotal role in this variability, contributing to differences not only between men and women [12], but also among women themselves [13].

Quantitative sensory testing (QST) is a diagnostic method to evaluate somatosensory function through various tests [10]. One QST method is pressure pain threshold (PPT) testing, which measures the minimum force required to elicit pain [14]. This semi-objective and efficient technique is a valuable and reliable tool for assessing pain thresholds [15].

Although previous studies have suggested that hormonal fluctuations during the MC may influence pain perception in women, findings remain inconsistent [16]. Therefore, this study aimed to evaluate differences in PPT between the FP and LP of the MC. Additionally, the study sought to assess variations in emotional status during these phases.

Material and Methods

This prospective case series study received approval from the Ethics Committee of the Faculty of Medicine at the University of Novi Sad (no. 01-39/7). Informed consent was obtained from all participants prior to their inclusion in the research. The study included women aged 18 years and older with no current pain. Exclusion criteria were: current use of analgesics, benzodiazepines, or antidepressants, presence of acute or chronic pain (headaches, injuries, etc.), severe cardiac, metabolic, or kidney diseases, neurological disorders, serious psychiatric conditions (schizophrenia, bipolar disorder, etc.), menopause, irregular menstrual cycles. Demographic and anthropometric data were collected including age, height, weight and educational level (years of education). Data on menstrual cycle duration and the number of days since the last menstruation were also gathered. Menstrual cycle phases we calculated using a tool provided by the U.S. Department of Health & Human Services [17].

Participants were instructed to abstain from sedatives, analgesics, alcohol, or intense physical activity 24 hours before testing. They were also advised to avoid consuming caffeine products within four hours of the test and ensure adequate sleep the night prior.

Testing followed the standard protocol routinely conducted in our laboratory [18–20]. Pressure pain threshold (PPT) was measured using a digital algometer (Wagner Instruments, FDX-50) with a 1 cm diameter rubber tip. After familiarizing participants with the procedure and equipment, testing commenced. PPT measurements were performed on the extensor carpi radialis longus muscle (5 cm distal to the lateral epicondyle of the humerus), and on the paraspinal musculature of the lumbar region (2-3 cm lateral to the L3 spinous process). The algometer pressure was gradually increased at a rate of 5 N/s, and participants were instructed to say "stop" when the sensation transitioned from pressure to pain, burning, stinging, or stabbing. The pressure value in N/cm^2 was recorded. Three measurements were taken at each site with a 10-second interval between them. The final result was the average of the three measurements. Both the left and right sides were tested alternately.

Participants completed the Depression, Anxiety, and Stress Scale (DASS-21), a tool for assessing levels of depression, anxiety, and stress. The DASS-21 contains 21 questions divided into three subscales, each comprising seven items. Participants rate their experiences over the previous week using a 4-point Likert scale. Higher scores indicated greater levels of depression, anxiety, and stress [21].

A priori analysis using G*Power 3.1 indicated a minimum required sample size of 82 participants to detect an effect size of 0.3, with $\alpha=0.05$ and a power of 0.8 [22, 23]. Data were analyzed using SPSS 23 software. Descriptive and inferential statistical methods were employed. Depending on the normality of the data distribution, the Student's t-test, the Mann-Whitney test, and χ^2 test were used for group comparisons. Statistical significance was set at $p \leq 0.05$.

Table 1. Demographic and anthropometric details of participants in relation to the menstrual cycle phase

	MC phase	Mean	Standard deviation	t/χ^2	p
Age (years)	FP	28.2	8.3	t=0.653	0.515
	LP	27.2	7.3		
Height (cm)	FP	168.4	5.5	χ^2=912.000	0.108
	LP	170.6	7.9		
Weight (kg)	FP	62.5	8.6	χ^2=1060.000	0.617
	LP	62.3	11.0		
Years of education	FP	16.03	2.495	t=0.678	0.501
	LP	15.65	1.495		

MC – menstrual cycle; FP –follicular phase; LP – luteal phase

Table 2. Pain threshold variations between participants in different phases of the menstrual cycle

	MC phase	Mean	Standard deviation	t	p
PPT Forearm (N/cm^2)	FP	33.55	12.15	t=0.509	0.979
	LP	33.55	13.65		
PPT Low back region (N/cm^2)	FP	56.85	19.95	t=0.982	0.619
	LP	58.93	21.20		

MC – menstrual cycle; FP –follicular phase; LP – luteal phase; PPT – pressure pain threshold

Table 3. Variations in depression, anxiety, and stress scores between participants in different phases of the menstrual cycle

	MC phase	Mean	Standard deviation	t/χ^2	p
DASS-21 Depression	FP	2.35	2.825	χ^2=1016.000	0.392
	LP	2.22	4.049		
DASS-21 Anxiety	FP	3.33	3.071	χ^2=972.500	0.243
	LP	2.85	3.353		
DASS-21 Stress	FP	7.08	5.408	t=-1.038	0.302
	LP	8.33	6.268		

MC – menstrual cycle; FP –follicular phase; LP – luteal phase; PPT – pressure pain threshold; DASS-21 – Depression Anxiety Stress Scale

Results

The study included 100 women. However, due to the small number of participants in the ovulation phase (n = 5), these individuals were excluded from further analysis. The final sample consisted of 95 participants (mean age 27.7 ± 7.8 years), with the majority being in the follicular phase (n = 49, 51.6%). The average menstrual cycle duration in the studied group was 28.99 ± 2.39 days. There were no significant differences between the follicular and luteal phase groups in terms of age (t=0.653; p=0.515), height (χ^2=912.000; p=0.108), weight (χ^2=1060.000; p=0.617), and years of education (t=0.678; p=0.501). Detailed demographic and anthropometric characteristics of the participants are presented in **Table 1.**

No significant differences were observed in PPT measurements for the forearm region (t=0.509; p=0.979) or the lower back region (t=0.982; p=0.619) between participants in the FP and LP of the MC. These results are summarized in **Table 2**.

Analysis of emotional status revealed no significant differences in levels of depression (χ^2=1016.000; p=0.392), anxiety (χ^2=972.500; p=0.243), or stress (t = -1.038; p=0.302) between the MC phases. Additional details are provided in **Table 3.**

Discussion

Pain is highly subjective and individual experience, influenced by a variety of physical, biological, and psychosocial factors [24–26]. Given the hormonal fluctuations during the menstrual cycle and their potential impact on pain perception [27], this study focused on healthy women without pain to investigate whether these changes affect pain thresholds.

The MC is regulated by hormones from the hypothalamus, pituitary gland, and ovaries [5]. Regularity and duration of the cycle serve as indicators of endocrine function and reproductive health [28]. Due to the small number of participants in the ovulation phase, this study compared only the FP and LP. The average MC length in our sample was 28.99 ± 2.39 days, aligning within the typical range of 25-30 days [29].

Hormonal fluctuations have been implicated in modulating women's pain thresholds, first reported by

Herren [30]. Since then, numerous studies have investigated the relationship between hormonal fluctuations and pain perception, with several suggesting that estrogen and progesterone lay significant roles [31, 32]. Estrogen, in particular, is recognized as a key pain modulator [33]. Smith et al. demonstrated a clear correlation between estradiol levels and pain thresholds, noting that high estrogen levels increase the number of opioid receptors, thereby enhancing the body's response to endorphins [34]. Progesterone, another critical sex hormone, acts synergistically with estrogen and exhibits both nociceptive and antinociceptive effects, depending on the MC phase [32]. Research on healthy women indicates heightened pain sensitivity during periods of low estrogen levels [35]. However, the prevailing consensus is that pain sensitivity is influenced not only by low estrogen levels but also by the dynamic fluctuations in these hormone levels, which can reduce pain thresholds [36, 37].

In contrasts to these findings, our results revealed no significant differences in PPT between the FP and LP. These results deviate from earlier studies that reported phase-dependent variations in pain perception. For example, Wiesenfeld-Hallin [38] and Riley et al. [39] observed higher pain thresholds during the FP and lower thresholds in the late LP. Stening [35] and Young-Hee et al. [40] also reported lower thresholds in the LP. Conversely, Martin et al. [41] reported greater pain sensitivity in the FP. These discrepancies may stem from methodological variations, including differences in pain stimuli, testing locations, and definitions of menstrual phases. Iacovides et al. [42] concluded in their review that current research does not provide conclusive evidence regarding the relationship between MC phases on pain perception.

Our study also assessed emotional status, finding no significant differences in depression, anxiety, or stress levels between the FP and LP. This is somewhat unexpected, as premenstrual syndrome (PMS), which is associated with the LP, affects up to 75% of reproductive-age women [6]. PMS includes both physical symptoms like fatigue, appetite changes, and low energy, as well as emotional symptoms such as irritability, depression, anxiety and impulsivity [43]. Hoyeret al. reported more pronounced symptoms of depression, anxiety, and stress during the LP, particularly in the late phase, which contrasts with our findings [44]. Recent literature supports the notion that emotional states are often exacerbated during the premenstrual and menstrual phases, with fluctuations in hormone levels playing a significant role [45]. Considering that previous studies suggest that depression, anxiety, and stress can lower pain thresholds and increase pain sensitivity [46–48], the question remains whether these psychological factors might have influenced the observed results in PPT during the FP and LP of the MC.

This study had several limitations. First, the small sample size for the ovulation phase prevented its inclusion in further analysis, even though this phase may exhibit the most pronounced decrease in pain threshold due to hormonal fluctuations [36]. Additionally, menstrual phases were determined using an online ovulation calculator, a simple and cost-effective method, but less accurate than more advanced techniques, such as blood hormone measurements or urine test strips [49, 50]. These methodological limitations may have influenced our findings and should be considered when interpreting the results.

Conclusion

Our study found no significant differences in pressure pain threshold or levels of depression, anxiety, and stress between the follicular phase and luteal phase of the menstrual cycle. These findings underscore the need for further research to elucidate the impact of menstrual cycle phases on pain thresholds.

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ANALYSIS OF SECULAR TRENDS IN THE USE OF MEDICATIONS AFFECTING VITAMIN B12 METABOLISM AND DEMENTIA IN PATIENTS RECEIVING PRIMARY HEALTHCARE

ANALIZA SEKULARNIH TRENDOVA UPOTREBE LEKOVA KOJI REMETE METABOLIZAM VITAMINA B12 I DEMENCIJE KOD PACIJENATA U PRIMARNOJ ZDRAVSTVENOJ ZAŠTITI

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Abstract

Introduction. This study aims to analyze secular trends in the use of medications that disrupt vitamin B12 metabolism and their association with increasing dementia rates in the Republic of Serbia. **Material and Methods**. A retrospective analysis was conducted using data from the *Health Statistical Yearbook* published by the Institute of Public Health, and information on drug turnover and consumption from the Medicines and Medical Devices Agency of Serbia, covering the period from 2006 to 2019. **Results**. The average annual incidence rate of dementia showed a continuous increase, rising from 0.95 in 2006 to 3.64 in 2019. The Mann Kendall trend test revealed a statistically significant upward trend for dementia (p=1.54), and non-sideropenic anemia (p=0.0002). The analysis of medication use demonstrated increasing trends for proton pump inhibitors (p=1.45), metformin (p=7.15), anticonvulsants (p=0.001), 5-aminosalicylic acid (p=5.35), antiepileptics (p=2.50), and clonazepam (p=1.95). Conversely, declining trends were observed for H2 antagonists (p=1.95) and phenobarbital (p=0.003). For carboxamide and valproate derivatives, no significant trend was observed. **Conclusion**. The rising use of medications that impair vitamin B12 metabolism - including metformin, proton pump inhibitors, 5-aminosalicylic acid, and clonazepam, as well as non-sideropenic anemias, parallels a continuous increase in dementia incidence.

Key words: Vitamin B 12; Dementia; Cognition; Metformin; Proton Pump Inhibitors; Clonazepam; Mesalamine; Primary Health Care; Risk Factors

Sažetak

Uvod. Cilj ove studije je analiza sekularnih trendova u podacima o potrošnji lekova koji remete metabolizam vitamina B12 sa trendom porasta demencija za teritoriju Republike Srbije. **Materijal i metode.** Studija predstavlja retrospektivnu analizu podataka dobijenih iz Zdravstveno-statističkog godišnjaka Instituta za javno zdravlje, kao i Podataka o prometu i potrošnji lekova Agencije za lekove i medicinska sredstva Srbije, a za period od 2006. do 2019. godine. **Rezultati.** Ukupna srednja vrednost stope obolevanja na 1.000 stanovnika na teritoriji Republike Srbije od demencije kontinuirano raste od 0,95 u 2006. godini do 3,64 u 2019. godini. Rezultati dobijeni Men-Kendelovim (*Mann Kendall*) testom govore da postoji porast trenda za demencije gde je p = 1,54 i statistički značajan porast trenda za nesideropenijske anemije gde je p = 0,0002. Rezultati dobijeni za lekove ukazuju na porast trenda upotrebe: inhibitora protonske pumpe (p = 1.45), metformina (p = 7,15), antikonvulziva (p = 0,001), 5-aminosalicilne kiseline (p = 5,35), antiepileptika (p = 2,50), klonazepama (p = 1,95), dok za H2 antagoniste postoji trend opadanja (p = 1,95) i (fenobarbitol p = 0.003), a za derivate karboksamida i valproata nije uočen porast ili opadanje trenda. **Zaključak.** Povećanje upotrebe lekova koji dovode do poremećaja metabolizma vitamina B12, uključujući metformin, inhibitore protonske pumpe, 5-aminosalicilnu kiselinu i klonazepam, kao i nesideropenijske anemije, praćeno je kontinuiranim porastom incidencije demencije.

