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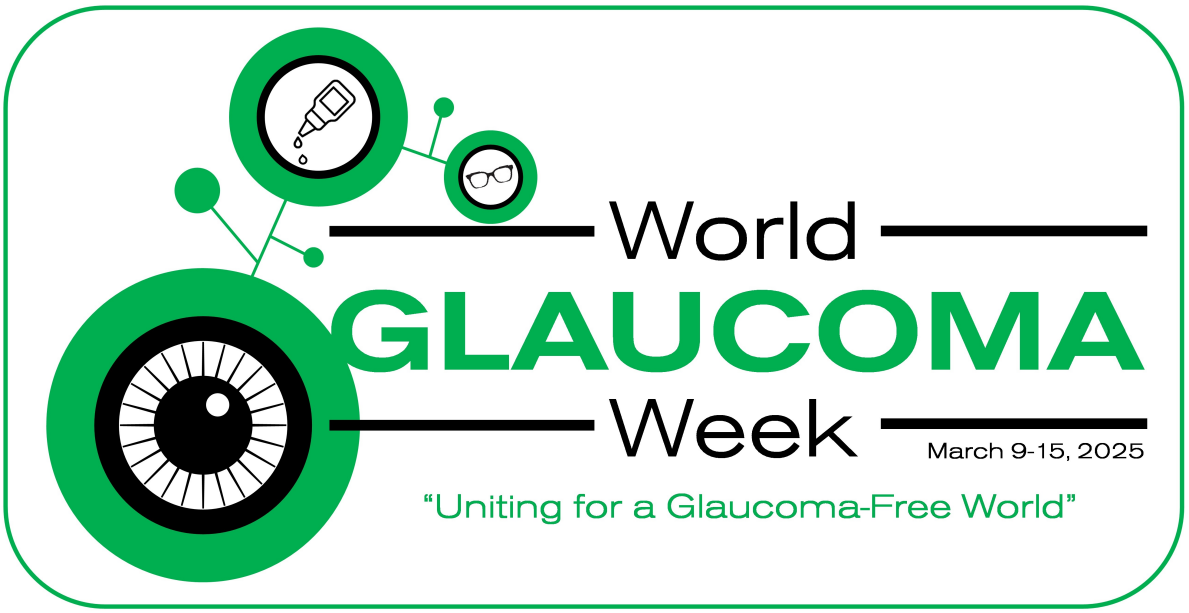

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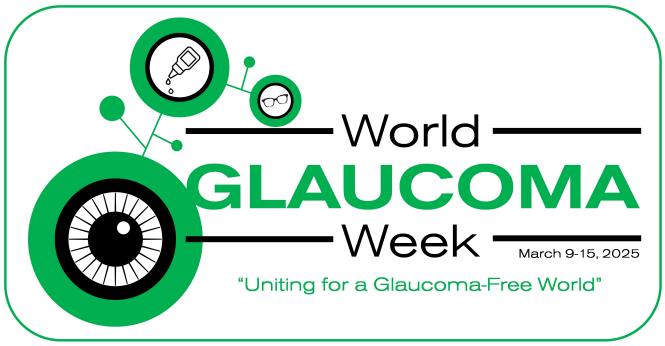

Glaucoma, the leading cause of irreversible blindness, is currently diagnosed in about 78 million people worldwide, and it is predicted that this number will rise to 111.8 million by 2040. Early detection of glaucoma and application of available treatment methods can prevent visual loss. The theme of this year's World Glaucoma Week, celebrated from March 9 to 15, is "Uniting for a Glaucoma-Free World".

Glaukom, vodeći uzrok ireverzibilnog slepila, je u ovom trenutku dijagnostikovan kod oko 78 miliona ljudi u svetu, a predviđa se da će ovaj broj porasti na 111,8 miliona do 2040. godine. Rano otkrivanje glaukoma i primena dostupnih načina lečenja mogu sprečiti gubitak vida. Tema ovogodišnje Svetske nedelje borbe protiv glaukoma, koja se obeležava od 9. do 15. marta je „Ujedinjeni za svet bez glaukoma".

GENERAL REVIEW

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Up-to-date approach in diagnosis and treatment of primary mediastinal B-cell lymphoma

Savremeni pristup u dijagnostici i lečenju primarnog medijastinalnog B-ćelijskog limfoma

Olivera Marković*†, Zorica Cvetković†‡, Ilija Bukurecki*, Anica Divac*, Nataša Stanisavljević*†, Milena Todorović Balint†§, Bela Balint‖¶

***Clinical Hospital Center "Bežanijska kosa", Clinic for Internal Medicine, Department of Hematology, Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia; ‡University Hospital Medical Center "Zemun", Clinic for Internal Medicine, Department of Hematology, Belgrade, Serbia; §University Clinical Center of Serbia, Clinic for Hematology, Belgrade, Serbia; ‖Serbian Academy of Sciences and Arts, Department of Medical Sciences, Belgrade, Serbia; ¶University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia**

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antineoplastic combined chemotherapy protocols; biomarkers; diagnosis; gene expression; histological techniques; lymphoma, large b-cell, diffuse; prognosis; tomography, emission-computed.

Ključne reči:
lečenje kombinovanjem antineoplastika, protokoli; bomarkeri; dijagnoza; geni, ekspresija; histološke tehnike; limfomi, b-krupnoćelijski, difuzni; prognoza; tomografija, kompjuterizovana, emisiona.

Introduction

Primary mediastinal B-cell lymphoma (PMBCL) is a rare type characterized by specific clinical and biological features [1]. It originates from thymic B lymphocytes. It was previously considered a subtype of diffuse large B-cell lymphoma (DLBCL), but the new classification recognizes it as a separate entity [2]. Molecular studies have determined that PMBCL shows increased expression of genes involved in nuclear factor (NF)-kB and Janus kinase (JAK) 2 signaling pathways, as well as amplification of 9p24 with frequent loss of functional human leukocyte antigen class II (HLA-II) complexes [3]. It is believed that these biological differences compared to classic DLBCL form the basis for a more favorable prognosis with fewer late relapses. PMBCL manifests as a mediastinal mass that spreads to surrounding organs and tissues, nearby lymph nodes, lungs, and pleural and pericardial spaces. Distant extranodal localizations, which are initially rare, are more frequently present in disease recurrence [4, 5].

At this time, there are no randomized clinical studies that define the optimal therapy for PMBCL. The foundation of therapy is immunochemotherapy based on rituximab and anthracyclines with the use of interim positron emission tomography (PET) scans, while in cases of disease recurrence, treatment is similar to that of relapsed DLBCL. Treatment in subsequent relapses includes the use of programmed death (PD)-1 inhibitors and chimeric antigen receptor-modified T (CAR-T) cells [6–9].

Pathohistological features

The histological picture of this lymphoma consists of a diffuse proliferation of large lymphoma cells with bands of fibrous tissue, along with cells that can vary in size and shape, sometimes resembling Reed-Sternberg cells [5]. Due to the presence of fibrosis, it can resemble carcinoma or thymoma, making it very important to obtain an adequate sample for pathohistological and immunohistochemical analysis. It can sometimes be very difficult to distinguish it from Hodgkin's lymphoma (HL) with nodular sclerosis and mediastinal gray zone lymphoma [4]. PMBCL and HL, nodular sclerosis type, have similar pathohistological features, as well as similar clinical characteristics (both occur in younger individuals as a large mediastinal mass), and share some biological and molecular characteristics [5]. The cellular composi

Correspondence to: Olivera Marković, Clinical Hospital Center "Bežanijska kosa", Clinic for Internal Medicine, Department of Hematology, Dr Žorža Matea bb, 11 080, Belgrade, Serbia. E-mail: markovic.olivera@bkosa.edu.rs

tion of the microenvironment in PMBCL varies and can resemble HL with fewer lymphoma cells and a diverse array of microenvironmental cells, than the one that more closely resembles DLBCL [6].

PMBCL cells express pan B-cell markers (CD20, CD79a, CD19, CD22) and B-cell transcriptional regulators (BOB.1, PU.1, OCT-2, PAX5, BCL6, MUM1/IRF4) [4, 10] with variable expression of BCL6, as well as BCL2, which is associated with a poor prognosis [11]. Expression of CD30 is present to varying degrees in about 80% of patients.

Similar to classic HL (cHL), PMBCL has been found to have amplification of 9p24.1, which results in overexpression of PD ligand 1 (PD-L1) and the formation of an immune "escape" phenotype [12, 13].

Clinical features

PMBCL most commonly occurs in young individuals, presenting as a large mediastinal mass. About 80% of cases are diagnosed at clinical stage I or II of the disease [5]. Local spread of the disease to surrounding structures (lungs, pericardium, pleura) is very often present. Unlike DLBCL, distant extranodal organ involvement is rare. Spread to distant organs is much more common in disease recurrence, but relapse of PMBCL in the central nervous system is rare (occurring in only about 3.0% of patients). The clinical symptoms of the disease are associated with the mediastinal mass. In cases of a large tumor mass, superior *vena cava* syndrome can develop, leading to thrombotic complications, which occur in about 30–40% of patients [14]. In some cases, PMBCL is not localized in the mediastinum, making the correct diagnosis more challenging for these patients [5].