Ključne reči: vitamin B12; demencija; kognicija; metformin; inhibitori protonske pumpe; klonazepam; mesalamin; primarna zdravstvena zaštita; faktori rizika

Introduction

In recent years, the growing number of newly diagnosed dementia cases has heightened the demand for early diagnosis, prevention, treatment and care by healthcare workers, medical institutions, social, and other state services. In advanced stages of dementia, patients often lose the ability to live independently as their functionality is significantly impaired by the symptoms of the condition [1]. Simultaneously, the stressful conditions of modern lifestyles have contributed to an increasing prevalence of diabetes, leading to greater reliance on biguanides, particularly metformin, as a first-line treatment, which, however, leads to reduced levels of vitamin B12 in the body [2–4]. In addition to metformin, the use of other drugs that interfere with cyanocobalamin metabolism is also becoming more frequent. For instance, focus on diagnosing and treating gastritis caused by *Helicobacter pylori*, has increased the use of medications such as proton pump inhibitors, H2 antagonists, antacids, and antibiotics – most commonly clarithromycin – for eradication of. The frequent or prolonged use of these drugs can disrupt vitamin B12 levels, which are essential for proper functioning of the nervous system and nerve cells

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Abbreviations

F00-F03 – dementia specified and unspecified
D51-D64 – nutritional, hemolytic and aplastic anemias
5ASA – 5-aminosalicylic acid
PPI – proton pump inhibitors
HBP – helicobacter pylori
ALIMS – Medicines and Medical Devices Agency of Serbia
DDD – Defined Daily Dose
ICD-10 – International Statistical Classification of Diseases and Related Health Problems, 10th Revision
PD – Parkinson's disease
MCI – mild cognitive impairment
PON1 – paraoxonase 1

[5–7]. Numerous studies have also highlighted an association between vitamin B12 deficiency and the use of anticonvulsants, which can elevate homocysteine levels [8–10].

Vitamin B12 (cyanocobalamin) primarily produced by microorganisms *Propionibacterium freudenreichii* and *Pseudomonas denitrificans* is predominantly obtained from animal-derived foods. Deficiency of vitamin B12 can lead to hematological, gastrointestinal, and neuropsychiatric disorders, including neuropathy, cerebellar ataxia, dementia and mood disturbances. This deficiency typically results from insufficient dietary intake, inadequate absorption, or reduced utilization [11].

Cyanocobalamin plays a vital role in amino acid metabolism, myelin renewal, and bone marrow cell formation. A deficiency can cause degeneration of the spinal cord's lateral and dorsal columns, resulting in neuropathy. Cognitive dysfunction of affected individuals is often accompanied by irritability, depression, and, in severe cases, psychosis [12].

Despite the increasing use of drugs that interfere with vitamin B12 metabolism, there has not been a corresponding increase in vitamin B12 intake or its supplementation. Consequently, the incidence of dementia continues to rise.

This study aims to analyze secular trends in the use of medications that disrupt vitamin B12 metabolism and their association with the rising incidence of dementia in the Republic of Serbia. It seeks to explore the potential connection between these medications and the observed increase in dementia cases.

Research hypotheses

1. There is a positive secular trend in the use of drugs that disrupt vitamin B12 metabolism and the frequency of cognitive impairment in the Republic of Serbia;

2. There is a positive secular trend in the incidence of dementia and megaloblastic anemia, but not sideropenic anemia, in Serbia.

Material and Methods

The study involves a retrospective analysis of data collected from the *Health Statistical Yearbook of the Republic of Serbia*, published by the Institute of Public Health of Serbia "Dr Milan Jovanovic Batut", and the information on drug turnover and consumption from the Medicines and Medical Devices Agency of Serbia (ALIMS). The analysis covered data from 2006 to 2019 for the territory of the Republic of Serbia [13, 14]. The strength and direction of the linear relationship between the increase in dementia incidence (per 1,000 inhabitants) and the use of medications affecting vitamin B12 metabolism (expressed as Defined Daily Dose (DDD) per 1,000 inhabitants per day)were assessed using the Mann Kendall test for individual trend analyses. Disease data were obtained from the reports of the Institute of Public health, focusing on diagnoses coded according to the WHO's International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10). The selected codes included: F00-03 (Dementia specified and unspecified), D50 (Iron deficiency anemia), and D51-64 (Nutritional, hemolytic, and aplastic anemias. These data were reported as the number of patients per 10,000 individuals in the general population. Medication usage data were collected from reports on drug consumption, presented as DDD per 1,000 inhabitants per day. The analyzed medications included: H2 receptor blockers, proton pump inhibitors, mesalazine (5-aminosalicylic acid), metformin, oral contraceptives, estrogens, tetracyclines, penicillins, erythromycin, phenobarbital, methotrexate, loop diuretics, iron supplements, vitamin B1, vitamin B12, folic acid, and drugs prescribed for dementia.

Results

The analysis of secular trends revealed a continuous increase in the total average incidence rate of dementia per 1,000 inhabitants in the Republic of Serbia, rising from 0.95 in 2006 to 3.64 in 2019 **(Figure 1).**

Using the Mann-Kendall test, a significant upward trend was observed for dementia (F00-F03) with

Figure 1. Mean incidence rate of dementia per 1,000 inhabitants per day in the period from 2006 to 2019.

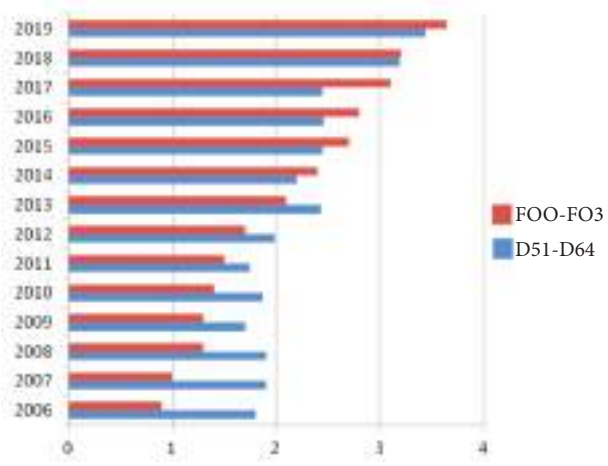

Figure 2. Increase in the incidence rate of dementia and anemias due to vitamin B12 deficiency and other non-sideropenic anemias

p=1.54, and for non-sideropenic anemias (D51-D64: nutritional anemias, hemolytic anemias, and aplastic erythrocyte disorders) with p=0.0002. Conversely, no significant trend was found for sideropenic anemias (D50), with p=0.77. Medication usage trends, analyzed through the Mann-Kendall test, indicated statistically significant increases in the use of: proton pump inhibitors (p=1.45), metformin (p=7.15), anticonvulsants (p=0.001), 5-ASA (p=5.35), antiepileptics (p=2.50), clonazepam (p=1.95). A decreasing trend was observed for H2 antagonists (p=1.95) and phenobarbital (p=0.003), while no significant changes were detected for carboxamide and valproate derivatives.

The increase in dementia incidence is paralleled by a faster rise in anemia cases caused by vitamin B12 deficiency and other anemias. This disparity in the incidence rates between dementia and these anemias has steadily narrowed over time, decreasing from 0.95 for dementia and 1.81 for anemias in 2006 to near equivalence in 2018, with rates of 3.64 and 3,44 respectively **(Figure 2).**

Figure 3 illustrates the mean usage rates of medications documented in the literature as affecting

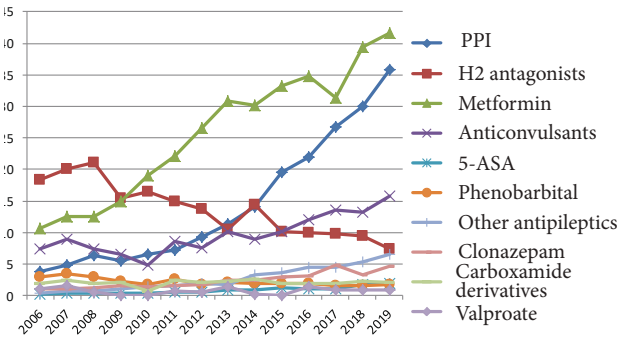

Figure 3. Drug utilization rate in the Republic of Serbia in the period from 2006 to 2019

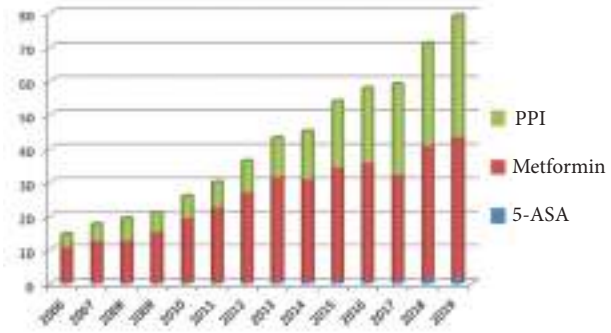

Figure 4. Increase in the utilization rate of drugs affecting vitamin B12 metabolism in the Republic of Serbia

vitamin B12 metabolism, based on data from the ALIMS database for the Republic of Serbia.

Figure 4 highlights the secular trends in drug use:

– The mean value for metformin usage increased from 10.71 in 2006 to 41.57 DDD/1,000 patients/day in 2019.

– The mean value for anticonvulsants increased from 7.45 to 15.71 DDD/1,000 patients/day.

– H2 antagonist use significantly decreased from 18.34 to 7.35 DDD/1,000 patients/day, contrasting with proton pump inhibitors, which increased from 3.74 to 35.85 DDD/1,000 patients/day during the same period.

– The use of mesalazine (5 aminosalicylic acid) increased from 0.19DDD/1,000 patients/day in 2006 to 1.91 in 2019.

Discussion

Our study revealed three key findings: (1) growing trend in dementia incidence in the Republic of Serbia between 2006 and 2019, (2) an increasing use of medications that disrupt B12 metabolism, and (3) a potential link between these trends [15].

However, the study's limitations must be acknowledged, as the observed trends cannot establish causality due to the influence of numerous long-term factors.

Due to various cultural and ethnic characteristics, and various diagnostic criteria in data collection, dementia prevalence increases significantly with age, ranging from 1.1% in individuals aged 65-69 to 45.1% in those over 85.

Garre-Olmo J. highlights that the prevalence and incidence of dementia are exponentially rising after the age of 65, and that dementia cases will increase in the decades to follow due to aging populations and longer life expectancy, suggesting that primary prevention strategies may reduce the public health burden on dementia [16].

Medications such as metformin and proton pump inhibitors (PPIs) have been shown to impair vitamin B12 absorption, potentially causing vitamin B12 de-

Table 1. Incidence rates of dementia and sideropenic and other anemias per 1,000 inhabitants per day in the Republic of Serbia

Year	Disease code	General Medicine Service	Disease rate per 1,000 inhabitants	Disease code	General Medicine Service	Disease rate per 1,000 inhabitants	Disease code	General Medicine Service	Disease rate per 1,000 inhabitants
2006	F00-F03	5506	0.9	D50	75615	13.0	D51-D64	10477	1.8
2007	F00-F03	5673	1.0	D50	79943	13.8	D51-D64	10963	1.9
2008	F00-F03	7517	1.3	D50	84964	14.7	D51-D64	10895	1.9
2009	F00-F03	7388	1.3	D50	87022	15.1	D51-D64	9755	1.7
2010	F00-F03	8053	1.4	D50	96538	16.7	D51-D64	10806	1.87
2011	F00-F03	8538	1.5	D50	99279	17.3	D51-D64	9984	1.74
2012	F00-F03	9856	1.7	D50	91450	15.9	D51-D64	11501	1.99
2013	F00-F03	12016	2.1	D50	95647	16.6	D51-D64	13948	2.43
2014	F00-F03	13997	2.4	D50	101424	17.7	D51-D64	12585	2.20
2015	F00-F03	15573	2.7	D50	94388	16.5	D51-D64	13975	2.45
2016	F00-F03	16163	2.8	D50	91229	16.0	D51-D64	14000	2.46
2017	F00-F03	17531	3.1	D50	84161	14.9	D51-D64	13866	2.45
2018	F00-F03	18093	3.2	D50	86663	15.4	D51-D64	17985	3.19
2019	F00-F03	20384	3.64	D50	87142	15.56	D51-D64	19282	3.44

ficiency that lead to gastroenterological, hematological, neurological, and cognitive impairments [17–20].

Miller JW, in his review, examined the mechanisms of vitamin B12 absorption and biochemistry, as well as the ways in which PPIs and metformin affect these processes. He argued that testing multiple analytes in serum improves the sensitivity and specificity of diagnosing vitamin B12 deficiency. Miller concluded that randomized controlled trials employing multiple analytes testing strategies are essential to determine whether PPIs and metformin significantly increase the risk of vitamin B12 deficiency [21].

Our study confirmed that the most significant increase in drug usage in Serbia over the study period was observed in for proton pump inhibitors and metformin.

The increasing prevalence of diabetes, driven by modern lifestyle factors such as chronic stress, diets high in carbohydrates, and improved diagnostic efforts by primary care physicians, likely explains the growing use of metformin. Early recognition of symptoms and timely initiation of therapy have also contributed to this trend.

A study by Almatrafi et al., involving 206 individuals with type 2 diabetes using metformin for six months, investigated the prevalence of vitamin B12 deficiency and its relationship with drug usage duration and dietary intake. The study found that 17.5% of patients using metformin had vitamin B12 deficiency, with significant differences in the duration of diabetes between those with deficiencies and those with normal vitamin B12 ranges [22].