Since PMBCL is a clinicopathological diagnosis, the certainty of the diagnosis can affect the comparison of studies. So far, only a few studies have used molecular diagnostics as the gold standard for diagnosis. For histological diagnosis, excisional biopsy is preferred. However, excisional biopsy is not feasible if the patient has respiratory and cardiac manifestations. In such situations, percutaneous needle biopsy is appropriate.

Molecular features

PMBCL shares significant clinical and histological similarities with HL, particularly with nodular sclerosis, and these similarities have been confirmed by gene profile analysis of the two types of lymphoma [13]. In PMBCL, genes involved in the JAK-STAT pathway and NF-kB activation show increased expression, while genes involved in B-cell receptor signaling pathways have reduced expression [13].

The key molecular characteristics of PMBCL include the amplification of 9p24.1, which leads to increased expression of PD-L1 and PD-L2, and recurrent genetic abnormalities in *B2M, CIITA, CD58, CD274,* and *PDC1LG2*, which contribute to an immunosuppressive tumor microenvironment [3]. A better understanding of the molecular pathways involved in the pathogenesis of PMBCL has opened up opportunities for the development of new targeted approaches in the treatment of this type of lymphoma.

Prognostic factors

The International Prognostic Index (IPI) used for determining the prognosis in DLBCL is not relevant for assessing the prognosis in patients with PMBCL, as these are typically young patients with a limited stage of disease [15–17]. Significant predictive factors in PMBCL include the presence of pleural or pericardial effusion, large tumor mass, extranodal disease localization, and doubled levels of lactate dehydrogenase (LDH) [15–18]. Patients without extranodal localization of the disease and normal LDH levels represent an ultra-low-risk subgroup with an 11% risk of poor therapeutic response and only 1% to 4% five-year mortality from lymphoma [19].

Zhou et al. [15] found that the absence of MUM1 expression and a low lymphocyte-to-monocyte ratio were associated with shorter progression-free survival (PFS) and overall survival (OS). However, another study found that low PD-L1 expression and high MUM1 expression were linked to a shorter time to disease progression [20].

In 2024, Noerenberg et al. [21] analyzed genetic changes in 340 patients with *de novo* PMBCL using whole-genome, whole-exome, and targeted sequencing. They found that several genetic aberrations significantly impacted therapy response and survival in PMBCL patients, which could be used for risk assessment and determining the optimal therapeutic approach [21]. In this study, *CD58* mutations were identified as the most significant adverse prognostic parameter, while patients with *DUSP2* mutations had long-lasting therapeutic responses and extended OS.

The Lymphoma Study Association (LYSA) group showed that a total metabolic tumor volume (TMTV) $\geq$ 360 cm^3 was associated with poor prognosis, regardless of treatment [22]. This group also found that total lesion glycolysis (TLG) $\geq$ 2,500 at the onset of the disease was associated with poorer PFS (p = 0.023). The International Extranodal Lymphoma Study Group (IELSG) 26 study found that combining initial TLG with the Deauville score at the end of treatment provided a better positive predictive value than TLG alone, with patients having TLG > 5.8 and an interim Deauville score of 4–5 experiencing poorer outcomes [23].

New biomarkers in PMBCL

Several studies have analyzed the significance of circulating tumor deoxyribonucleic acid (ctDNA) as a biomarker with potential applications in diagnosis, prognosis, response assessment, and disease remission monitoring. Currently, there is limited data on ctDNA in PMBCL, but studies published so far have revealed a high degree of concordance between the molecular profile of primary tumor biopsies and ctDNA in patients. Analyzing 197 patients with PMBCL, Schroers-Martin et al. [24] found higher ctDNA levels in patients with PMBCL and mediastinal gray zone lymphoma compared to DLBCL, cHL, and trans-

formed low-grade lymphoma. A subsequent multicenter study analyzing samples from 217 patients with DLBCL and PMBCL showed that pre-treatment ctDNA levels have prognostic significance both in *de novo* patients and those with relapsed disease. In the group of patients receiving initial therapy, a reduction in these levels after initial therapy was associated with better outcomes. Patients who achieved a 2-log or greater reduction in ctDNA after the first cycle of therapy had better outcomes after 24 months, with a longer time to adverse events (83% vs. 50%, respectively), and similar results were observed after two cycles of therapy (82% vs. 46%) [25].

First-line treatment

At present, there are no randomized clinical trials that define the optimal therapy for PMBCL. The reason for this is the rarity of this type of lymphoma and the necessity for urgent treatment in most patients, which complicates the inclusion in clinical trials. Treatment data primarily come from non-randomized prospective and retrospective studies [22, 26–37] (Table 1). Studies conducted before using rituximab showed that better treatment outcomes were achieved with more aggressive protocols. Today, several immunochemotherapy protocols are used: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) administered every 14 (R-CHOP14) or 21 (R-CHOP21) days; rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone (R-ACVBP) with consolidation therapy depending on PET findings; dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin (DA-EPOCH) [22, 27–34]. In the rituximab era, one of the most well-known studies conducted by Dunleavy et al. [27] showed that treatment with the DA-EPOCH-rituximab (DA-EPOCH-R) protocol eliminates the need for radiation therapy in patients with chemosensitive PMBCL. Following the publication of this study, the use of DA-EPOCH-R has increased significantly, while the use of radiation therapy has decreased significantly. In this series of patients, a higher percentage of patients achieved complete therapeutic response after DA-EPOCH-R compared to the R-CHOP21 protocol (84% vs. 70%, $p = 0.046$) [27]. The efficacy of these two protocols has been evaluated in several subsequent studies, but the results have been inconsistent [28, 30]. One of the largest recently published retrospective studies by an Italian group of authors involved 891 patients with PMBCL [31]. This study compared the efficacy of R-CHOP21,

Table 1

Studies in patients with PMBCL receiving first-line therapy

Authors	Therapy	Study type	n	PFS (95% CI)	OS (95% CI)
Camus et al. [22]	ACVBP14* R or O	retrospective	313	89.4	92.4
	CHOP14* R or O			89.4	100.0
	CHOP21* R or O			74.7	87.5
Hayden et al. [26]	R-CHOP	retrospective	159	80.0	89.0
Dunleavy et al. [27]	DA-EPOCH-R	prospective	51	93.0 (81–98)	97.0
Shah et al. [28]	R-CHOP	retrospective, multicentric	56	76.0 (64–88)	89.0
	DA-EPOCH-R		76	85.0 (75–94)	91.0
Santarsieri et al. [29]	DA-EPOCH-R	retrospective		3-year 92.8	97.2
Malenda et al. [30]	R-CHOP	retrospective	53	1-year 87.0	100.0
	DA-EPOCH-R			73.9	92.0
Iannitto et al. [31]	R-CHOP21*	retrospective, multicentric	891	71.0	91.0
	R-CHOP14*			89.0	
	R-megaCHOP			93.0	
	R-VACOP-B			83.0	
	R-MACOPB			86.0	
	DAEPOCH-R			77.0	
Rieger et al. [32]	R-CHOP-like	prospective, (subanalysis MInT)	44	78.0 (61.0–88.0)	88.5
Moskowitz et al. [33]	R-CHOP-14*-ICE	prospective	54	78.0	88.0
Gleeson et al. [34]	R-CHOP-14*	prospective	50	80.0	84.0
	R-CHOP21*				
Giulino-Roth et al. [35]	DA-EPOCH-R	retrospective, multicentric	118	87.5	97.1
Halahleh et al. [36]	R-CHOP	retrospective	49	60.0	71.0
Casadei et al. [37]	R-MACOP-B	retrospective	151	69.3	82.6

PMBCL – primary mediastinal B-cell lymphoma; n – number; PFS – progression-free survival; CI – confidence interval; OS – overall survival; R – rituximab; O – obinutuzumab; ACVBP – doxorubicin, cyclophoshamide, vindesine, bleomycin, prednisone; CHOP – cyclophosphamide, doxorubicin, vincristine, prednisone; R-CHOP – rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; DA-EPOCH-R – dose-adjusted etoposide, prednisone, vincristine, cyclophospamide, doxorubicin rituximab; R-megaCHOP – rituximab plus high-dose chemotherapy CHOP; R-VACOP-B – rituximab, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin; R-MACOP-B – rituximab, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin; R-CHOP-14-ICE – R-CHOP-ifosfamide, carboplatin, etoposide; MInT – Mabthera International Trial Group.

Note: * – immunochemotherapy protocols are used and administered every 14 or 21 days.

R-CHOP14, rituximab plus high-dose chemotherapy cyclophosphamide, doxorubicin, vincristine, prednisone (R-megaCHOP), rituximab, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin (R-VACOP-B), rituximab, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin (R-MACOP-B), and DA-EPOCH-R protocols. The study results indicated that R-CHOP is a suboptimal treatment for patients with PMBCL, whereas survival outcomes with other protocols were comparable to those achieved with the original R-MACOP-B protocol, which resulted in a five-year PFS of 86% [95% confidence interval (CI): 81–90] and a five-year OS of 91% (95% CI: 86–94) [31]. It should be noted that the interpretation of these study results is challenging because the criteria for selecting patients for each regimen are unclear, and most studies do not include a large enough number of patients to detect small differences. Moreover, the DA-EPOCH-R protocol has not been widely adopted in many centers, likely due to its complicated administration and hematologic toxicity compared to R-CHOP14/21. Just a few months ago, the largest meta-analysis of studies involving 4,068 patients was published, focusing on the efficacy of first-line therapy. The results of this study showed that more intensive chemotherapy protocols yield better results compared to the standard R-CHOP protocol, with a reduced need for radiation therapy [38].

Consolidation mediastinal radiotherapy (RT) is often used after the R-CHOP protocol, but it is unclear whether RT affects the risk of disease relapse and OS, especially in those who achieve complete remission after immunochemotherapy. Retrospective studies have shown conflicting results, likely due to different criteria for applying RT. For instance, a Surveillance, Epidemiology, and End Results (SEER) study of 250 patients with stage I or II PMBCL diagnosed between 2001 and 2012 reported a better five-year OS in patients who underwent RT (90% vs. 79 %; $p = 0.029$) [39]. Conversely, another SEER study involving 258 cases in all stages of PMBCL (diagnosed between 2006 and 2011) showed no difference in five-year survival (82.5% vs. 78.6%; $p = 0.470$), although the group not receiving RT had a numerically higher proportion of patients with an advanced stage of the disease (31% vs. 19%) [40].

The Role of PET-CT in PMBCL

PET-computed tomography (CT) – (PET-CT) plays a significant role in assessing the extent of the disease at diagnosis, evaluating the therapeutic response, and determining the need for radiation therapy after immunochemotherapy. CT is insufficient in sensitivity and specificity in PMBCL because patients may have a certain degree of sclerosis in the mediastinum after treatment, resulting in the persistence of residual tumor mass in the mediastinum after therapy in most patients. PET-CT has greater specificity than CT, but there is still the possibility of false-positive findings after therapy due to inflammatory changes post-treatment [41]. Should additional therapy be considered in such cases, a biopsy of the mass positive on PET-CT is necessary.

The results of published studies highlight the importance of assessing the metabolic therapeutic response based on various quantitative PET-CT parameters: tumor volume, including maximum standardized uptake value (SUVmax), TMTV, and TLG, which correlate with the prognosis of PMBCL patients [22, 42, 43]. TLG, which reflects both tumor volume and metabolism determined by PET-CT scan, has proven to be the strongest predictor of outcome, where the five-year PFS was 64% in those with high and 99% in those with low values [22, 41]. Similar results were obtained in a study where patients were treated with the DA-EPOCH-R protocol (two-year PFS was 60% in patients with high TLG and 95% in those with low TLG, $p = 0.006$) [42]. In the IELSG study, multivariate analysis showed that both tumor metabolic heterogeneity and TLG were independently associated with shorter PFS. The combination of these two factors identified a subgroup of 10% of patients with a five-year PFS of only 11% [22, 41]. Thus, quantitative PET-CT parameters are a potential tool for determining optimal therapy for each patient.

The role of mediastinal RT is not fully defined due to the potential for long-term adverse events on one hand and the favorable disease course on the other. A rational approach would be to apply RT based on PET-CT findings, i.e., omitting RT in patients who achieve PET-CT negativity after R-CHOP. The results of the largest prospective study of primary mediastinal B-cell lymphoma, IELSG37, support this approach. The results of this study show that radiation therapy can be omitted in patients who have a complete metabolic response after chemoimmunotherapy [44].

In any case, there is currently no consensus on the optimal therapeutic approach for PMBCL patients in the first-line therapy. However, the cornerstone of therapy consists of rituximab and anthracycline-based immunochemotherapy with interim PET-CT evaluation [5, 38]. Some authors recommend considering DA-EPOCH-R for patients with aggressive presentations and advanced-stage disease that has spread beyond the chest [15, 38].

Given that favorable results are achieved with intensive immunochemotherapy, consolidation therapy with autologous stem cell transplantation is no longer standard in first-line therapy.

Relapsing/refractory PMBCL

Nearly all PMBCL relapses occur within the first two years in approximately 2.5% to 4.5% of patients, except for rare late central nervous system relapses [1, 5]. The treatment of relapsing/refractory (R/R) PMBCL is similar to that of DLBCL, involving salvage therapy followed by high-dose chemotherapy and autologous hematopoietic stem cell transplantation (HSCT). However, few studies have evaluated the outcomes of R/R PMBCL, especially in the rituximab era.

In the Memorial Sloan Kettering Cancer Center study that included 60 patients with R/R PMBCL, a three-year PFS of 57% and OS of 61% were achieved, with a transplant rate of 85% [45]. Other series have also confirmed favorable outcomes after autologous HSCT, with a five-year OS ranging

from 57% to 70% [46, 47]. However, patients with refractory disease had poorer outcomes, making them candidates for the application of new therapeutic modalities.

New therapeutic approaches in PMBCL treatment

Amplification or translocation of the 9p24.1 region, found in over 50% of PMBCL cases, leads to increased expression of PD-L1. This finding has formed the basis for using PD-1 inhibitors in treating PMBCL, especially given the limited treatment options for patients with R/R disease. The first evidence of the efficacy of PD-1 inhibitors in PMBCL came from the phase 1 study KEYNOTE-013, which analyzed the effectiveness of pembrolizumab in patients with R/R lymphoma, including PMBCL [48]. In an updated analysis of this study, a therapeutic response in patients with R/R PMBCL was achieved in 48% (33% complete response – CR) of patients, with similar results observed in the subsequent phase II study, KEYNOTE-170, with a therapeutic response of 45% and CR of 13% after a follow-up of 29.1 months (KEYNOTE-013) [48] and 12.5 months (KEYNOTE-170) [49], respectively. The median PFS was 10.4 and 5.5 months, respectively, and the median duration of response was not reached in either study. Pembrolizumab was used as a bridging therapy to transplantation in nine patients. Grade 3/4 adverse events occurred in 23% of patients, with neutropenia being the most common adverse event. As a result of these findings, the Food and Drug Administration (FDA) approved pembrolizumab for the treatment of adult and pediatric patients with PMBCL who have relapsed after two or more lines of therapy.

Zinzani et al. [12] presented the final results of KEYNOTE-170 after a median patient follow-up of 48.7 months. This study provided the longest follow-up of PMBCL patients treated with pembrolizumab; 24% of patients completed two years of treatment. The overall therapeutic response was recorded in 41.5% (20.8% CR), and the four-year PFS and OS were 33% and 45%, respectively. Grade 3/4 treatment-related adverse events occurred in 22.6% of patients, and 7.5% discontinued treatment due to adverse reactions. Some of the most severe adverse effects were immune-mediated [12]. This study solidified the role of PD-1 inhibitors in the third line of PMBCL therapy but also opened the possibility of using PD-1 inhibitors in earlier lines of treatment. Studies are currently underway that gather data on the use of PD-1 inhibitors in previously untreated PMBCL patients.

To improve the treatment outcomes of patients with R/R PMBCL, there are attempts to combine PD-1 inhibitors with other drugs. For instance, in the CheckMate 436 study, which included a group of patients with R/R PMBCL, nivolumab was administered in combination with brentuximab vedotin (BV) [9]. Although a previous study using BV monotherapy achieved CR in only 13% of patients with R/R PMBCL [50], in this study, CR was achieved in 37% of patients (the overall therapeutic response was 73%), indicating the synergistic effect of PD-1 inhibitors and BV [9]. Half of the responding patients (n = 11) underwent autologous HSCT, and none of the patients relapsed. Grade 3 and 4 treatment-related adverse events were higher (53%) than after PD-1 inhibitor monotherapy. Neutropenia was observed in 30% of patients, and peripheral neuropathy in 27% of patients [9].

CAR-T therapy in PMBCL

CAR-T cell therapy has brought significant changes in the treatment of R/R aggressive B-cell lymphomas, including PMBCL. The ZUMA-1 and TRANSCEND NHL001 studies tested axicabtagene ciloleucel (axi-cell) and tisagenlecleucel (tisa-cel) in patients with R/R B-cell lymphoma (including PMBCL) after failure of at least two lines of therapy [7, 51]. In the ZUMA-1 study, which included 101 patients, the therapeutic response was 83%, with 58% CR; the median OS was 25.8 months, and the five-year OS rate was 42.6% [51]. Similarly, in the TRANSCEND study, a therapeutic response was achieved in 73% (with 53% CR) of all included patients, and in 15 patients with R/R PMBCL, the therapeutic response was 79% [7]. Estimated two-year PFS and OS rates were 40.6% and 50.5%, respectively [7]. The United FDA has approved axi-cell for treating R/R large B-cell lymphoma after the failure of the first lines of therapy, including autologous stem cell transplant. Tisa-cel was approved for the treatment of adults with R/R large B-cell lymphoma after two lines or more of systemic therapy.