Similarly, a cross-sectional study by Khan TU et al. aimed to confirm the prevalence of vitamin B12 deficiency in patients with type 2 diabetes using metformin. Results showed that 27.33% of metformin users had vitamin B12 levels below 150 pg/ml, emphasizing the importance of screening for vitamin B12 deficiency before initiating metformin therapy [23].

Increased accessibility to microbiological diagnostics for Helicobacter pylori has facilitated timely treatment and more frequent use of eradication therapies. This has driven a notable rise in the use of medications, particularly PPIs, between 2006 and 2018. Additionally, self-medication by patients reporting various gastrointestinal symptoms has contributed to the significant increase in PPI consumption.

In a descriptive cross-sectional study by Gökışık MT et al., which included 421 T2D patients using metformin, it was found that the rate of vitamin B12 deficiency was significantly higher among those with Helicobacter pylori infection [24].

Patients' awareness and insight to their health condition lead to more frequent visits to doctors and more significant use of medications that help them recover and heal their weakened health.

Owen MD et al. in their thesis suggest that metformin is associated with maternal vitamin B12 deficiency similarly to antifolate. Vitamin B12 and folate balance is vital for one-carbon metabolism, which is essential for deoxyribonucleic acid methylation and purine/pyrimidine synthesis. Metformin use during pregnancy can lead to genomic instability and aberrant gene expression. They believe that it is very important to improve our understanding of the transgenerational effects of metformin in order to provide more effec-

tive prophylaxis for the prevention of unwanted fatal outcomes [25].

Carrillo ÑL et al., in their case report, described an elderly patient with a 40-year history of gastric ulcer who presented with behavioral changes, pallor, and multiple ecchymosis. The patient was diagnosed with reversible dementia caused by pernicious anemia, accompanied by pancytopenia and neurological changes. The patient's condition improved with vitamin B12 supplementation [26].

Vitamin B12 deficiency typically first manifests as hematological, gastrointestinal, and neurological abnormalities, while psychiatric symptoms often appear as later consequences. Similarly, thiamine (vitamin B1) plays a crucial role as a catalyst in energy generation through the decarboxylation of amino acids and alpha-keto acids and serves as a coenzyme for transketolase reactions in its pyrophosphate form. Thiamine is also essential for the propagation of nerve impulses and maintenance of myelin sheath. Deficiencies in thiamine, like those of cobalamin, can adversely affect the cardiovascular, nervous, and immune systems.

In their study, Ayako M et al. highlighted the importance of vitamin B1 in preventing dementia in women. Interestingly, they found no significant association between vitamin B1 and dementia in men as assessed by the Mini-Mental State Examination. This discrepancy suggests the need for longitudinal studies to determine whether declining vitamin levels occur before or after cognitive impairment and whether maintaining adequate vitamin levels can prevent the progression of cognitive decline and the onset of dementia [27].

In the research conducted by McCarter SJ et al., it was found that patients with Parkinson's disease (PD) who did not develop dementia exhibited higher levels of vitamin B12 at the time of diagnosis compared to those who eventually developed dementia. These researchers noted that lower vitamin B12 levels and elevated homocysteine concentrations were associated with cognitive impairment in PD patients. Recent evidence suggests that vitamin B12 may influence the accumulation of alpha-synuclein, potentially offering a disease–modifying effect. Using a cohort of 25 PD patients, the study revealed that 15 (60%) developed dementia, while those who did not develop dementia had higher-than-elemental levels of vitamin B12 at the time of diagnosis [28].

Nalder L et al. examined individuals aged 60-85 years with available vitamin B12 biomarker data (n=1347). Their study detected vitamin B12 deficiency in 17.2% of participants and folate deficiency in 1%. Notably, low vitamin B12 levels were associated with poorer memory performance in men [29].

Perła-Kaján J et al. explored the potential role of paraoxonase 1 (PON1) status in the development of neurological diseases, including Alzheimer's disease. The study included individuals with mild cognitive impairment (MCI) (n = 196; mean age 76.8 years; 60% female). The researchers conclude that DPON1 is a novel factor associated with cognitive impairment, and its effects may be improved through vitamin B supplementation in individuals with MCI [30].

The importance of vitamin B6, B12, and folic acid supplementation is critical for patients with MCI. Olaso-Gonzalez G et al. assessed the effects of treatment with vitamins B6, B12, and/or folic acid in their review for MCI patients. The review included eight primary studies with a total of 1,140 participants. The findings demonstrated a reduction in plasma homocysteine levels among MCI patients taking vitamins B6, B12 and/or folic acid supplements, with a statistically significant decrease observed after just one month of supplementation [31].

Cognitive impairment is one of the leading causes of disability and dependence among older adults globally [32]. Elevated homocysteine levels are a recognized risk factor for cognitive decline. Antioxidant vitamins offer a protective effect by reducing oxidative stress [33]. Positive outcomes following cobalamin supplementation have been reported in various clinical and epidemiological studies, suggesting potential improvements in cognitive functions [34, 35].

Conclusion

The rising use of medications that disrupt vitamin B12 metabolism – such as metformin, proton pump inhibitors, 5-aminosalicylic acid, and clonazepam - correlates with an increase in non-sideropenic anemias and the incidence of dementia.

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REVIEW ARTICLES
PREGLEDNI ČLANCI

CONTEMPORARY METHODS OF THE IMPROVEMENT OF RESIN-DENTIN BONDS – A LITERATURE REVIEW

SAVREMENE METODE ZA UNAPREĐIVANJE VEZE IZMEĐU ADHEZIVNIH SMOLA I DENTINA – PREGLED LITERATURE

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Abstract

Introduction. Despite significant advancements in materials for permanent restorations, there is still no material that fully replicates the physical, chemical, and biological properties of dental tissues. Contemporary research in adhesive dentistry focuses on achieving a reliable and durable bond between restorative materials and dental substrates. This paper aims to present recent innovations in dental technology focused on improving the challenging bond between restorative materials and dentin. Currently, several key strategies address this issue, including the application of collagen cross-linkers, antioxidants, inhibitors of endogenous proteinases, reinforcement with inorganic fillers and remineralization agents, modifications in bonding techniques, and substrate treatment using lasers. **Conclusion.** Current knowledge and experimental findings suggest that modern methods of modifying the dentin substrate can achieve a satisfactory initial adhesive bond. However, the degradation of the interfacial contact surface remains a significant challenge, compromising the long-term durability of restorations.

Key words: Dentin-Bonding Agents; Adhesives; Dentin; Collagen; Matrix Metalloproteinases

Sažetak

Uvod. Uprkos raznovrsnosti i unapređenju materijala za definitivne ispune, još uvek ne postoji materijal koji u potpunosti odgovara fizičkim, hemijskim i biološkim osobinama zubnih tkiva. Savremena istraživanja u oblasti adhezivne stomatologije usmerena su na postizanje adekvatne i predvidljive veze između restaurativnih materijala i zubnog supstrata. Cilj ovog rada je da predstavi novija dostignuća dentalne tehnologije kojima bi se unapredila, još uvek, kontroverzna veza restaurativnih materijala sa dentinom. Trenutno postoji nekoliko glavnih strategija za rešavanje ovog problema koji uključuju: umreživače kolagena, antioksidante, inhibitore endogenih proteinaza, ojačavanje matriksa smole neorganskim puniocima i/ili agensima za remineralizaciju, modifikaciju tehnike adhezivnog vezivanja i tretiranje supstrata laserom pre adhezivnog vezivanja. **Zaključak.** Na osnovu trenutnih znanja i eksperimentalnih istraživanja može se zaključiti da se primenom savremenih metoda modifikacije dentinskog supstrata postiže adekvatna inicijalna adhezivna veza. Međutim, degradacija međufazne kontaktne površine još uvek predstavlja čest problem, koji kompromituje dugovečnost restauracija.

Ključne reči: dentinski adhezivni agensi; adhezivi; dentin; kolagen; matriks metaloproteinaze

Introduction

The primary objective and greatest challenge in adhesive dentistry is achieving a predictable and durable bond between dental substrates and restorative materials. A reliable bond ensures strong retention, minimal microleakage, and restoration stability [1, 2]. The strength and effectiveness of this bond are influenced by several factors, including the physical and chemical properties of dental tissues and restorative materials. Additionally, external factors such as the oral environment – humidity, mastication, diet, temperature fluctuations, and variations in saliva pH – exert significant effects and should not be overlooked.

Micro-mechanical bonding to dentin relies on forming a resin-reinforced dentin layer known as the "hybrid layer". This layer is created when adhesive resin penetrates the extracellular dentinal matrix, primarily composed of collagen fibrils. Consequently, the stability of the collagen matrix is critical for maintaining the integrity and stability of the hybrid layer, which improves the mechanical properties and longevity of the bond [3–5]. However, numerous studies have revealed that mechanical and ultrastructural changes within the hybrid layer occur over time, indicating

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Abbreviations

MMPs – matrix metalloproteinases
PA – proanthocyanidins
DMA – N-(3,4-dihydroxyphenethyl) methacrylamide
CHX – chlorhexidine
BAC – benzalkonium chloride
EDTA – ethylenediaminetetraacetic acid
DMSO – dimethyl sulfoxide
Er:YAG – Erbium-doped Yttrium Aluminium Garnet

its limited durability. Factors contributing to these changes include deficient infiltration of adhesive resin into demineralized dentin and the elution, or separation of residual monomers from the polymerized adhesive resin. Exposed collagen fibers within the hybrid layer that remain unprotected by resin are susceptible to degradation over time.

Matrix metalloproteinases (MMPs) are zinc and calcium-dependent endopeptidases whose activity can be observed in many biological and pathological processes related to the degradation of the extracellular matrix. Various MMPs, such as stromelysin-1 (MMP-3), collagenase (MMP-8), and gelatinases A and B (MMP-2 and MMP-9), have been identified in dentin substrates [6]. More recently, matrilysin-1 (MMP-7) has been also been detected [7]. While MMPs contribute to dentin maturation, they become inactive after the mineralization of the collagen matrix [6]. Endogenous MMPs are inactive in dentin; however, they are reactivated in certain conditions such as reduced pH in the environment caused by caries or etching. Proteases gradually degrade collagen fibers, leading to the deterioration of the bonding surface and a reduction in bond strength, which can range from 36% to 70% within 12 to 14 months [8, 9].

In recent years, numerous studies have been published about the deterioration of bond strength in adhesively bonded restorations. According to research, advances in dental technology have provided several approaches to address this issue, which are explored in this paper [6]. The objective of this study is to review and highlight recent developments in dental technology aimed at improving the still-challenging adhesive bond between restorative materials and dentin.

I Collagen Cross-Linkers

In biological sciences, the term "cross-linking" refers to the formation of chemical bridges during chemical reactions between proteins or other molecules, resulting in altered physical properties of biological molecules [10].

Collagen fibers are composed of myofibrils interconnected by endogenous covalent cross-links. Cross-links formed using external cross-linkers are notably stable over extended periods, as the formation of covalent bonds is irreversible. Demineralization of tissue leads to a significant reduction in collagen matrix stiffness, decreasing from 18,000 MPa to 1-3 MPa. This low modulus of elasticity allows free movement of collagen fibers, including lateral and rotational shifts, which expose active binding sites for MMPs [2]. Additionally, cross-linking agents have been shown to inhibit MMP actively by reducing molecular mobility, inducing conformational changes in collagen structure, or converting negatively charged carboxyl groups into positively charged amides [2]. Recent studies suggest that pretreating dentin or enriching adhesive systems with natural or synthetic collagen cross-linking agents improves the mechanical properties of collagen and enhances the adhesive bond [2].

Several natural substances exhibit collagen cross-linking properties, including proanthocyanidins (PA), polyphenols from green tea extract (EGCG) [11], naringin [2], chitosan [12, 13], riboflavin [14], genipin, bromelain enzyme [6], curcumin [15, 16], black tea flavonoids [17], eucalyptus lignin, cardanol from Indian cashew nutshells [18], and urushiol [19]. Among these, chitosan stands out for its unique properties and versatility. Chitosan, a semi-synthetic molecule derived from an amino-polysaccharide and created through the partial acetylation of chitin [12, 13], is valued for its biocompatibility, biodegradability, and low toxicity. In dentistry, chitosan has applications in caries prevention, mechanical integrity enhancement, antimicrobial treatments, and tissue regeneration. Research indicates that chitosan improves bond strength and longevity in dental restorations, particularly in etch-and-rinse or self-etch adhesive systems. However, its effectiveness depends on its concentration and specific application. While lower concentrations (0.12% to 0.25%) enhance bonding strength, higher concentrations (0.5% and 1%) may reduce bonding efficacy.

Among synthetic cross-linkers, commonly used agents include glutaraldehyde, carbodiimides, and DMA monomers etc. [6, 9, 20–24]. One notable compound is N-(3,4-dihydroxyphenethyl) methacrylamide (DMA), inspired by adhesive proteins of mussels. These shellfish found worldwide exhibit unique wet adhesion properties that enable them withstand harsh marine environments, such as turbulent currents and protease degradation. This behavior parallels the demands on the resin-dentin interface in the oral cavity, which must endure daily mechanical stresses, temperature fluctuations, pH variations, and proteolytic attacks. Researchers have sought to translate the adhesion properties of mussels into dentin bonding treatments, yielding promising in vitro results. One such compound, N-(3,4-dihydroxyphenethyl) DMA, is incorporated into dental adhesives due

to its ability to polymerize with adhesive monomers and cross-link with dentin collagen via non-covalent bonds. This significantly enhances the durability and mechanical properties of the resin-dentin interface. Furthermore, DMA monomers exhibit hydrolytic stability and inhibit MMPs, thereby protecting the integrity of the hybrid layer. The incorporation of DMA monomers has demonstrated potential in improving bond strength, reducing nanoleakage, and extending the longevity of dental restorations, making them a valuable addition to modern adhesive systems. However, challenges remain, such as the limited shelf life of these monomers due to the oxidation of catechol groups, necessitating further research to optimize their application. Notably, one-minute pretreatment with a DMA-EtOH primer has been shown to enhance resin-dentin interface integrity, inhibit endogenous MMP activity, and prolong resin-dentin bond durability [20].