Other new therapeutic approaches

Given the presence of CD30 positivity in the majority of PMBCL patients, these patients were included in phase 1/2 studies with $CD30^+$ B-cell lymphomas where a combination of BV and chemotherapy [cyclophosphamide, doxorubicin, prednisone (CHP) + BV] was used. In 22 patients with PMBCL, the two-year PFS was 86%, and the two-year OS was 100%, with no difference observed when consolidative RT was added ($p = 0.950$). However, the molecular profile of PMBCL was confirmed in 11 out of 14 (79%) patients, indicating a diagnostic challenge due to similarities with DLBCL, HL, and mediastinal gray zone lymphoma [52].

The efficacy of the antibody-drug conjugate, loncastuximab (ADCT-402), was also evaluated in patients with R/R B-cell non-HL, including 7 patients with PMBCL. A therapeutic response was achieved in 70 patients (48.3%), including CR in 36 patients (24.8%) [53]. Among 11 patients with a complete therapeutic response, the response was maintained for ≥ 2 years. These results led to FDA approval of the drug.

Bispecific antibodies have been evaluated across various B-cell lymphomas but have not specifically targeted PMBCL. Despite evidence of aberrant JAK/STAT pathway activation in PMBCL, JAK2 inhibitors have not been effective in this patient population [54]. In a pilot study where ruxolitinib was administered to patients with R/R HL and R/R PMBCL, none of the PMBCL patients demonstrated a favorable therapeutic response [54].

Conclusion

There is currently no consensus on the optimal first-line therapeutic approach for patients with PMBCL. Excellent results are achieved with immunochemotherapy based on rituximab and anthracycline, with the use of interim PET scans. DA-EPOCH-R regimen should be considered in those with aggressive presentations and advanced-stage disease spreading beyond the chest. Treatment of R/R PMBCL is similar to that of DLBCL, involving salvage therapy followed by high-dose chemotherapy and autologous HSCT if a favorable response is achieved. Bispecific antibodies have been studied in various B-cell lymphomas but have not specifically included PMBCL. PD-1 inhibitors and CAR-T therapy have found a place in the third-line treatment of PMBCL, with questions arising about their use in earlier lines of treatment.

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Effects of sufentanil in combination with dexmedetomidine for patient-controlled intravenous analgesia after renal transplantation

Efekti sufentanila u kombinaciji sa deksmedetomidinom za intravensku analgeziju koju kontroliše bolesnik posle transplantacije bubrega

Jun Ma*, Yawen Yang†, Linhao Sun†, Tingting Wu†

***Wuhan Wuchang Hospital, Department of Anesthesiology, Wuhan, China; †Soochow University, First Affiliated Hospital, Department of Anesthesiology, Suzhou, China**

Abstract

Background/Aim. Nowadays, the most convenient analgesic method is patient-controlled intravenous injection of one or more adjuvant drugs. The aim of our study was to evaluate the effects of sufentanil plus dexmedetomidine for patient-controlled intravenous analgesia (PCIA) after renal transplantation. **Methods**. Seventy-eight patients receiving living-related renal transplantation under general anesthesia were selected. Radioisotope scanning was performed, and the single glomerular filtration rate of the unilateral kidney was ≥ 40 mL/min/1.73 m². The control group (CG) and observation group (OG) (39 patients in each group) were analgesic with sufentanil and sufentanil plus dexmedetomidine, respectively. When the Visual Analogue Scale (VAS) score exceeded 4 points, 0.05 mg/kg oxycodone was intravenously injected for remedial analgesia. Plasma levels of endothelin, urea nitrogen, and creatinine were measured by radioimmunoassay. Heart rate (HR), mean arterial pressure, oxygen saturation, VAS score, and sedation score were recorded before anesthesia and after surgery. Analgesic remediation rate, number of effectively pressing the PCIA pump, and incidence rate of adverse reactions within 48 hrs after surgery were recorded. **Results**. HR of OG was significantly lower than that of CG 6 and 12 hrs after surgery ($p < 0.05$). VAS score of OG was lower than that of CG 6, 12, and 24 hrs after surgery ($p < 0.05$). OG had a lower postoperative remedial rate, number of effectively pressing the PCIA pump, and incidence rates of nausea and vomiting ($p < 0.05$) compared to CG. Endothelin, urea nitrogen, and creatinine levels significantly decreased after surgery compared with those before anesthesia ($p < 0.05$). The levels of OG were lower than those of CG at each time point after surgery ($p < 0.05$). **Conclusion**. Sufentanil plus dexmedetomidine can be safely and effectively used for PCIA after renal transplantation, with superior outcomes to those of sufentanil alone.

Key words:
analgesia; analgesia, patient-controlled; anesthesia, intravenous; kidney transplantation; postoperative period.

Apstrakt

Uvod/Cilj. Intravenska injekcija jednog ili više pomoćnih lekova, koju kontroliše bolesnik, najpouzdanija je analgetska metoda u današnje vreme. Cilj rada bio je da se proceni efekat sufentanila u kombinaciji sa deksmedetomidinom za intravensku analgeziju koju kontroliše bolesnik (*patient-controlled intravenous analgesia* – PCIA), nakon transplantacije bubrega. **Metode.** Izvršena je selekcija 78 bolesnika kojima je u opštoj anesteziji izvršena transplantacija bubrega živog davaoca. Urađeno je skeniranje radioizotopima, a pojedinačna stopa glomerulske filtracije jednog bubrega bila je ≥ 40 mL/min/1.73 m². Ispitanici iz kontrolne grupe (KG) i posmatrane grupe (PG) (po 39 bolesnika u obe grupe) primili su u cilju analgezije sufentanil i sufentanil sa deksemedetomidinom, redom. Kada je skor Vizuelne analogne skale (VAS) bio iznad 4 poena, intravenski je injektirano 0,05 mg/kg oksikodona za dodatnu analgeziju. Nivoi endotelina, uree i kreatinina u plazmi mereni su radioimunoesejem. Frekvencija srčanog rada (*heart rate* – HR), srednji arterijski pritisak, saturacija kiseonikom, skor VAS i skor sedacije beleženi su pre anestezije i posle operacije. Zabeleženi su stopa dodavanja analgetika, broj efektivnog pritiska PCIA pumpe i stopa incidencije neželjenih reakcija tokom 48 sati posle operacije. **Rezultati.** Nađena je značajno niža HR kod PG u odnosu na KG 6 i 12 sati posle operacije ($p < 0,05$). Skor VAS u PG bio je niži nego u KG 6, 12 i 24 sata posle operacije ($p < 0,05$). Kod PG utvrđena je niža stopa postoperativnog dodavanja leka, broja efektivnih pritisaka na PCIA pumpu i stope incidencije mučnine i povraćanja, u odnosu na KG ($p < 0,05$). Nivoi endotelina, uree i kreatinina su se značajno snizili posle operacije, u poređenju sa nivoima pre anestezije ($p < 0,05$). Nivoi u PG bili su niži od nivoa u KG na svakoj posmatranoj tački posle operacije ($p < 0,05$).

Correspondence to: Yawen Yang, First Affiliated Hospital of Soochow University, Department of Anesthesiology, No. 188 Shizi Road, Suzhou 215000, Jiangsu Province, China. Email: wrrdr5725zlxa@163.com

Zaključak. Sufentanil u kombinaciji sa deksmedetomidinom se može bezbedno i efikasno koristiti za PCIA posle transplantacije bubrega, sa uspešnijim ishodom u odnosu na primenu samog sufentanila.

Ključne reči:
analgezija; analgezija, kontrolisana od strane bolesnika; anestezija, intravenska; transplantacija bubrega; postoperativni period.

Introduction

Chronic renal failure is an irreversible disease that develops over a long period of time. With the prolongation of life expectancy and the increasing incidence of chronic diseases, such as hypertension and diabetes mellitus, the incidence rate of chronic renal failure is on the rise [1]. Kidney transplantation is the best therapy for patients with end-stage renal disease. This method has lower costs and fewer complications than long-term hemodialysis, and it can also improve patients' survival and quality of life [2]. Most patients suffer from end-stage renal disease, i.e., uremia. In addition to renal failure, the patients also suffer from other complications concerning other organ dysfunction and serious complica-tions, such as hypertension, water retention, electrolyte imbalance, acid-base imbalance, cardiac insufficiency, blood coagulation abnormality, metabolic and endocrine disorders, and nervous system diseases. Given the pathological, physiological, pharmacokinetics, and pharmacodynamics characteristics of end-stage renal failure, patients are less tolerant to anesthesia and analgesia in surgery [3, 4]. Therefore, it is of great significance to select appropriate anesthetic methods and drugs for patients receiving renal transplantation.

Sufentanil exerts a central analgesic effect by acting on μ and K receptors [5]. Nie et al. [6] used postoperative patient-controlled intravenous analgesia (PCIA) with sufentanil for women undergoing cesarean section. Although the analgesic effects were satisfactory, patients had adverse reactions (AR) such as nausea, vomiting, lethargy, and respiratory depression during follow-up because the dose of sufentanil was too high, but a lower dose led to insufficient analgesia. Currently, the most convenient and reasonable analgesic method is patient-controlled intravenous injection of one or more adjuvant drugs [7]. Dexmedetomidine (DEX) binds α-2 adrenergic receptors in the brainstem *nucleus coeruleus* to inhibit the transmission of pain signals to the brain and produce analgesic effects at the central level [8, 9]. It is now an adjuvant drug for anesthesia and pain management [10, 11]. The combination of sufentanil and DEX (SDEX), although the dose of each drug is lower than that of the single-use, takes a synergistic effect [12]. Meanwhile, sufentanil can enter the central nervous system through active transport. The concentration of unbound sufentanil in brain tissue is higher, and the pharmacodynamic effect is stronger, which makes up for the defect of low affinity for μ opioid receptor [13].

This study was designed to evaluate the efficacy of SDEX for PCIA after renal transplantation.

Methods

Baseline clinical data

Seventy-eight patients receiving living-related renal transplantation under general anesthesia from December 2020 to December 2022 were selected. This study has been approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (from December 5, 2020), and written informed consent has been obtained from all donors and recipients. Kidney donors were determined eligible based on the Consensus Statement of the Amsterdam Forum on the Care of the Live Kidney Donor (2004). Absolute and relative contraindications of living donors were excluded. Radioisotope scanning was performed, and the single glomerular filtration rate of the unilateral kidney was ≥ 40 mL/min/1.73 m^2.

Inclusion criteria were as follows: 1) patients aged 20–50 years, the American Society of Anesthesiologists class II–III, and body weight not exceeding or lower than 20% of the standard one; 2) patients receiving regular preoperative hemodialysis, with the time from last dialysis to surgery of 12–36 hrs; 3) patients receiving preoperative treatment with diabetic agents, and preoperative fasting blood glucose level of < 10 mmol/L; 4) patients without abnormalities of other routine examinations or heart and lung functions before surgery.

Exclusion criteria were: 1) patients with severe cardiac and pulmonary dysfunction, vital organ insufficiency, difficulty in tolerating surgery, severe anemia (hemoglobin < 7 g/dL), or diseases related to nerve or neuromuscular transmission function; 2) patients with blood coagulation abnormality, respiratory failure and history of renal transplantation; 3) patients with communication difficulties (language/physical defects/culture) affecting information collection.

The patients were divided into two groups (39 patients each) using the random number table method. The control group (CG) was analgesic with sufentanil, and the observation group (OG) was given SDEX. Medical staff who did not participate in this study connected and set the PCIA pump and observed related postoperative indicators. PCIA was performed by the researchers of this study immediately after surgery.

Anesthesia methods

Penehyclidine hydrochloride (0.5 mg) was intramuscularly injected 30 min before anesthesia. The venous access of the upper extremity was opened, and sodium acetate – Ringer's solution was infused at 5 mL/min. The opposite iliac artery was catheterized under local anesthesia. Blood pressure, heart rate (HR), central venous pressure, electrocardiogram,

and oxygen saturation (SpO_2) were routinely monitored. The bispectral index (BIS) was continuously monitored using the Aspect A-1000 BIS monitor.

Anesthesia induction: each patient was sequentially intravenously (i.v.) injected with 0.05 mg/kg midazolam, 1.5–2.0 mg/kg propofol, 0.3 μg/kg sufentanil, and 0.2 mg/kg cisatracurium besylate. After tracheal intubation, mechanical ventilation was performed to maintain partial pressure of end-tidal (P_{ET}) CO_2 of 35~40 mmHg (1 mmHg = 0.133 kPa) and SpO_2 of 95%~98%

Anesthesia maintenance: each patient was subjected to inhalation of 1–2% sevoflurane as well as i.v. infusion of 4–6 mg/kg/h propofol, 5–10 μg/kg/h remifentanil, and 0.1 mg/kg/h cisatracurium besylate to maintain the fluctuations of HR and mean arterial pressure (MAP) within 20% of the baseline values. BIS was maintained at 40–60. Sevoflurane, remifentanil, and cisatracurium besylate were discontinued 30 min before surgery, and propofol was stopped at the end of surgery. After surgery, 5 mg tropisetron and 0.1 mg/kg oxycodone were i.v. administered for both groups. A PCIA pump was connected immediately after surgery. The endotracheal tube was removed after the patients woke up naturally and had indications for extubation. CG was given 1.5 μg/kg sufentanil and 5 mg tropisetron dissolved in 100 mL of normal saline, and OG was given 1.5 μg/kg sufentanil, 2 μg/kg DEX hydrochloride, and 5 mg tropisetron dissolved in 100 mL of normal saline. The background infusion rate was 2 mL/h, the PCIA dose was 0.5 mL/time, and the lockout time was 15 min. The PCIA pump was used continuously for 48 hrs to maintain a VAS score of ≥ 4 points. When the VAS score exceeded > 4 points, 0.05 mg/kg oxycodone was i.v. injected for remedial analgesia.

Observation indices

Venous blood was collected before anesthesia and 24, 48, and 72 hrs after surgery. The levels of plasma endothelin, urea nitrogen, and creatinine were measured by radioimmunoassay. HR, MAP, SPO_2, VAS score, and sedation score were recorded before anesthesia and 2, 6, 12, 24, and 48 hrs after surgery.

The analgesic remediation and number of effectively pressing the PCIA pump within 48 hrs after surgery were recorded. AR within 48 hrs after surgery, such as oversedation (Ramsay score: ≤ 4 points), nausea, vomiting, dizziness, respiratory depression, and catheterization-induced bladder irritation, were recorded.

Statistical analysis

All data were analyzed by SPSS 16.0 software. The normally distributed measurement data were represented as mean ± standard deviation. Intergroup comparisons at various time points were performed by the repeated measures analysis of variance data. In case of significant intergroup differences, intergroup comparisons at each time point were conducted with the Independent Samples *t*-test. The time differences between groups were compared using the Student-Newman-Keuls q (SNK-q) test. The numerical data were subjected to the Chi-square (χ^2) test. The ranked data were compared with the rank sum test; $p < 0.05$ was considered statistically significant.

Results

Baseline clinical data and surgical outcomes

The two groups had similar baseline clinical data and surgical outcomes, including surgical time, anesthesia time, intraoperative blood loss, and intraoperative fluid transfusion volume ($p > 0.05$) (Table 1).

MAP, HR, and SpO_2 at different time points

The MAP values of the two groups decreased after surgery compared with those before anesthesia ($p > 0.05$). HR of OG was significantly lower than that of CG 6 and 12 hrs after surgery ($p < 0.05$). At each time point after surgery, the SpO_2 values of the two groups were maintained at above 95%, without significant intergroup differences ($p > 0.05$) (Table 2).

VAS and Ramsay scores at different time points

The VAS score of OG was significantly lower than that of CG 6, 12, and 24 hrs after surgery ($p < 0.05$). There was no statistically significant difference in Ramsay's score between the two groups ($p > 0.05$) (Table 3).

Table 1

Baseline clinical data and surgical outcomes in patients who underwent renal transplantation

Parameter	Control group	Observation group	*t* or χ^2 value	*p*-value
Age, year	45.67 ± 5.67	45.78 ± 5.98	$t = 0.431$	0.667
Body weight, kg	58.35 ± 4.38	58.29 ± 4.41	$t = 0.512$	0.613
BMI, kg/m^2	22.88 ± 2.48	23.02 ± 2.51	$t = 0.601$	0.549
Gender ratio (male/female)	26/13	25/14	$\chi^2 = 0.325$	0.567
ASA class II/III	33/6	32/7	$\chi^2 = 0.247$	0.618
Surgical time, min	153.78 ± 19.87	154.37 ± 20.22	$t = 1.379$	0.170
Anesthesia time, min	157.13 ± 24.38	156.96 ± 23.28	$t = 1.245$	0.216
Intraoperative blood loss, mL	189.23 ± 19.29	192.35 ± 21.29	$t = 1.315$	0.191
Intraoperative fluid transfusion volume, mL	1568.97 ± 109.97	1571.78 ± 112.45	$t = 1.110$	0.269

ASA – American Society of Anesthesiologists. n = 39 patients in each group.
All values are given as mean ± standard deviation or numbers.

Analgesic remediation, number of effectively pressing the PCIA pump, and incidence rate of adverse reactions within 48 hrs after surgery

Compared with the CG, the postoperative remedial rate, number of effectively pressing the PCIA pump, and incidence rates of nausea and vomiting were significantly lower in OG than in CG within 48 hrs after surgery ($p < 0.05$). However, they had similar incidence rates of catheter-related bladder irritation and dizziness ($p > 0.05$) (Table 4).