II Antioxidants

The polymerization process of resin-based materials can be disrupted by oxidizing agents, which are generated in dental substrates during clinical procedures such sodium hypochlorite and tooth whitening with hydrogen peroxide-based products [6]. Contemporary research suggests effective strategies to overcome this issue, including the use of proanthocyanidins (PA), noni fruit juice (Morinda citrifolia), vitamins C [15] and E, as well as quercetin. When applied to dentin treated with sodium hypochlorite before the adhesive system is used – or when incorporated into the primer or adhesive resin - these oxidants have demonstrated the ability to neutralize the oxidative effects of sodium hypochlorite. By restoring the redox potential of oxidized dentin, they create conditions conductive to effective polymerization [6].

III Inhibitors of Endogenous Proteinases

The most commonly used inhibitors of MMPs include:

1. Chlorhexidine

Chlorhexidine (CHX) is the most widely used MMP inhibitor. In addition to its antimicrobial properties, CHX inhibits MMPs through a chelating mechanism. It remains bound in dentin for at least 12 weeks or longer, especially in demineralized dentin. A study by Breschi et al. [23] demonstrated the presence of CHX ions in the hybrid layer even after 10 years of aging in artificial saliva, originating from the primer applied before adhesive bonding. This prolonged binding preserved MMP inhibition and provided ongoing protection of the hybrid layer. However, sodium chloride can displace CHX from both mineralized and demineralized dentin, indicating that CHX's binding mechanism is purely electrostatic [6]. CHX can be applied after acid etching or incorporated into any component of the adhesive system. Low concentrations (0.5-5%) are effective, while concentrations below 0.1% have been found to be ineffective [6]. The most common method of CHX application is in the form of a primer due to its widespread availability in dental offices and its rapid action, taking effect within 30 seconds [23].

2. Tetracyclines

Minocycline has been shown to enhance the strength and stability of the dentin-adhesive bond when applied after acid etching. A 2% minocycline solution applied post-etching effectively prevents the breakdown of the interfacial contact surface for more than two years [25]. As a chemically modified tetracycline (CMT), minocycline retains its ability to chelate calcium (Ca) and zinc (Zn) ions, which underlies its mechanism for inhibiting endogenous proteases. Similarly, doxycycline was demonstrated effectiveness [6]. However, its application involves a more complex technological process. Specifically, incorporating doxycycline into adhesive systems can result in phase separation, resulting in the absence of the desired effect of doxycycline [6, 26]. Care must be taken when using tetracyclines, as they may cause dark discoloration of dental tissues due to photodegradation process [23].

3. Quaternary ammonium compounds

Quaternary ammonium compounds, such as benzalkonium chloride (BAC), are positively charged at physiological pH, enabling them to inhibit proteases through a cationic mechanism similar to CHX. BAC binds strongly to demineralized dentin, providing an immediate inhibitory effect and maintaining the strength of the adhesive bond over time [23]. Commercially, BAC is available as a 1% antimicrobial conditioning agent incorporated into phosphoric acid etchant. In this form, it exhibits antiproteolytic properties but does not enhance the initial adhesive bond strength. Additionally, the rinsing step in total-etch protocols reduces the BAC concentration in the hybrid layer, limiting its inhibitory potential. Incorporating BAC into the primer or adhesive resin could improve its efficacy without extending clinical procedures [9, 27].

4. Ethylenediaminetetraacetic acid

Due to its chelating properties, ethylenediaminetetraacetic acid (EDTA) inhibits MMP activity by binding zinc and calcium ions when applied for 1 to 5 minutes. Additionally, EDTA enhances collagen infiltration by adhesive resin, likely due to the re-

moval of the smear layer, which facilitates improved monomer interaction. Studies have shown that adhesive bonds formed on dentin treated with EDTA are stronger compared to those treated with phosphoric acid. However, EDTA is easily washed away during rinsing, which may diminish its inhibitory effect [6].

5. α-tomatine

Tomatine is a glycoalkaloid found in all parts of the tomato plant, consisting of two glycoalkaloids – alpha-tomatine and dehydrotomatine [28]. Recent studies have revealed that alpha-tomatine can inhibit MMP-2 and MMP-9 activity in certain cancer cells, exhibiting anti-metastatic properties. In a study by Ucuncu et al. [29], alpha-tomatine and chlorhexidine were shown to exhibit differing inhibitory effects on various MMPs.In vitro experiments demonstrated that alpha-tomatine achieved higher bond strength than chlorhexidine and control groups across all subgroups, irrespective of dentin type [29].

IV Strengthening the resin matrix with inorganic fillers and/or remineralization agents

Given the hybrid layer's susceptibility to both enzymatic and chemical degradation of adhesive components, reinforcing the resin matrix is a promising approach to enhancing hybrid layer resistance. Functionalized nanoparticles of titanium dioxide, copper, silver, and zinc oxide, modified with carboxylic acid, have been utilized to strengthen the organic resin matrix [6]. Copper nanoparticles, in particular, serve as MMP inhibitors and exhibit cross-linking properties. Studies have demonstrated that adding copper nanoparticles at concentrations of 0.1% and 0.2% to total-etch adhesive systems significantly improves adhesive bonding to both healthy and caries-affected dentin [30]. Similarly, zinc (Zn) plays a dual role by promoting dentin remineralization and inhibiting demineralization. Zinc further contributes to bond durability through competitive enzyme inhibition, while also offering anti-inflammatory and antibacterial effects [31]. However, adhesive resins containing nanoparticles are prone to agglomeration and uneven filler dispersion due to their low viscosity, which can compromise the adhesive's strength and stability. Carbon nanotubes, composed of a hexagonal network of carbon atoms rolled into cylindrical nanostructures, present a promising solution. These structures are strong and rigid, with notable thermal and electrical properties. Recent findings suggest that carbon nanotubes can reinforce both the adhesive resin and the adhesive bond. Additionally, their ability to encapsulate various compounds provides a novel strategy for preventing adhesive bond degradation [6].

Biomimetic remineralization

A novel approach to preserving the hybrid layer involves biomimetic remineralization, a process that mimics natural tooth remineralization. This method aims to fill unoccupied spaces in the resin matrix with hydroxyapatite, facilitating the remineralization of collagen fibers and the fossilization of MMPs [22]. For this purpose, bioactive silicates and phosphoprotein analogs are employed, which can form apatite crystals within collagen fibers [6]. It is crucial, however, for remineralization agents to include MMP inhibitors. This is because the remineralization process requires time, during which the demineralized collagen fibrils must be protected from enzymatic degradation [22].

V Modification of the adhesive bonding technique

Strategies to improve the adhesive bond focus on eliminating unbound water or residual monomers from the hybrid layer.

1. Application of an additional layer of adhesive resin

One widely discussed approach involves applying a hydrophobic resin layer after completing the adhesive bonding protocol. This additional resin layer interacts with unbound surface monomers, reducing their presence on the interfacial contact surface. The results in a denser hybrid layer that offers greater resistance to tensile forces and degradation [32]. Oliveira et al. [33] demonstrated that applying a double resin layer using a self-etch adhesive systems lead to the formation of longer resin tags in both healthy and demineralized dentin [6].

2. Ethanol wet bonding

In clinical practice, ethanol-based adhesive systems are typically applied to water-saturated dentin following acid etching. However, this process is prone to microscopic phase changes within the adhesive [9]. To mitigate this risk, ethanol wet bonding has been proposed, substituting ethanol for water as the rinsing medium to eliminate residual water from the hybrid layer. This technique inhibits proteolytic enzymes through a dual mechanism: infiltration of voids between collagen fibers with adhesive resin, and absence of unbound water [9, 22, 34].

3. Dry bonding

Dry bonding eliminates residual water from the hybrid layer by utilizing 2-step self-etch adhesive systems. These systems employ a primer designed to gently etch dentin and infiltrate it with acidic monomers, followed by the application of an adhesive resin free of solvents. The primers contain a high concentration of acidic monomers with minimal water, sufficient to ion-

ize the monomers and dissolve the mineral phase of dentin. After self-etching for 10 seconds, the dentin surface is dried, and the adhesive resin is applied prior to light polymerization. The resulting hybrid layer contains a larger portion of the smear layer, including "smear plugs" that prevent the outflow of dentinal fluid during the adhesive bonding process [9, 22].

4. Application of dimethyl sulfoxide as a primer or solvent

Dimethyl sulfoxide (DMSO) is an organic sulfur compound with low surface energy, making it an effective solvent to facilitate polymerization during adhesive bonding [9, 22, 35]. DMSO enhances bond strength and durability by stabilizing the collagen matrix, inhibiting MMPs, and optimizing dentin moisture content [35]. Studies have demonstrated that incorporating DMSO into wet bonding protocols significantly improves the long-term stability of the adhesive bond [35].

VI Substrate treatment with lasers before adhesive bonding methods

Two of the most commonly used lasers are Er:YAG (Erbium-doped Yttrium Aluminium Garnet) lasers and plasma-based lasers. The Er:YAG laser induces specific topographical changes on the dentin substrate surface by removing the smear layer, evaporating water and organic content, and creating crater-like alterations [6]. These changes effectively increase the surface area available for bonding, which is critical for achieving durable adhesion [32, 36]. Laser irradiation has also been shown to have positive effects on dentin exposed to oxidizing agents [37]. Plasma lasers improve bond quality and stability over time, particularly during aging. Their mechanism of action involves generating carboxyl and carbonyl groups on the dentin surface, which enhances wetting and facilitates better monomer interaction [6, 38–40].

Synthesis of Current Knowledge and State of Investigations

Adhesive dentistry continues to evolve driven by advancements in both chemical and physical techniques. While chemical methods, particularly those involving natural agents, have been extensively investigated, physical techniques remain underexplored but exhibit considerable potential for further development.

A comparison of chemical agents and techniques reveals that each offers unique benefits and challenges. Chemical approaches, such as collagen cross-linkers and proteinase inhibitors, effectively strengthen the collagen matrix and inhibit enzymatic degradation, making them valuable tools for enhancing the biochemical stability of adhesive bonds. CHX and EDTA are particularly valuable due to their established clinical use, widespread availability, and ease of application in dental practice. Conversely, remineralization agents aim to enhance the mechanical and structural integrity of the bond by promoting natural dentin repair processes. Despite their potential, these agents often require extended treatment times and controlled conditions, which can be challenging to achieve in clinical settings. Physical techniques, such as laser treatments, offer a novel approach by inducing topographical changes on the dentin surface, thereby increasing the bonding area and improving adhesion strength and stability. Although promising, laser-based methods are limited by factors such as the cost, equipment accessibility, and the need for practitioner expertise. The most effective strategy for achieving the bond between restorative materials and dental tissues lies in the synergistic application of the various agents and techniques. For instance, combining collagen cross-linkers with laser treatments could optimize both chemical stability and physical adhesion of the bond.

This synthesis underscores the need to diversify research efforts, placing greater emphasis on physical techniques, particularly laser-based methods, while continuing to explore the use of chemical agents.

Conclusion

Current knowledge and experimental research demonstrate that modern methods for dentin substrate modification significantly enhance the initial adhesive bond. Collagen cross-linkers and proteinase inhibitors play a pivotal role in improving bond stability, while antioxidants and remineralization agents offer protection against degradation. Furthermore, modifications in adhesive techniques and laser treatments contribute to bond strength but require careful clinical application. The optimal approach to achieving long-lasting resin-dentin bonds involves a tailored combination of these methods. However, the degradation of the interfacial contact surface remains a persistent challenge that compromises the longevity of restorations.

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SURGICAL TREATMENT OF ECTOPIC MAXILLARY THIRD MOLARS INVOLVING MAXILLARY SINUS – A SYSTEMATIC LITERATURE REVIEW

HIRURŠKO LEČENJE EKTOPIČNIH GORNJIH UMNJAKA UNUTAR MAKSILARNOG SINUSA – SISTEMATSKI PREGLED LITERATURE

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Summary

Introduction. This article aims to evaluate surgical procedures for the extraction of ectopic maxillary third molars involving the maxillary sinus. **Systematic Review.** A systematic review of case reports and case series of ectopic maxillary third molars involving the maxillary sinus was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement guidelines. **Results.** A total of 45 articles reporting 79 cases of ectopic third molars met the eligibility criteria. Among the patients, 45 were male (58%) and 34 were female (42%), with a median age of 25.5 years. Dentigerous cysts were found in 75% of cases. The conventional Caldwell-Luc procedure was employed in 39% of reported cases, while the Caldwell-Luc technique with intraoral endoscopy was utilized in 15% of cases. Endoscopic sinus surgery was performed in 36% of cases, with postoperative complications reported in 10%. **Conclusion.** Based on extensive review of the literature, we can conclude that the Caldwell-Luc procedure remains the preferred method for removing ectopic teeth in the maxillary sinus, whether or not associated with dentigerous cysts. Further refinement of intraoral endoscopy linked with the Caldwell-Luc technique should be particularly considered as it may lead to its establishment as the gold standard for such interventions.