Levels of plasma endothelin, urea nitrogen, and creatinine

The levels of endothelin, urea nitrogen, and creatinine significantly decreased at all-time points after surgery

Table 2

MAP, HR, and SpO_2 at different time points

Parameter	Before	Postoperative				
		2 hrs	6 hrs	12 hrs	24 hrs	48 hrs
MAP, mmHg						
CG	117.94 ± 18.35	108.79 ± 17.37	104.78 ± 16.01	99.23 ± 15.89	98.64 ± 16.78	98.79 ± 16.93
OG	117.61 ± 18.29	110.68 ± 17.24	102.78 ± 15.79	98.25 ± 16.94	98.57 ± 17.01	97.16 ± 17.02
t-value	0.503	0.773	0.204	0.088	0.201	0.006
p-value	0.614	0.443	0.837	0.769	0.652	0.947
HR, bpm						
CG	79.46 ± 12.04	78.82 ± 12.43	98.45 ± 13.24	99.35 ± 13.12	79.45 ± 11.28	78.23 ± 12.34
OG	78.79 ± 11.89	77.81 ± 11.78	75.48 ± 12.23*	76.27 ± 12.47*	78.29 ± 11.76	77.69 ± 12.04
t-value	0.452	0.172	3.445	3.246	0.228	0.017
p-value	0.651	0.865	0.001	0.003	0.643	0.896
SpO_2, %						
CG	96.82 ± 1.17	96.76 ± 1.39	96.24 ± 1.25	96.52 ± 1.31	96.42 ± 1.54	96.56 ± 1.43
OG	96.78 ± 1.23	96.85 ± 1.25	97.48 ± 1.23	96.29 ± 1.19	96.54 ± 1.29	96.82 ± 1.34
t-value	0.474	0.197	0.351	0.242	0.373	0.021
p-value	0.635	0.842	0.724	0.811	0.708	0.793

MAP – mean arterial pressure; HR – heart rate; SpO_2 – oxygen saturation; CG – control group; OG – observation group. n = 39 patients in each group. All values are given as mean ± standard deviation.
***Intergroup comparison at the same time point, $p < 0.05$.**

Table 3

VAS and Ramsay scores at different time points

Parameter	Postoperative				
	2 hrs	6 hrs	12 hrs	24 hrs	48 hrs
VAS score					
CG	1.25 ± 0.23	2.97 ± 0.38	2.71 ± 0.36	2.53 ± 0.38	1.71 ± 0.41
OG	1.14 ± 0.18	1.26 ± 0.35*	1.09 ± 0.32*	1.34 ± 0.31*	1.48 ± 0.39
t-value	0.351	4.341	4.769	4.947	0.375
p-value	0.724	0.001	0.001	0.001	0.710
Ramsay score					
CG	2.18 ± 0.43	2.11 ± 0.39	2.36 ± 0.34	2.27 ± 0.32	2.21 ± 0.29
OG	2.17 ± 0.42	2.24 ± 0.41	2.29 ± 0.41	2.22 ± 0.33	2.19 ± 0.31
t-value	0.240	0.476	0.197	0.454	0.170
p-value	0.809	0.637	0.842	0.653	0.863

VAS – Visual Analog Scale; For other abbreviations, see Table 2. n = 39 patients in each group.
All values are given as mean ± standard deviation. *Intergroup comparison at the same time point, $p < 0.05$.

Table 4

Analgesic remediation, number of effectively pressing the PCIA pump, and incidence rate of adverse reactions within 48 hrs after surgery

Parameter	Control group	Observation group	*t* or χ^2 value	*p*-value
Analgesic remediation rate, %	20.51	2.56*	$\chi^2 = 4.805$	0.001
Number of effectively pressing the PCIA pump	7.82 ± 1.19	1.84 ± 0.43*	$t = 3.971$	0.008
Incidence rate, n				
of nausea	20.51	7.69*	$\chi^2 = 0.851$	0.048
of vomiting	12.82	2.56*	$\chi^2 = 5.651$	0.001
of catheter-related bladder irritation	2.56	0	–	–
of dizziness	12.82	10.26	$\chi^2 = 0.211$	0.644

PCIA – patient-controlled intravenous analgesia. n = 39 patients in each group.
Values for the number of effectively pressing the PCIA pump are given as mean ± standard deviation.
***Intergroup comparison at the same time point, $p < 0.05$.**
Note: *t* or χ^2 and *p*-value for incidence rate of catheter-related bladder irritation cannot be calculated due to zero value.

Table 5

Levels of plasma endothelin, urea nitrogen, and creatinine

Parameter	Before	Postoperative		
		24 hrs	48 hrs	72 hrs
Endothelin, ng/L				
CG	129.76 ± 18.57	98.71 ± 13.39#	91.71 ± 11.23#	87.56 ± 11.85#
OG	128.74 ± 18.47	86.45 ± 13.45*#	79.65 ± 11.54*#	72.29 ± 12.76*#
Urea nitrogen, mmol/L				
CG	17.41 ± 1.74	10.45 ± 1.92#	9.49 ± 1.75#	6.98 ± 1.48#
OG	17.31 ± 1.84	7.74 ± 1.86*#	6.89 ± 1.22*#	4.67 ± 0.37*#
Creatinine, mmol/L				
CG	977.12 ± 81.43	467.72 ± 81.45#	376.21 ± 61.21#	196.89 ± 51.31#
OG	976.14 ± 81.25	334.81 ± 82.23*#	219.41 ± 54.17*#	104.33 ± 49.13*#

CG – control group; OG – observation group. n = 39 patients in each group.
All values are given as mean ± standard deviation. *Intergroup comparison at the same time point after surgery, $p < 0.05$; #compared with the same group before surgery, $p < 0.05$.

compared with those before surgery ($p < 0.05$). The levels of OG were significantly lower than those of CG at each time point after surgery ($p < 0.05$) (Table 5).

Discussion

Postoperative pain (PP) is a complex physiological and psychological reaction caused by noxious stimulation [14, 15]. Multimodal analgesia is a combined use of analgesic drugs with different mechanisms of action or different analgesic measures to produce analgesic effects through multiple routes to obtain better effects and reduce AR [16]. Notably, PCIA can better alleviate PP using combined analgesic drugs [17]. In this study, SDEX was used for PCIA, and the results showed that the VAS scores at 6, 12, and 24 hrs after combined use were significantly lower than those of sufentanil alone, which is consistent with previous literature [18].

When used for postoperative analgesia, opioids, such as sufentanil, may often cause AR-like respiratory depression, nausea and vomiting, and excessive sedation despite good analgesic effects [19]. If the dosage of the drugs is not controlled well, it may further increase postoperative risk. Unlike opioids, DEX can work effectively without inducing respiratory depression [20]. In this study, the postoperative remediation rate and PCIA effective compression times also decreased. In addition, the incidence rate of nausea and vomiting was also significantly lower, and the HR values at 6 and 12 hrs after surgery of the SDEX were significantly lower than those of the sufentanil group. Likewise, Tang et al. [21] reported that SDEX worked better than sufentanil alone in alleviating PP, inflammation, and delirium after esophageal cancer surgery. Plasma endothelin is synthesized by endothelial cells and is significantly elevated under stress conditions such as pain and anxiety, which, therefore, is considered to be a sensitive index of stress response [22]. When renal function is severely impaired, plasma endothelin increases, further causing contraction of renal blood vessels, reduced renal blood flow, and glomerular filtration rate, thereby reducing urine output [23]. Besides, the abundance of urea nitrogen is a well-established substitute marker for renal function. During a stress response, the production of urea nitrogen is promoted, finally leading to the increase in blood level [24]. Moreover, creatinine is excreted by renal metabolism, so its level in plasma is elevated when the renal function is damaged, especially in the case of stress response [25]. PCIA can effectively alleviate pain and reduce stress response in renal transplant patients [26]. Herein, plasma endothelin, urea nitrogen, and creatinine levels in OG were significantly lower than those in CG 24 hrs after surgery. The findings are in agreement with those of a previous study [27].

Conclusion

In conclusion, sufentanil combined with dexmedetomidine can be safely and effectively used for PCIA after renal transplantation, with an effect better than that of sufentanil alone.

Conflict of interests

The authors declare no conflict of interest.

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Factors associated with physician burnout syndrome: a comparative analysis

Faktori povezani sa sindromom sagorevanja kod lekara: komparativna analiza

Marko Stojanović, Nataša Rančić, Miodrag Stojanović

University of Niš, Faculty of Medicine Niš, Niš, Serbia; Institute for Public Health Niš, Niš, Serbia

Abstract

Background/Aim. Burnout syndrome (BS) can occur in doctors at different levels of health care. The aim of this study was to identify the prevalence of BS and compare the differences among physicians in health institutions in Southeast Serbia (SES). **Methods.** A multicenter, questionnaire-based cross-sectional study was conducted among 373 physicians (252 female and 121 male) from primary, secondary, and tertiary care institutions in SES from 2023 to 2024. The Maslach Burnout Inventory-Human Services Survey (MBI-HSS) was applied. **Results.** A significantly higher score on the emotional exhaustion (EE) subscale was observed in physicians older than 55 years ($p < 0.001$) and those with more than 25 years of work experience and more than 14.5 years of specialist experience. Higher EE scores were also observed in doctors with specialization ($p < 0.001$), then, in those in managerial positions ($p = 0.031$), in doctors with additional engagement in private practice ($p = 0.019$), those with more than two duties *per* week ($p = 0.008$), and physicians who assessed that their greatest job burden was dealing with a large number of patients during the workday ($p < 0.001$). A significant increase in depersonalization (DP) subscale values was associated with age above 53.5 years, additional engagement in private practice ($p = 0.015$), shift work ($p = 0.049$), and physicians who assessed that their greatest job burden was dealing with a large number of patients during the workday ($p < 0.001$). A significant increase in personal achievement subscale values was observed among doctors in managerial positions ($p = 0.004$). The highest percentage of physicians from tertiary care institutions had high EE and low personal achievement values, while the highest percentage of physicians from primary care institutions had high DP values. **Conclusion.** Doctors in health institutions in SES have a moderate degree of BS. Additional research into BS and the factors that contribute to its occurrence is needed, as well as taking appropriate preventive and corrective measures at each of the three levels of health care.