Key words: Molar, Third; Maxillary Sinus; Tooth Eruption, Ectopic; Dentigerous Cyst; Endoscopy; Treatment Outcome

Sažetak

Uvod. Cilj ovog rada bio je da se procene tehnike hirurškog lečenja ektopičnih gornjih umnjaka unutar maksilarnog sinusa. **Sistematski pregled.** Sistematski pregled prikaza slučajeva i serija slučajeva ektopičnih maksilarnih trećih molara koji zahvataju maksilarni sinus sproveden je prema *Preferred Reporting Items for Systematic Reviews and MetaAnalyses* protokolu. **Rezultati.** Ukupno 45 članaka koji prikazuju 79 slučajeva ektopičnih trećih molara ispunili su kriterijume podobnosti. Ukupno je registrovano 45 muškaraca (58%) i 34 žene (42%). Srednja starost bila je 25,5 godina. Prisustvo odontogene ciste nađeno je u 75% slučajeva. Konvencionalni *Caldvell Luc* pristup je korišćen u 49% prijavljenih slučajeva, dok je kombinovani *Caldvell Luc* pristup, uz intraoralnu endoskopiju, korišćen u 15% slučajeva. Endoskopska hirurgija sinusa rađena je u 36% slučajeva. U 10% slučajeva zabeležene su postoperativne komplikacije. **Zaključak.** Na osnovu obimnog pregleda literature možemo zaključiti da je postupak *Caldvell Luc* i dalje tretman izbora za evakuaciju ektopičnih zuba u maksilarnom sinusu sa odontogenim cistama ili bez njih. Posebna pažnja se može posvetiti daljim modifikacijama intraoralne endoskopije povezane sa *Caldvell Luc* tehnikom koja može postati zlatni standard za ovu intervenciju.

Ključne reči: treći umnjak; maksilarni sinus; ektopični zub; odontogena cista; endoskopija; ishod lečenja

Introduction

Ectopic teeth most commonly involve mandibular third molars and maxillary canines [1]. However, ectopic maxillary third molars within the maxillary sinus (MTMMS) are a rare clinical presentation. When located in the sinus cavity, these teeth may manifest with classic sinusitis symptoms such as facial pain, swelling, headache, and nasal obstruction, or they may remain asymptomatic [1]. Symptoms usually occur when a dentigerous cyst is present in the sinus cavity. The development of dentigerous cysts in the maxillary sinus related to an ectopic tooth is rare. These cystic lesions are characterized by constant growth, leading to bone resorption and the displacement of surrounding structures and teeth [1].

The diagnosis of ectopic maxillary third molars and dentigerous cysts in the maxillary sinus is made through clinical and radiological examinations. Preoperative planning for the treatment of maxillary sinus pathologies is important due to the close proximity of vital anatomical structures [2]. Surgical extraction of ectopic third molars in the maxillary sinus is indicated for symptomatic patients as well as asymptomatic patients with dentigerous cysts [1–3]. The Caldwell-Luc procedure (CL), first described in 1893, was the treatment of choice for approaching maxillary sinus lesions until the introduction of endoscopic sinus surgery [2]. The CL technique has been used for enucleation of tumors, cysts, polyps, foreign bodies, and chronic sinusitis, as well as for the reduction of facial fractures, orbital floor decompression, closure of oroantral fis-

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Abbreviations

MTMMS– ectopic maxillary third molars involving maxillary sinus
CL – Caldwell-Luc procedure
ESS – endoscopic sinus surgery
PPF – pterygopalatine fossa
CBCT – cone-beam computed tomography
CT – computed tomography
MRI – magnetic resonance imaging
PRISMA – Preferred Reporting Items for Systematic Reviews and MetaAnalyses
DC – dentigerous cyst
AM – ameloblastoma
OKC – odontogenic keratocyst

tula, access to pterygomaxillary fossa, and approaches to the sphenopalatine ganglion [1–3]. However, the CL approach is associated with frequent complications such as postoperative swelling, infraorbital nerve paresthesia, damage to the roots of adjacent teeth, oroantral fistula formation, and postoperative anterior maxillary wall defects [4, 5]. Since the introduction of endoscopic sinus surgery (ESS), many authors have reported successful endoscopic management of ectopic teeth in the maxillary sinus, highlighting the reduced risk of injury to adjacent anatomical structures and less invasive nature of the procedure [4, 5]. Common complications of ESS procedures include damage to the lacrimal duct, nasal synechiae, and orbital trauma [3–5]. Clinical trials comparing the treatment outcomes between CL and ESS in maxillary sinus surgery have reported low complication rates for both approaches in the surgical treatment of chronic sinusitis [1–5]. Although the CL procedure is no longer considered the first-line treatment for maxillary sinus pathology, it remains in use for selected cases. In the era of endoscopy, the CL procedure combined with nasal and intraoral endoscopy is used with good outcomes.

The aim of this article is to evaluate surgical procedures for the extraction of ectopic maxillary third molars involving the maxillary sinus, with or without the presence of a dentigerous cyst.

Review of the Literature

A systematic review of case reports and case series involving ectopic maxillary third molars within the maxillary sinus was conducted following the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. In June 2022, electronic searches were performed in the PubMed (National Library of Medicine), Web of Science (Clarivate Analytics), and Scopus (Elsevier) databases using the search terms "ectopic third molar", "ectopic wisdom tooth", "maxillary sinus" and "dentigerous cyst". The retrieved references were exported to the EndNote software (Clarivate Analytics, Philadelphia, USA) to identify and eliminate duplicates.

Articles written in Latin script that mentioned ectopic maxillary third molars in the maxillary sinus, either as case reports or case series, were included in the review. Titles and abstracts of the retrieved references were screened by the authors. If the title or abstract met the inclusion criteria, the full text of the article was reviewed. Articles were excluded if the full text was unavailable. After evaluation of the full texts, only references that satisfied the eligibility criteria were included. Quality assessment and data evaluations were performed by all authors, and any disagreements were resolved through discussion.

For each selected article, the following data were extracted: type of study (case report or case series), sex and age, symptoms, diagnostic method, presence of an associated dentigerous cyst, localization of the ectopic tooth in the sinus cavity, type of anesthesia, surgical treatment, and postoperative complications.

Results

The search of the selected electronic databases revealed 250 references. Of these, 85 papers were selected for full-text evaluation, and 45 articles, reporting 79 cases of MTMMS, met the eligibility criteria [3–47] (**Figure 1**). Detailed characteristics of the studies included in this systematic review are presented in **Table 1**.

Demographic data are summarized in **Table 2**. There were 45 males (58%) and 34 female patients (42%), with a median age of 25.5 years (range 10 to 78

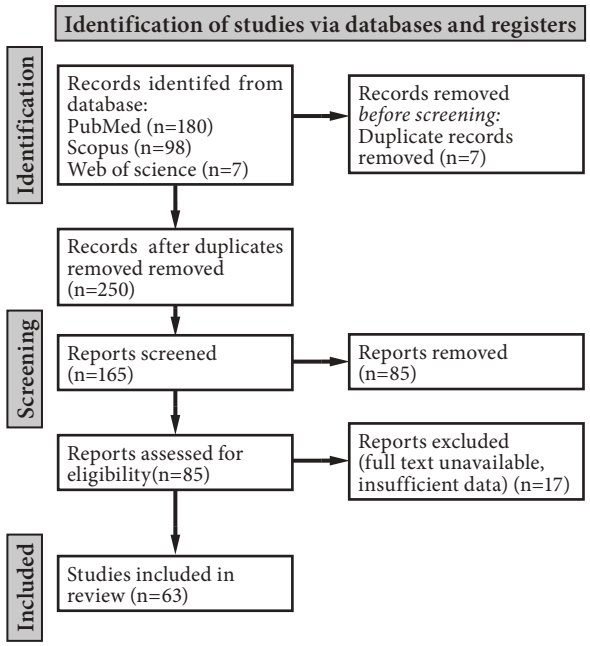

Figure 3. Study inclusion flow chart

Table 1. Publications on maxillary sinus cyst with ectopic third molars

Article	Type of study	Sex/ Age	Symptoms	Imaging	Maxillary sinus cyst	Teeth localization in maxillary sinus	Treatment	Complications
Arici et al. (2022)	Case report	F/32	Pain PR Swelling	CBCT	Dentigerous cyst	Bilateral Superior wall	CL	No
Mavriqi et al. (2022)	Case report	F/38	Pain	CBCT	No	Inferior and posterior wall	Transsinusal extraction and antrostomy + LA	No
El Fattah et al. (2021)	Case series (11 patients)	7M/4F 25-48y	4 asymptomatic 1 proptosis 3 PR + swelling 2 tooth loosening	CT	11 cysts	5 inferior wall 2 medial wall 2 superior wall 1 anterior wall 1 lateral wall	ESS + GA 5 transoral sublabial approach 4 endonasal through middle meatal antrostomy 2 endonasal prelacrimal	No
Lo Faro et al. (2021)	Case report	F/60	UNO PR Facial swelling	CBCT	Dentigerous cyst	Postero superior	CL + GA	No
Sigdel et al. (2021)	Case report	F/10	Facial swelling	CT	Dentigerous cyst	Lateral	CL + intranasal and intraoral endoscopy + GA	No
Seo et al. (2021)	Case series	M/21 F/26 F/65 M/54	Asymptomatic Asymptomatic Tooth loosening Tooth loosening	CT	No	Bilateral posterior Bilateral posterior Posterosuperior Posterosuperior	Modified intraoral endoscopic assisted sinus surgery (MESS)	No
Masalha et al. (2021)	Case series	10 patients 6 M/4 F 16-61 y	7 sinonasal symptoms (UNO, PR, swelling) 2 asymptomatic 1 oroantral fistula	CT	8 dentigerous cysts	1 inferior wall 2 inferior and medial wall 3 superior (orbital floor) 1 anterior 1 lateral	Nasal endoscopy + GA	1 Periorbital emphysema 1 numbness to the cheek 1 Postoperative bleeding
Allen et al. (2020)	Case report	M/14	Asymptomatic	CT	No	Inferior and posterior	Nasal endoscopy + GA	No
AlMomen et al. (2020)	Case report	M	UNO PR Sinusitis	CT	Dentigerous cyst	Medial wall	Transnasal endoscopy + GA	No
Dhivare et al. (2020)	Case report	M/17	Facial swelling Pain	Panoramic	Dentigerous cyst	Superior wall	CL + intranasal and intraoral endoscopy + GA	No
Balaji et al. (2020)	Case report	M/42	Pain	CT	Dentigerous cyst	Superior wall	CL	No
Moldovan et al. (2020)	Case report	F/22	UNO PR	CBCT	Dentigerous cyst	Postero superior	CL + GA	No
Elmorsy et al. (2020)	Case report	F/13	UNO PR	CBCT	Dentigerous cyst	Postero superior	CL + GA	No
Li et al. (2020)	Case series	F/56	Pain PR Swelling	CT	Dentigerous cyst	Posteromedial	CL + intraoral endoscopy + GA	No
		M/21	Pus swelling	CT	Dentigerous cyst	Posteromedial		No
Ngoc et al. (2019)	Case report	M/15	UNO	CBCT	Follicular cyst	Superior	CL	No
Alkhudair et al. (2019)	Case report	M/19	Recurrent sinusitis	CT	Dentigerous cyst	Bilateral ectopic teeth 1 lateral wall 1 medial wall	Nasal endoscopy + GA	No
Bernardi et al. (2018)	Case series	F/16	Exophtalamos Facial swelling Diplopia	CT + MRI	Follicular cysts in sinus and PPF	Posterosuperior wall	CL + intraoral endoscopy + GA	No
		F/20	Facial swelling Diplopia UNO			Posterosuperior wall	CL + intraoral endoscopy + GA	No
Sharma et al. (2019)	Case report	F/27	PR Facial swelling	CBCT	Follicular cyst	Bilaterally ectopic third molars in inferior wall	CL + GA	No
Emanuelli et al. (2018)	Case report	M/40	UNO PR Facial swelling	CT	Follicular cyst	Inferior wall	Nasal endoscopy + GA	No
Lombroni et al. (2018)	Case report	F/37	Asymptomatic	CT	Dentigerous cyst	Inferior and posterior	No	No
Barbieri et al. (2017)	Case report	M/42	PR	CT	Follicular cyst	Superior wall and infraorbital canal	CL + intraoral endoscopy + GA	No
Liau et al. (2017)	Case report	M/63	PR Facial swelling	CBCT	Follicular cyst	Postero superior	CL + endoscopy + GA	No
Marini et al. (2017)	Case report	F/40	UNO PR Facial swelling Headache Trismus	CT	Follicular cyst	Posterosuperior	CL + GA	No

Sinha et al. (2017)	Case report	F/25	UNO PR Facial swelling	CT	Follicular cyst	Superior and medial	CL	No
Jendi et al. (2017)	Case report	F/24	Swelling PR	CT	Odontogenic keratocyst	Lateral	CL + GA	No
Kumar et al. (2016)	Case report	M/22	Pain Headache	CT	Follicular cyst	Posterolateral wall	CL + GA	No
Aydin et al. (2016)	Case report	F/21	Chronic sinusitis Oroantral fistula	CT	Osteoma	Posterior wall	CL + intranasal and intraoral endoscopy + GA	Oroantral fistula
Kara et al. (2015)	Case report	F/16	UNO PR Facial swelling	CT	Follicular cyst	Superior	CL +GA	No
Kayabasoglu et al. (2015)	Case report	F/46	UNO PR Facial swelling	CT	Follicular cyst	Inferior	CL + intraoral endoscopy + GA	No
Bello et al. (2014)	Case report	M/17	UNO PR Facial swelling	CT	No	Posterolateral	CL + GA	No
Demirtas et al. (2014)	Case report	M/19	Pain Blurred vision	CT	Follicular cyst	Superior	CL	No
Mamatha et al. (2014)	Case report	M/17	UNO PR Facial swelling	CBCT	Follicular cyst	Lateral wall	CL + GA	No
Mermod et al. (2014)	Case report	M/74	Pain	CT	No	Posterolateral	CL + GA	No
Sivolella et al. (2014)	Case series	3 M/ 3W 14-65y	5asymptomatic 1 UNO PR	CT	6 dentigerous cysts	5 inferior and lateral 1 superior and lateral	CL + LA	No
Sogur et al. (2014)	Case series	M/19 M/24	Swelling Intraoral pus	CBCT	2 Denigerous cyst	Inferior and medial	CL + LA	No
Datil et al. (2014)	Case report	M/41	UNO PR Swelling	CT	Dentigerous cyst	Posterior and medial	CL + GA	No
Ramanojam et al. (2013)	Case series	F/48	Hypoesethesia of infraorbital nerve	CT	Dentigerous cyst	Inferior Posterior and lateral	CL	Hypoesethesia of infraorbital nerve
		M/22	Asymptomatic	CT	No	Anterior and lateral	CL + GA	No
		F/32	Pain	CT	No		CL	No
Abdollahifakhim et al. (2013)	Case report	M/17	UNO PR Facial swelling Headache Halitosis	CT	Follicular cyst	Superior and medial	CL + nasal endoscopy + GA	No
Vidya et al. (2013)	Case report	M/23	Facial pain and swelling	CBCT	Follicular cyst	lateral nasal wall	CL + GA	No
Guruprasad et al. (2013)	Case report	F/21	UNO PR Facial swelling	CT	Follicular cyst	Floor and medial wall	CL + GA	No
Rai et al. (2013)	Case report	F/46	Pain swelling	CT	Follicular cyst	Suerior wall	CL + GA	No
Viterbo et al. (2013)	Case report	M/29	Asymptomatic	CT		Posterior	CL + GA	No
Asnani et al. (2012)	Case report	F/28	UNO PR Facial swelling	CT	Follicular cyst	Superior and medial wall	CL + GA	No
Garde et al (2012)	Case report	M/19	Pain Diplopia	CT	Follicular cyst	Superior	CL + GA	No
Kasat et al (2012)	Case report	M/22	UNO PR Facial swelling	CT	Follicular cyst	Posterosuperior	CL	No
Thakur et al. (2011)	Case report	M/25	Pain	CT	Follicular cyst	Inferior wall	CL + LA	No
Mohan et al. (2011)	Case report	F/28	UNO PR Facial swelling	CT	Follicular cyst	Superior and medial	CL	
Beriat et al. (2011)	Case report	F/24	Pain	CT	Dentigerous cyst	Inferior wall	CL	No
Buyukkurt et al. (2010)	Case series	M/30	Facial swelling	CBCT	Follicular cyst	Posterolateral wall	CL + LA	No
		F/19	Facial swelling	CT	Follicular cyst	Superior and medial wall	CL + LA	No
Prabhu et al. (2009)	Case report	M/14	Facial swelling Pain	CT	Follicular cyst	Superior	CL	No
Lamb et al. (2009)	Case report	M/41	Asymptomatic	CT	Mucocela	Anterior and medial	CL	No
Micozkadioglu et al. (2007)	Case report	F/24	UNO PR Facial swelling Headache	CT	Follicular cyst	Inferior	transnasal endoscopy + GA	No
Prasad et al. (2007)	Case report	M/45	PR Facial swelling	CT	Follicular cyst	Anterior	CL + GA	No

Srinivasa et al. (2007)	Case report	M/45	UNO PR Facial swelling	CT		Anterior	CL + GA	No
Di Pasquale et al. (2006)	Case report	F/14	Asymptomatic	CT	Follicular cyst	Anterior and medial	Nasal endoscopy + GA	No
Buyukkurt et al. (2005)	Case report	M/31	UNO PR Facial swelling Headache	CT	Follicular cyst	Medial wall	CL + LA	No
Kamei et al. (2001)	Case report	F/38	PR	OPG	No cyst	Superior and posterior	CL	No
Hasbini et al. (2001)	Case report	M/21	UNO PR Facial swelling Headache	CT	Follicular cyst	Superior and medial	Endoscopy + GA	No
Bodner et al. (1997)	Case series	F/51 M/25	Asymptomatic Pain	CT	Dentigerous cyst	Inferior and lateral Inferior	CL + GA	No No
Vele et al. (1996)	Case report	M/30	UNO PR Facial swelling	2D	Dentigerous cyst	Posterosuperior wall	CL	No
Meara et al. (1996)	Case report	M/78	UNO PR Facial swelling Headache	CT	Follicular cyst	Posterolateral	CL	Recurrence of cyst after 3 months with oroantral fistula
Funderburg et al. (1992)	Case report	F/18	UNO PR Pain	CT		Posterior	CL + endoscopy + GA	No
Chuong et al. (1984)	Case report	M/16	Halitosis	CT	Follicular cyst	Posteromedial	CL	No
Golden et al. (1981)	Case report	F/37	UNO PR Facial swelling Headache	CT	Follicular cyst	Superior and medial	CL + GA	No
Present Case	Case report	M/13	Intraoral swelling	CBCT	Follicular cyst	Third molar displaced in pterygopalatine fossa	Modified CL + GA	No

M – male; F – female; UNO – unilateral nasal obstruction; PR – purulent rhinorrhea; CT – computed tomography; CBCT – cone beam computed tomography; MRI – magnetic resonance imaging; PPF – pterygopalatine fossa; CL – Caldwell-Luc operation; LA – local anesthesia; GA – general anesthesia

years). The most commonly reported symptoms were pain, swelling, unilateral nasal obstruction, and purulent discharge, while 20% of cases were asymptomatic. Rare symptoms included orbital proptosis, exophthalmos, diplopia, blurred vision, and inferior orbital nerve paresis. In terms of diagnostic imaging, CT was performed in 64 cases (82%), while CBCT was performed in 13 cases (14%). Dentigerous cysts were present in 78% of cases, while rarer findings included osteomas and odontogenic keratocysts associated with ectopic teeth. The most frequent ectopic positions of the third molars within the sinus cavity were in the superior and posterior regions. The conventional Caldwell-Luc approach was utilized in 39 cases (49%), while a modified Caldwell-Luc procedure with intraoral endoscopy was used in 11 (15%) cases. Endoscopic sinus surgery was performed in 29 cases (36%). General anesthesia was administered in 57 cases (71%), while local anesthesia was used in 12 cases (15%) treated with the CL approach. Postoperative outcomes were largely favorable, with 90% of patients experiencing no postoperative complications. However, postoperative sequelae were reported in six cases, including periorbital emphysema, cheek numbness, bleeding, and recurrent oroantral fistula.

Discussion

The most commonly reported surgical procedures for the extraction of ectopic teeth and maxillary sinus cysts in this review were the Caldwell-Luc procedure, endoscopic sinus surgery (ESS), and ESS combined with the Caldwell-Luc approach, accounting for 49%, 15%, and 36% of reports respectively [3–47]. The CL procedure has long been considered the classic surgical approach to the maxillary sinus due to its easy intraoral access to the sinus cavity through canine fossa, which can be performed under local anesthesia. The traditional CL operation involves accessing the sinus cavity through the canine fossa, removing the diseased sinus membrane, and drainage through an artificial aperture on the inferior nasal meatus. However, in the era of endoscopic surgery, the CL approach is considered aggressive due to common complications, including postoperative swelling, infraorbital nerve paresthesia, trauma to adjacent teeth, oroantral fistula, and postoperative anterior maxillary wall defects [1, 2]. In addition, postoperative maxillary cysts may develop years after the CL procedure due to reduced sinus volume [4, 5]. Also, the removal of the entire sinus mucosa during the CL procedure leads to its replacement with non-functional mucosa, perma-

nently altering the physiological functions of the sinus [4, 5]. Further complications may arise when an inferior meatal antrostomy is performed to sinus drainage, such as extended surgery time, nasolacrimal duct injury, and epistaxis [15]. Several modified, less aggressive versions of the CL procedure have been described, omitting the inferior meatotomy operation while still achieving good postoperative outcomes [15]. Endoscopic sinus surgery was introduced as a less invasive technique to traditional sinus surgery, offering reduced risk of damage to tooth roots and the infraorbital nerve, while also promoting sinus function recovery by preserving physiological mucosal lining [12]. In this review, 32% of ectopic third molars in the maxillary sinus were extracted using ESS, with the percentage of reported cases increasing in recent years. Many clinicians consider ESS as the first-line approach for treating maxillary sinus pathologies, especially maxillary sinusitis. However, even in the era of endoscopic surgery, the CL procedure remains the treatment of choice in treating sinusitis with irreversible mucosal damage, oroantral fistulas, odontogenic lesions, orbital floor fracture evaluations, and orbital decompression [1, 2]. Compared to the CL procedure, nasal endoscopy is performed under general anesthesia. Our review found significant differences in both surgical technique and anesthetic procedure. All cases utilizing local anesthesia involved the CL procedure, which may be an advantage of this approach. About 80% of cases were performed under general anesthesia, which although more comfortable for the patient and the surgeon, carries inherent risks. Thus, for situations where local anesthesia in indicated, the CL operation remains the treatment of choice for ectopic teeth in the maxillary sinus.

To reduce the complications associated with the two standard techniques, several authors have reported modifications to these approaches. Li et al. [15] used an endoscopy-assisted CL operation to treat dentigerous cysts and ectopic third molars involving the maxillary sinus, demonstrating that this approach provides clear visibility during operation, less bleeding and nerve injury, minimal incisions, and minimal bone loss. The combination of ESS and CL operation has also been shown to be effective for the removal of foreign bodies in the sinus and even for the enucleation of maxillary sinus tumors such as nasal inverted papilloma. Sigdel et al. [9] proposed a combined endoscopic transantral and endonasal approach for treating large maxillary sinus dentigerous cysts with impacted tooth. El Fattah et al. [4] reported three endoscopic-assisted surgical techniques for managing ectopic maxillary third molars with dentigerous cysts, tailoring the approach based on the location of the ectopic tooth. The authors utilized an endoscopic transoral sublabial approach for managing teeth in the inferior sinus wall, an endoscopy through middle meatal antrostomy for teeth located in the medial and superior walls, and an endonasal pre-lacrimal approach for teeth localized in the anterior wall. All approaches provided excellent visibility, access, and successful outcomes. Seo et al. [5] reported a modified ESS technique that integrates elements of both the conventional Caldwell-Luc procedure and functional endoscopic sinus surgery. This approach includes repositioning the buccal bony window and enlarging the maxillary ostium via an endonasal approach, which enables postoperative reduction of the maxillary sinus resulting from postoperative cyst formation or scar tissues.

Radiological examinations are essential in the preoperative diagnosis and treatment planning for enucleation of ectopic teeth and cysts in maxillary sinus. Conventional panoramic radiographs are useful due to their low level of radiation and diagnostic accuracy for assessing lesions and location of the ectopic tooth [1, 2]. However, 2D panoramic imaging has limitations in interpreting the exact location of the ectopic tooth, especially when displaced in the posterosuperior direction, due to superimposition of different bony structures [1, 2]. Specialized imaging modalities like CT, MRI, and CBCT play a vital role in managing and definitely diagnosing dentigerous cysts involving the maxillary sinus. CBCT offers several advantages over CT and MRI, including multiplanar volume reconstructions, 3D volumetric planning, a significantly lower radiation dose, lower costs, shorter scan times, and fewer imaging artifacts [1]. CBCT is a useful diagnostic tool for evaluating maxillary sinus pathologies due to high sensitivity and specificity for detecting jaw bone pathologies compared with panoramic, CT and MRI.