Key words:
burnout, psychological; physicians; primary health care; serbia; specialization; surveys and questionnaires; tertiary care centers.

Apstrakt

Uvod/Cilj. Sindrom sagorevanja (*burnout syndrome* – BS) se može javiti kod lekara u različitim nivoima zdravstvene zaštite. Cilj rada bio je da se utvrdi prevalencija BS i uporede razlike među lekarima u zdravstvenim ustanovama u Jugoistočnoj Srbiji (JIS). **Metode.** Multicentrična studija preseka, zasnovana na upitnicima, sprovedena je među 373 lekara (252 žene i 121 muškarca) iz ustanova primarne, sekundarne i tercijarne zaštite u JIS od 2023. do 2024. godine. Primenjena je anketa *Maslach Burnout Inventory-Human Services Survey* (MBI-HSS). **Rezultati.** Značajno veći skor na subskali emocionalne iscrpljenosti (*emotional exhaustion* – EE) zabeležen je kod lekara starijih od 55 godina ($p < 0,001$) i onih sa više od 25 godina radnog iskustva i više od 14,5 godina specijalističkog staža. Viši rezultati EE zabeleženi su i kod lekara specijalista ($p < 0,001$), zatim kod onih na rukovodećim pozicijama ($p = 0,031$), kod lekara sa dodatnim angažovanjem u privatnoj praksi ($p = 0,019$), onih sa više od dva dežurstva nedeljno ($p = 0,008$), kao i kod lekara koji su ocenili da im je najveće opterećenje na poslu veliki broj pacijenata tokom radnog dana ($p < 0,001$). Značajno povećanje u vrednostima subskale depersonalizacije (DP) bilo je povezano sa životnim dobom iznad 53,5 godina, dodatnim angažovanjem u privatnoj praksi ($p = 0,015$), radom u smenama ($p = 0,049$), kao i kod lekara koji su procenili da im je najveće opterećenje na poslu bio veliki broj pacijenata tokom radnog dana ($p < 0,001$). Značajno povećanje vrednosti subskale ličnog postignuća primećeno je među lekarima na rukovodećim pozicijama ($p = 0,004$). Najviši procenat lekara iz tercijarnih zdravstvenih ustanova imao je visoke vrednosti EE i niske vrednosti ličnog postignuća, dok je najviši procenat lekara iz primarnih zdravstvenih ustanova imao visoke vrednosti DP. **Zaključak.** Lekari u zdravstvenim ustanovama u JIS imaju srednji stepen BS.

Correspondence to: Marko Stojanović, University of Niš, Faculty of Medicine, Bulevar dr Zorana Djindjića 81, 18 000 Niš, Serbia.
E-mail: 100janovic.marko86@gmail.com

Potrebna su dodatna istraživanja BS i faktora koji doprinose njegovom nastanku, kao i preduzimanje odgovarajućih preventivnih i korektivnih mera na svakom od tri nivoa zdravstvene zaštite.

Ključne reči:
sagorevanje na radu, sindrom; lekari; zdravstvena zaštita, primarna; srbija; specijalnosti, medicinske; ankete i upitnici; zdravstvene ustanove, tercijarne.

Introduction

Burnout syndrome (BS) is a major psychosocial problem that affects professionals from different areas, and it is particularly prevalent among physicians, where the incidence of burnout is even on the rise [1]. The syndrome is defined as a state resulting from prolonged stress, marked by ongoing fatigue, negative feelings towards one's work, and reduced effectiveness in professional tasks [2]. Among physicians, BS is associated with decreased well-being and worse quality of patient care [3]. It is characterized by three dimensions: a feeling of lack of energy or emotional exhaustion (EE); increased mental distance of the worker from the work he or she is engaged in or a sense of negativism and/or cynicism in relation to work, i.e., depersonalization (DP); reduced professional efficacy or lower personal achievement (PA) [4]. EE refers to the worker's impression that his or her emotional and physical resources are exhausted beyond their limits, with symptoms that often manifest as fatigue, headache, insomnia, and changes in appetite. DP refers to the appearance of distance and a cynical attitude toward the people to whom the service is provided, a negative attitude towards work, and the loss of a sense of personal identity. A reduced sense of PA refers to the negative self-assessment of competencies and achievements at work, with symptoms that are visible as a loss of work motivation, declining self-esteem, and overall productivity [2, 4].

According to the 11th Revision of the International Classification of Diseases (ICD-11), BS is defined as a syndrome resulting from chronic workplace stress that has not been successfully managed [5]. Burnout is not defined as a mental disorder or illness. In some European countries with developed social security systems (especially in Sweden and the Netherlands), BS is recognized and established as an official medical diagnosis [6].

In a study comparing incidences of burnout among physicians from the United States of America and a sample of workers from the general population, Shanafelt et al. [7] reported an incidence of BS of 37.9% in physicians compared to 27.8% in the general population ($p < 0.001$). The frequency of the presence of burnout varies around the world. For instance, in Europe, there is a difference between the European Union (EU) countries (10%) and the non-EU countries (17%). In EU countries, the frequency of burnout ranges from 4.3% in Finland to 20.6% in Slovenia, and within the non-EU countries, from 13% in Albania to 25% in Turkey [8]. This study also showed that a higher level of burnout among country employees is positively related to a higher workload.

In a study from 2022, data on the presence of BS in selected countries were presented based on a survey conducted among primary care (PC) physicians. Based on the obtained data, more than half of all surveyed doctors stated they were under a lot of stress but did not feel the presence of BS. According to this research, less than 5% of the total surveyed physicians in Australia, the Netherlands, Germany, Switzerland, the United Kingdom, and the United States of America felt a higher level of BS and questioned their ability to continue quality work. In this 2022 study, only 18% of New Zealand doctors and just over 18% of Canadian physicians surveyed reported significant burnout. Percentages in other countries were significantly lower, with the lowest percentage of BS being among the Swiss doctors [9].

According to the results of a review paper that included 182 studies published between 1991 and 2018, involving 109,628 individuals from 45 countries, significant variability was observed in the prevalence of BS among physicians, ranging from 0% to 80.5%. According to this research, physicians at the front line of patient care, such as family medicine doctors, internal medicine specialists, and those employed in the fields of emergency medical care, are at the highest risk of developing BS [10].

The incidence of BS varies among physicians of different specialties, but it is important that it shows an increasing tendency. Physicians from all three institutions, primary, secondary, and tertiary care, are almost at equal risk, although the contributing factors differ. To date, no study has been conducted in Southeast Serbia (SES) that compares the risk of developing BS among doctors from different levels of healthcare institutions. Therefore, the aim of the study was to determine the prevalence of BS among physicians in state health institutions of the SES, as well as the factors that significantly influence this phenomenon.

Methods

Study design

A multicenter cross-sectional study was performed from 2023 to 2024. The study included physicians from different state health institutions of five districts in SES. The size of the representative sample was determined based on official statistical data regarding the number of physicians in SES [11] and the previously defined research parameters: a study power of 80%, a type I error probability (α) of 0.05 for two-tailed hypothesis testing, and the assumption that the physician population would complete at least 60% of the total number of distributed questionnaires. The final sample

consisted of 373 physicians who completed all the questionnaires.

Physicians of both sexes, employed full-time in state health institutions, who gave their written consent to participate in the research were included in the study. Criteria for exclusion from the study were as follows: interruptions in work longer than one year or multiple job changes in the last five years, emotional suffering related to problems in private life (recent death and/or illness of a close family member), and refusal to provide written consent to participate in the research.

Questionnaires

The basic sociodemographic characteristics of the respondents (sex, age, work experience, level of health care, managerial positions, shift work, and fieldwork), as well as specific characteristics of the workplace, were collected by an epidemiological questionnaire specially prepared for this study, which contained 20 questions [12]. There was a question that allowed respondents to express their level of satisfaction with working conditions at the workplace and a question without predefined answers, where the respondents could freely define the greatest burden at the workplace.

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Niš, Serbia (No. 12-8818-27/7, from September 23, 2020, and No. 12-4370-1/2-7, from April 19, 2024).