Regarding the surgical approaches, there were no differences based on the presence of a dentigerous cyst associated with ectopic teeth. Given that dentigerous cyst (DC) are found in about 80% of cases involving ectopic maxillary third molars, differential diagnosis with other cystic radiolucent lesions in the maxillary sinus, such as ameloblastoma (AM) and odontogenic keratocystic tumor (OKC), is essential due to varying treatment approaches for each lesion [48, 49]. Treatment of AM requires radical resection with clear margins and long-term follow-up, while OKC and DC are usually enucleated [49]. Comparative studies of the radiological features of these lesions are well-documented for mandibular lesions, while comparative studies for maxillary lesions remain limited. Meng et al. [49] used CBCT to evaluate the three-dimensional radiological features of maxillary AM, OKC and DC,

Table 2. Summarized data from the assessed articles

Data	Total n (%)	CL	CL + ESS	ESS
Total	105 (100%)	54 (51%)	18 (17%)	33 (32%)
Sex M/F	60 /45	32 /22	7/9	18/10
Age	31.21 ± 15.00	32.43±17.87	35.23±11.21	30.11±15.23
Symptoms				
Asymptomatic	21 (20%)	10 (18%)	5 (27%)	11 (30%)
Swelling	53 (52%)	33 (61%)	10 (59%)	14 (43%)
Pain	19 (16%)	11 (23%)	4 (23%)	11 (33%)
PR	52 (45%)	16 (31%)	7 (42%)	13 (40%)
UNO	35 (33%)	21 (41%)	6 (34%)	11 (33%)
Headache	15 (10%)	5 (11%)	3 (18%)	3 (9%)
Radiological diagnosis				
2D	2 (2%)	1 (2%)	1 (6%)	0
CT	86 (82%)	39 (72%)	14 (88%)	28 (100%)
CBCT	15 (14%)	14 (26%)	1 (6%)	0
CT + MRI	2 (2%)	0	0	0
Dentigerous cyst				
Yes	80 (76%)	45 (83%)	12 (66%)	25 (75%)
No	23 (22%)	8 (15%)	6 (34%)	7 (22%)
Other				
Osteoma	1 (1%)	1 (2%)	-	-
OKC	1 (1%)	-	-	1 (3%)
Position of the tooth in the sinus				
Superior	35 (33%)	16 (24%)	6 (33%)	10 (31%)
Posterior	17 (15%)	10 (21%)	9 (50%)	1 (3%)
Inferior	21 (20%)	11 (22%)	1 (4%)	11 (33%)
Lateral	11 (12%)	6 (11%)	1 (4%)	4 (11%)
Medial	11 (12%)	5 (10%)	1 (4%)	4 (11%)
Anterior	9 (8%)	6 (11%)	0	4 (11%)
Anesthesia				
Local	12 (11%)	12 (22%)	0	0
General	75 (71%)	29 (54%)	16 (100%)	28 (100%)
Unknown	18 (17%)	13 (24%)	0	0
Complications				
Yes	6 (6%)	2 (4%)	1 (6%)	3 (11%)
No	94 (90%)	52 (96%)	15 (94%)	25 (89%)
Unknown	5 (4%)	0	0	0

UNO – unilateral nasal obstruction; PR – purulent rhinorrhea; CT – computed tomography; CBCT – cone beam computed tomography; MRI – magnetic resonance imaging; CL – Caldwell-Luc operation; ESS – endoscopic sinus surgery; OKC – odontogenic keratocyst

and they identified significant differences among these lesions in terms of selected radiological features, such as radiopacity, location, size, bone expansion, internal structure, external borders, tooth envelopment, and tooth resorption. AM typically presents in the posterior maxilla, predominantly as oval shaped, unilocular or honeycombed internal structure with scalloped borders, and is frequently associated with adjacent tooth displacement and root resorption. OKC, by contrast, appears predominantly in the posterior maxilla as radiolucent, irregularly shaped lesion, with smooth borders and unilocular or multilocular internal structure, often enveloping adjacent teeth. OKC has higher tendency to fill the entire maxillary sinus compared to AM and DC. DC typically presents as an oval, radiolucent lesions with smooth borders and enveloped teeth.

This review identified significant differences in the location of ectopic teeth within the maxillary sinus and the choice of surgical technique. It appears that the CL procedure was preferred for teeth positioned in superior and posterosuperior locations, likely due to its good visualization and access to the posterior and superior walls of the maxillary sinus. Three-dimensional reconstruction of tooth localization is important in treatment planning. It is worth noting that ESS was used for the treatment of superiorly and posterosuperiorly positioned teeth in nine cases without any intraoperative or postoperative complications. Therefore, the observed differences in the choice of technique may be explained by the individual skills of the surgeon.

Conclusion

Based on the extensive literature review, we can conclude that the Caldwell-Luc procedure remains the preferred treatment for the removal of ectopic teeth associated with the maxillary sinus, with or

without the presence of dentigerous cysts. However, recent reports on the use of endoscopic sinus surgery for the removal of ectopic teeth favor this less invasive approach. Special should be given to further modifications of intraoral endoscopy in combination with the Caldwell-Luc technique, which may become the gold standard for this intervention.

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CASE REPORTS
PRIKAZI SLUČAJEVA

MULTI-ORGAN COMPLICATIONS ASSOCIATED WITH ADULT VARICELLA ZOSTER VIRUS INFECTION – CASE REPORT

MULTIORGANSKE KOMPLIKACIJE INFEKCIJE VIRUSOM VARICELLA ZOSTER U ODRASLOM DOBU – PRIKAZ SLUČAJA

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Case report
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Abstract

Introduction. The varicella-zoster virus is the causative agent of chickenpox, a disease that predominantly affects the pediatric population. Although the condition typically follows a benign course, it can lead to prolonged and multisystem involvement in both immunocompromised and immunocompetent, adults. This paper aims to present a case of an adult patient with pneumonia, pulmonary embolism, antiphospholipid syndrome, liver lesions, and thrombocytopenia following exposure to the virus, highlighting the increased risk of complications in adulthood. **Case Report.** A 25-year-old female was admitted to the Institute for Pulmonary Diseases of Vojvodina with fever, fatigue, malaise, and the onset of exanthema accompanied by dyspnea. The patient had no history of chronic diseases but was previously hospitalized during pregnancy due to eclampsia. Clinical findings and serological analyses confirmed a primary varicella-zoster virus infection. Chest radiography revealed pathognomonic bilateral nodular pneumonia, warranting further radiological evaluation due to the severity of the findings. Elevated D-dimer levels raised suspicion of pulmonary embolism, which was subsequently confirmed through computed tomography pulmonary angiography. While adult age and history of smoking were identified as risk factors, the extent of the clinical findings prompted immunological testing, leading to a diagnosis of antiphospholipid syndrome. **Conclusion.** This case underscores the importance of early recognition and comprehensive management of varicella-zoster pneumonia. A multidisciplinary approach is essential to ensure favorable outcomes in patients with severe complications.

Key words: Varicella Zoster Virus Infection; Multiple Organ Failure; Chickenpox; Antiphospholipid Syndrome; Pneumonia; Pulmonary Embolism

Sažetak

Uvod. Varičela zoster virus izaziva ovčije boginje, bolest koja pogađa dečiju populaciju. Mada bolest najčešće ima benigni tok, može biti i protrahovana i zahvatati više tkiva i organa kod imunokompromitovanih, ali i imunokompetentnih, zaraženih odraslih osoba. Cilj rada je prikaz slučaja bolesnice obolele od pneumonije i plućne embolije, sa antifosfolipidnim sindromom, lezijom jetre i trombocitopenijom, izazvanom ekspozicijom virusu u odraslom dobu, kada je incidencija nastanka komplikacija najveća. **Prikaz slučaja.** Pacijentkinja starosti 25 godina hospitalizovana je na Institutu za plućne bolesti Vojvodine povodom tegoba u vidu temperature, slabosti i malaksalosti, praćenih razvojem egzantema i dispnoičnih tegoba. Bolesnica je bez istorije hroničnih bolesti, tokom trudnoće bila hospitalizovana zbog eklampsije. Na osnovu kliničke slike i seroloških analiza potvrđena je primoinfekcija varičela zoster virusom. Radiogramom grudnog koša verifikovana je patognomonična obostrana nodularna pneumonija. S obzirom na masivnost nalaza, dijagnostički tok zahtevao je detaljniju radiološku obradu. Budući da je registrovana višestruko povišena vrednost D-dimera sumnjalo se na plućnu emboliju, koja je potvrđena kompjuterizovanom tomografijom sa pulmoangiografijom. Faktori rizika za nastanak komplikacija bili su adultni uzrast i pušački staž, međutim s obzirom na opsežnost nalaza postavljena je sumnja na imunokompromitovanost, te su urađena i imunološka ispitivanja, kojima je utvrđen antifosfolipidni sindrom. **Zaključak.** Kroz prikaz slučaja naglašavamo značaj pravovremenog prepoznavanja i sveobuhvatnog lečenja varičela zoster pneumonije, uz multidisciplinarni pristup koji je ključan u postizanju povoljnog ishoda.

Ključne reči: infekcije varičela zoster virusom; multiorganska disfunkcija; varičela infekcija; antifosfolipidni sindrom; pneumonija; plućna embolija

Introduction

Disseminated varicella infection, although rare, has been documented in both immunocompromised and immunocompetent adults [1, 2]. The report aims to present a rare case of disseminated varicella-zoster virus infection in an adult patient complicated by pneumonia, pulmonary embolism, antiphospholipid syndrome, liver lesions, and thrombocytopenia.

Case Report

We report the case of a 25-year-old female admitted to the Institute for Pulmonary Disease of Vojvodina.

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Abbreviations

AST – aspartate transaminase
ALT – alanine transaminase
LDH – lactate dehydrogenase
GGT – gamma-glutamyltransferase

Her symptoms began five days prior to hospitalization with high fever, weakness, and malaise, followed by the development of exanthema. Over the next few days, her condition worsened, and two days before admission, she developed dyspnea. The patient's medical history was notable only for a previous pregnancy complicated by eclampsia. She had no known chronic illnesses, allergies, alcohol abuse, or family history of disease. A smoker with a 5 pack-year history, she was unvaccinated against varicella. Epidemiologically, her son was diagnosed with varicella two weeks prior. On admission, she was afebrile, dyspneic, normocardic, and normotensive, with a diffuse polymorphous rash. Auscultation revealed normal breath sounds with bilateral crackles.

Clinical evaluation and serological analyses confirmed a primary varicella-zoster virus infection.

Chest X-ray revealed bilateral multiple confluent nodules, predominantly in the lower lobes. Blood gas analysis indicated hypoxemic respiratory insufficiency. Electrocardiography showed sinus rhythm at 80 bpm, with a Q wave and inverted T wave in lead DIII. Laboratory tests revealed leukocytes within reference range, mild erythrocytosis, thrombocytopenia, eosinopenia, elevated C-reactive protein, and significantly raised D-dimer levels. Elevated aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), and gamma-glutamyltransferase (GGT) enzyme levels was also noted **(Figure 1).**

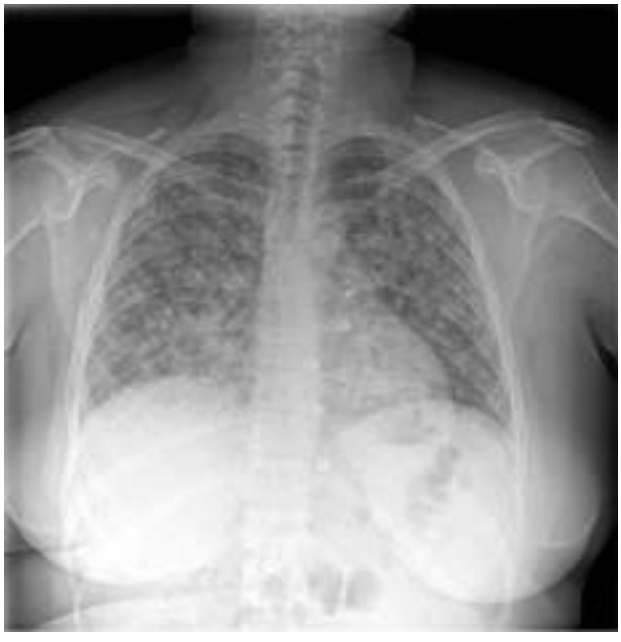
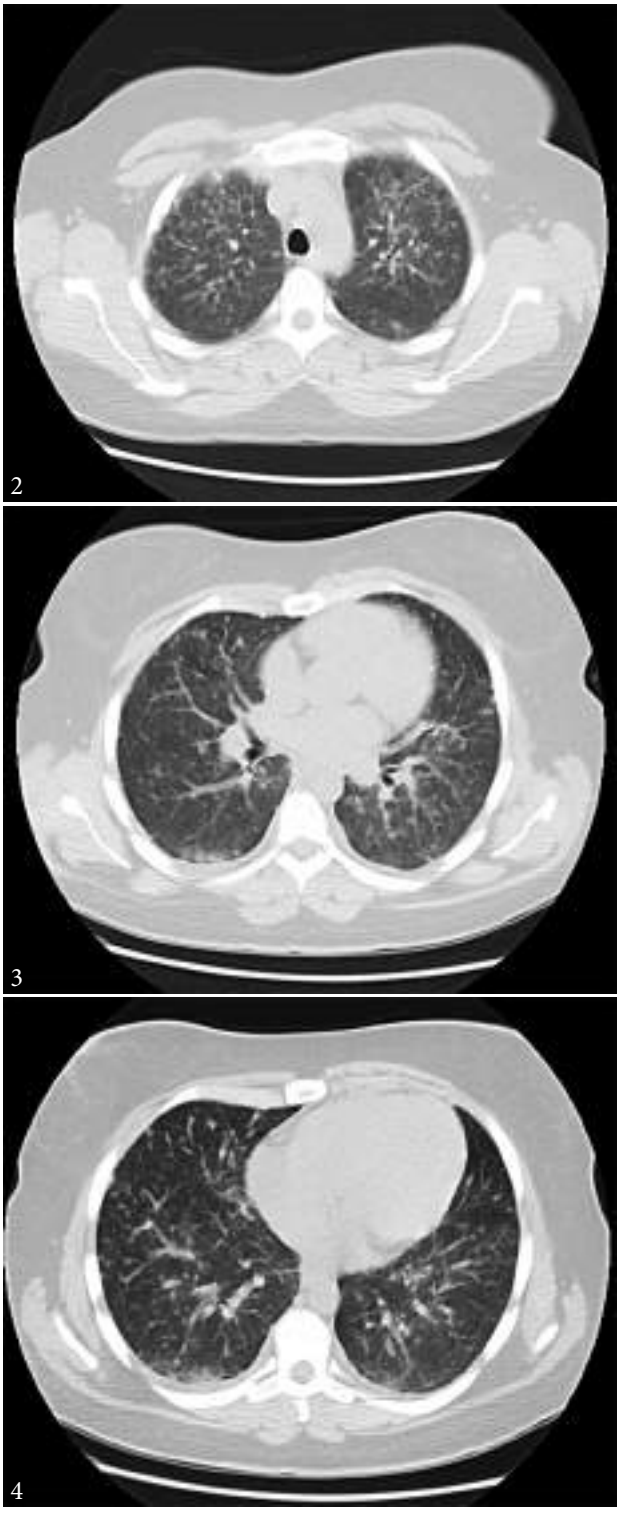


Figure 1. Chest X-ray on admission

Computed tomography pulmonary angiography (CTPA) demonstrated increased lung parenchyma density across all segments with bilateral polygonal ground-glass opacities and numerous semi-solid to solid nodules (up to 8 mm in diameter); enlarged mediastinal lymph nodes at positions 7-12 mm, 10R-14 mm; bilateral pleu-



Figures 2, 3 and 4. Chest CT scan

ral effusions (7 mm on the right, 8 mm on the left); filling defects in bilateral basal segmental and S1 pulmonary arteries on the right. Cranial computed tomography findings were normal **(Figures 2, 3 and 4)**.

Abdominal ultrasonography showed hyperechoic structure suggestive of diffuse fatty infiltration of the liver, with otherwise normal findings.

The patient initially received outpatient treatment with oral first-generation cephalosporins. Upon hospital admission, intravenous acyclovir, third-generation cephalosporins, glucocorticoid, oxygen therapy, and supportive care were initiated. Consultation with infectious disease specialists led to a 10-day course of antiviral and antibiotic therapy, alongside corticosteroid therapy with gradual dose tapering.

Parenteral anticoagulants were administered during hospitalization and transitioned to direct oral anticoagulants prior to discharge.

On the seventh hospital day, inflammation markers, blood counts, and D-dimer levels showed significant improvement. Liver enzyme levels AST, ALT, GGT, while still elevated, demonstrated a partial decrease.

Immunological testing came negative for HBs antigen, anti-HCV, anti-HIV, and multiple seromarkers. However, it identified a borderline positive beta-2 glycoprotein 1 IgM antibody and a positive anticardiolipin IgM antibody.

A follow-up chest X-ray at discharge showed complete regression of parenchymal shadows.

The treatment resulted in the disappearance of symptoms, complete radiological regression, normalization of inflammation markers, and partial decrease in hepatic enzyme values, and the patient was discharged in good general condition, cardiorespiratory compensated, and advised on further home care **(Figure 5)**.

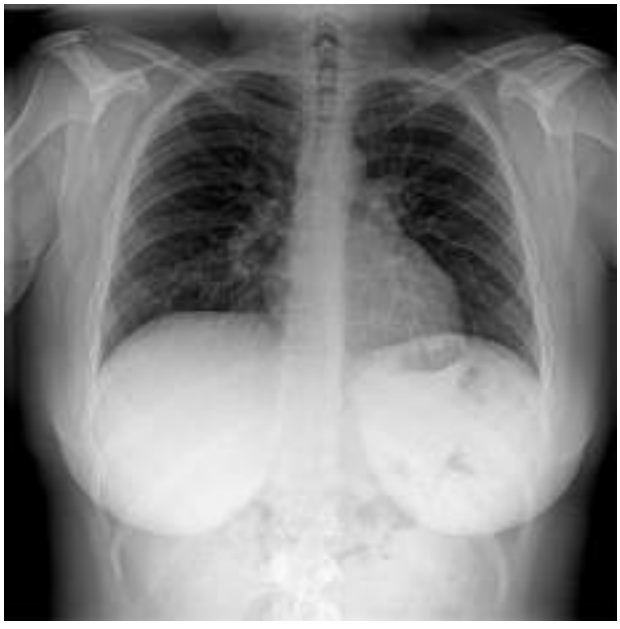


Figure 5. Chest X-ray at discharge

Discussion

Varicella-zoster virus primarily causes varicella (chickenpox) during its primary activation and herpes zoster (shingles) upon reactivation [3, 4]. Varicella predominantly affects pediatric populations, often occurring in collective settings during winter and spring. The disease's severity increases when transmission occurs within households, as seen in our patient [5].

Vaccination significantly reduces household transmission. Adults who are vaccinated contribute to herd immunity, protecting vulnerable populations such as young children or individuals with certain medical conditions. The varicella-zoster vaccine not only prevents chickenpox but also reduces the risk of shingles later in life. For immunocompromised individuals, varicella-zoster immunoglobulin is used as post-exposure prophylaxis [1, 6, 7]. Considering our patient's environment, which included a child in a collective setting, vaccination is highly advisable.

Adults face a higher risk of severe varicella complications, including respiratory complications affecting 5-15% of cases, with pneumonia occurring in approximately 1 in 400 individuals. Risk factors include pregnancy, lung disease, smoking, and immunosuppression. A higher number of skin lesions (>100) also correlates with increased viremia and pneumonia risk. Respiratory symptoms such as dyspnea, fever, cough (sometimes with hemoptysis), and chest pain typically appear within a week of symptom onset, as observed in our patient who was also a smoker. In rare cases, respiratory disease may progress to acute respiratory distress syndrome, often requiring invasive ventilation and hemodynamic monitoring in intensive care units [8–10].

Our patient also developed pulmonary embolism, a rare but documented complication of varicella-zoster infection [11–16]. Notable thrombotic complications such as purpura fulminans, necrotizing vasculitis, cerebral venous thrombosis, deep vein thrombosis, and pulmonary embolism have been reported. The mechanisms underlying these thrombotic complications are multifactorial, primarily involving endothelial injury, protein S deficiency, and the formation of antiphospholipid antibodies, as seen in our case [11, 17]. The presence of beta-2 glycoprotein 1 IgM and anticardiolipin IgM antibodies, along with the patient's history of pulmonary embolism, preeclampsia and eclampsia, fulfilled the diagnostic criteria for antiphospholipid syndrome [18]. The link between varicella-zoster virus and antiphospholipid syndrome is rarely discussed in

the literature, but immune responses triggered by the virus may enhance antiphospholipid antibody production, increasing thrombotic risk in susceptible individuals [19–21].

Hepatotoxicity in varicella infection usually presents as elevated liver enzymes and liver lesions, but fulminant hepatic failure due to extensive viral replication and hepatocellular destruction due to virus-induced cell lysis, a complication of progressive varicella, has also been reported [22, 23]. Another possible cause of liver damage may be clotting issues related to antiphospholipid syndrome. In our case, the patient exhibited a 16-fold elevation of liver enzymes and fatty liver infiltration.

Thrombocytopenia, often asymptomatic, can manifest as petechiae or bruises. Severe hemorrhagic complications, although rare, include intracranial hemorrhage [24]. In this case, thrombocytopenia was mild and asymptomatic.

Diagnosis is based on clinical presentation, isolation of viral antigens or serological tests for viral antibodies. Disease severity and risk factors guide further diagnostic strategies. Other authors followed a similar approach, conducting radiological imaging such as X-ray, computed tomography with pulmonary angiography, serological tests for vasculitis, connective tissue disorders (antinuclear antibodies, anticardiolipin antibodies), and antiphospholipid antibodies, protein S deficiency, human immunodeficiency virus, hepatitis antigens and antibodies [12].

First-line antiviral treatment involves acyclovir, administered orally or intravenously, with the latter preferred for severe and disseminated cases, including pneumonia, encephalitis, thrombocytopenia, and hepatitis, and immunocompromised patients. The use of acyclovir is recommended within the first 24 hours of rash onset. The use of acyclovir in pregnant women is permitted in indicated cases. Antiviral treatment is recommended for 7-10 days, depending on the clinical presentation, extending to 14 days for severe cases. Other antivirals include valacyclovir, famciclovir, brivudine, and foscarnet [3, 5, 8, 25].

The use of corticosteroids remains controversial. While they may reduce inflammation, their immunosuppressive effect can lead to adverse outcomes [25].

Despite a reported mortality rate of 10–30% for varicella pneumonia, rising to 50% for cases requiring mechanical ventilation [5], our patient achieved full recovery and was discharged in good general condition for further home care.

Conclusion

While varicella typically follows a benign course in childhood, complications and severe outcomes are more common in adults. Early identification of at-risk individuals, timely diagnosis, and appropriate treatment are critical. Exclusion of potential multi-organ complications through thorough evaluation ensures better outcomes in complex cases.

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BOOK REVIEWS
PRIKAZI KNJIGA

The 12th Balkan Congress of Arthroscopy, Sports Traumatology, and Knee Surgery, held in Novi Sad from November 15 to 17, 2024, served as the platform for the presentation of the book *The Anterior Cruciate Ligament of the Knee*. This comprehensive 455-page publication, organized into seven chapters, was edited by Vukadin Milankov, Vladimir Krstić, and Radmila Matijević. It features contributions from 69 authors across Serbia, North Macedonia, Montenegro, and Bosnia and Herzegovina, who collectively share 25 years of advancements and expertise in the study and management of anterior cruciate ligament injuries. This collaborative effort provides invaluable insights for practitioners in the field of orthopedic surgery and sports medicine.

Over 400 million years of tetrapod evolution have culminated in the human knee, the body's most complex joint. Distinguished by its intricate anatomy and multifaceted functionality, the knee provides static support, remarkable dynamic capabilities, and protection against injury. More than any other joint, its proper function depends on the harmonious interplay of its components, enabling movement, stability and upright posture.

Knee injuries, particularly those involving the anterior cruciate ligament (*Ligamentum Cruciatum Anterius* – ACL), have become increasingly prevalent due to rising participation in sports, heightened physical activity, and more frequent traffic accidents. Notably, these injuries often afflict young individuals, the most active segment of the workforce and athletic population, thus posing a significant public health concern.

In the 20th century, sports emerged as a sociological phenomenon and a symbol of modern living, often regarded as a benchmark for societal progress. Philosophers have argued that professional sports represent a new ideology of the 20th century and perhaps its greatest illusion. Integrated into contemporary society, sports are deeply entwined with media, politics, economics, and sociological constructs such as religion, race, gender, and age, significantly shaping the behaviors of large populations.

The anterior cruciate ligament (ACL) is the most frequently injured ligament in the knee and has been the subject of extensive research. A significant proportion of ACL injuries occur during sports activities often referred to as the "beginning of the end" for the knee due to the subsequent damage to the soft tissues

and bone structures, as well as the eventual onset of secondary degenerative changes. Therefore, it is crucial to consider all potential etiological factors and thoroughly assess injury risks to reduce the overall incidence of ACL injuries. Beyond physical limitations, ACL injuries impose substantial economic and emotional burdens.

Anterior cruciate ligament injuries remain a major concern in orthopedic surgery due to their long-term consequences, including persistent pain, joint instability, and the early onset of degenerative changes. Despite the identification of numerous risk factors, there is no consensus on which are the most influential. A systematic approach to identifying these risk factors is essential for developing effective prevention strategies. Key variables include gender, age, participation in sports involving frequent knee twisting and abrupt changes in direction and pace, anatomical and physiological characteristics, training regimens, playing surfaces, and sports equipment. Investigation of these etiological determinants not only sheds light on their contribution to injury occurrence but also to identifies modifiable factors that can inform targeted prevention measures.

Advancements in diagnostic methods, surgical techniques, and rehabilitation protocols have improved both the detection of ACL injuries and treatment outcomes. In most instances, conservative management fails to yield satisfactory results, making surgical intervention the preferred option, particularly for younger individuals and active or recreational athletes.

The primary objectives of surgical treatment for anterior cruciate ligament (ACL) injuries are to restore knee stability, improve functional capacity, and facilitate a timely return to sports and daily activities. Moreover, ACL reconstruction is widely regarded as a "quality-of-life" procedure, allowing young individuals to resume their professional, personal, and athletic pursuits while minimizing the risk of irreversible degenerative changes. The integration of contemporary scientific achievements in biology, biomechanics, and technical sciences has substantially improved treatment options and outcomes for ACL injuries.

Beyond its vital role in maintaining knee stability and function, the ACL delineates the boundary between an active, fulfilling life and the limitations imposed by injury. An ACL tear is more than a mere physical setback; it introduces a range of economic and emotional challenges that can profoundly impact patients' lives. Prolonged work absences may result in reduced income or even unemployment, affecting both individuals and employers who must manage decreased productivity and potential workforce adjustments.

The economic toll, however, is only part of the broader impact of ACL injuries. Emotional repercussions can be equally, if not more, profound. Patients frequently experience frustration and a sense of helplessness due to restricted mobility and the inability to participate in previously enjoyable activities. Among athletes, the fear of re-injury can undermine self-confidence and motivation, particularly when striving to return to competitive sports. Furthermore, chronic pain and ongoing instability may lead to depression, social isolation, and a diminished quality of life.

The complexity of ACL injuries places them at the forefront of challenges in orthopedic surgery. In light of this, it is essential to provide both experienced clinicians and trainees across multiple disciplines with a comprehensive resource addressing prevention, diagnostics, and therapeutic options for ACL injuries. This book aims to offer practical guidance through every phase of care, providing a framework to enhance patient well-being, with the ultimate goal of restoring mobility, resilience, and confidence in a brighter recovery path.

In addition, this text serves as a valuable reference for undergraduate and postgraduate students in medicine and physical education, as well as practitioners in orthopedics, sports medicine, physical medicine, rehabilitation, and related fields. By synthesizing the latest research and clinical experience, it seeks to improve patient outcomes and advance progress in orthopedic surgical practice.

Prof. dr Miroslav Milankov

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