In our study, we used the Maslach Burnout Inventory (MBI) – Human Services Survey (HSS) – (MBI-HSS) questionnaire, an internationally accepted measuring instrument created from the original MBI questionnaire. It was specially designed to assess the presence of BS among employees in the healthcare field. In a book published in 1998, authors Schaufeli and Enzmann [13] provide a comprehensive guide to BS research, critically analyzing the various tools and methodologies used to investigate the syndrome, including the MBI. The authors discuss that the MBI was used in around 90% of burnout research studies at the time and the MBI-HSS was particularly used in healthcare and human service fields.

The MBI-HSS questionnaire consists of 22 individual statements (or items/questions) [14] about physicians' personal feelings related to work, to which respondents provide answers later calculated on three subscales that measure different dimensions of professional BS. The used subscales are as follows: (a) EE – measures the feeling of emotional exhaustion caused by the burden on the workplace; (b) DP – measures the loss of interest and impersonal attitude towards the recipients of services and/or treatment and teaching; (c) PA – measures the degree of satisfaction with PA in the workplace and success in working with health care users.

Values for the EE, DP, and PA subscales are generated from answers to a total of 9, 5, and 8 questions, respectively. Depending on the level of agreement or disagreement with the views expressed in the questions, the answers are evaluated on a 7-point Likert scale, starting from 0 (never), once a year or less (1), once a month or less (2), a few times a month (3), once a week (4), a few times a week (5), to every day (6).

The total score for each subscale was obtained by summing the results of the answers to the precisely defined questions (22 in total from the questionnaire) according to the instructions of the authors of the MBI-HSS. After that, the subjects' scores were classified in each subscale according to the cut-off values. The cut-off values for the EE subscale are as follows: 27 points and more for the high level, 17 to 26 points for the medium level, and 16 points and less for the low level. The cut-off values for the DP subscale are 13 points and more for the high level, 7 to 12 points for the medium, and 6 and less for the low level. Finally, the values for the PA scale are as follows: 39 points and more for the high, 32 to 38 points for the medium, and 31 and less for the low level.

If the EE and DP subscale values are in the high-level category, there is a high risk of developing BS, while a high level on the PA subscale reduces such risk. The risk of developing BS is practically minimal if there is a low level on the EE and DP subscales and a high level on the PA subscale. It is stated that the PA subscale cannot independently indicate the presence and severity of BS but is only relevant if the results are confirmed by the findings of the remaining two subscales (EE and DP) [15].

Statistical analysis

Data entry, ranking, grouping, and both tabular and graphical representation were carried out using the commercial software Microsoft® Excel® 16.0, LTSC MSD Version 2018, and the statistical package SPSS version 18.0.

A comparison of the prevalence of specific categories of descriptive characteristics between groups was performed using the Chi-square test. The Shapiro-Wilkins test was used to assess the normality of the distribution of the values within groups with numerical values. For comparing two data groups, the Student's *t*-test was used if the distribution of values within the groups was normal; if the distribution of values was not normal, the non-parametric Mann-Whitney *U* test (Mann-Whitney rank sum test) was used. For comparing values among three or more groups of respondents with different levels of EE, DP, and PA subscales, one-way analysis of variance (ANOVA) was performed if the distribution of values within the groups was normal; if the distribution values were not normal, the Kruskal-Wallis test was used.

Statistical significance was determined for *p*-values less than 0.05.

Results

Sociodemographic characteristics of the respondents

Of the total number of physicians contacted (402), a total of 373 (252 females and 121 males) participated in the study with completely filled-out questionnaires. Overall, 135 (36.2%) respondents were from PC institutions, 175 (46.9%) from secondary care (SC), and 373 (16.9%) from tertiary care (TC) institutions (Table 1).

Table 1

Basic sociodemographic and professional characteristics of examined physicians according to sex

Characteristics	Male (n = 121)	Female (n = 252)	Total (n = 373)	*p*
Age (years)	46.9 ± 11.9	47.9 ± 10.5	47.6 ± 10.9	0.611**
Work experience (years)	19.2 ± 12.1	19.1 ± 11.6	19.1 ± 11.8	0.807***
Length of specialist residency (years)	16.5 ± 10.8	15.2 ± 10.0	15.6 ± 10.7	0.878***
Manager position, yes	26 (21.5)	40 (15.9)	66 (17.7)	0.183*
Shift work, yes	81 (66.9)	182 (72.2)	263 (70.5)	0.295*
Level of healthcare				
primary care institutions	30 (24.8)	105 (41.7)	135 (36.2)	0.002*
secondary care institutions	70 (57.8)	105 (41.7)	175 (46.9)	0.003*
tertiary care institutions	21 (17.4)	42 (16.6)	63 (16.9)	0.868*
Professional education				
specialization underway	16 (13.2)	30 (11.9)	46 (12.3)	0.717*
specialist	88 (72.7)	193 (76.6)	281 (75.4)	0.418*
general practitioner	17 (14.1)	29 (11.5)	46 (12.3)	0.485*
Additional work engagement				
medical faculty	9 (7.4)	11 (4.4)	20 (5.4)	0.217*
private sector	35 (28.9)	53 (21.0)	88 (23.5)	0.093*
other	8 (6.6)	11 (4.4)	19 (5.1)	0.356*
without	69 (57.1)	177 (70.2)	246 (66.0)	0.012*
Doctor on duty				
once a week	32 (26.4)	80 (31.7)	112 (30.0)	0.296*
twice a week	31 (25.6)	48 (19.0)	79 (21.2)	0.146*
more than twice a week	13 (10.7)	19 (7.5)	32 (8.6)	0.301*
without this work obligation	45 (37.3)	105 (41.8)	150 (40.2)	0.409*
The main workload at work†				
large number of patients	51 (42.1)	128 (50.8)	179 (48.0)	0.118*
huge administrative work	62 (51.2)	114 (45.2)	176 (47.2)	0.277*
demanding diagnostic and therapeutic procedures	12 (9.9)	13 (5.2)	25 (6.7)	0.085*
unresolved interpersonal relationships	15 (12.4)	44 (17.5)	59 (15.8)	0.210*
other burdens	11 (9.1)	17 (6.7)	28 (7.5)	0.421*
no load	7 (5.8)	15 (6.0)	22 (5.9)	0.949*

Values are presented as mean ± standard deviation and numbers (percentages).

†The possibility of multiple answers is offered; *Chi-square test; **Student's *t*-test; ***Mann-Whitney *U* test.

Among the studied physicians, a statistically significantly higher percentage of women worked in PC institutions compared to men (41.7% vs. 24.8%; Chi-square test: $p = 0.002$). On the other hand, a statistically significantly higher percentage of men worked in the SC institutions compared to women (57.8% vs. 41.7%; Chi-square test: $p = 0.003$).

Among the participants in this study, there were no significant differences between sexes in terms of age, length of employment, and length of specialist experience. Of the total number of physicians, 252 (67.6%) respondents were female, with an average age of 47.9 ± 10.5 years, ranging from 26 to 68, while 121 (32.4%) were male, with an average age of 46.9 ± 11.9 years, ranging from 27 to 65.

The average work experience of the female participants was 19.1 ± 11.6 years and their specialist experience was 15.2 ± 10.0 years, while for the male participants, the same parameters (average work/specialist experience) were 19.2 ± 12.1 years and 16.5 ± 10.8 years, respectively. An equal number of physicians were either in residency or without specialization (12.3% each). Among the female respondents, a significantly higher percentage had no additional engagement compared to the male respondents (70.2% vs. 57.1%; Chi-square test: $p = 0.012$). Shift work was the most common type of work organization, reported by 70.5% of the physicians, and on-call duty is present among 59.8%.

Among the physicians with an on-call duty, 50.2% had one on-call duty a week. However, considering all respondents, this workload level affects 30.0%. Physicians with two on-call duties twice a week experienced a significantly higher workload, comprising 21.2% of participants. The greatest burden was seen in those with more than two on-call duties a week, representing 8.6%. Of the total number, 150 (40.2%) physicians did not have on-call duties as a work obligation. The two most common responses to the question about the greatest work burden were a high number of patients to see during working hours, reported by 48.0% of physicians, and a large number of administrative forms to complete during patient examinations and/or treatment, reported by 47.2% of physicians. Only 5.9% indicated that they do not experience significant work-related stress (Table 1).

Results of the burnout syndrome subscales analysis

The sociodemographic and professional characteristics of the respondents in relation to the EE subscale values (the ANOVA analysis) are shown in Table 2.

The median age of respondents with low EE subscale scores was 42 years, while for those with high EE subscale

Table 2

Sociodemographic and professional characteristics of respondents in relation to the level of emotional exhaustion subscale values

Characteristics	Low (n = 123)	Moderate (n = 100)	High (n = 150)	*p*
Age (years)	42 (35–52)	49.5 (42.7–57)	53 (44–58)	0.000**
Work experience (years)	13 (3–26)	20 (10–30.2)	25 (15–30)	0.000**
Length of specialist residency (years)	4 (0–18)	13 (3–21.2)	14.5 (4–22)	0.000**
Manager position, yes	13 (10.6)	19 (19.0)	34 (22.7)	0.031*
Shift work, yes	84 (68.3)	71 (71.0)	108 (72.0)	0.794*
Level of healthcare				
primary care institutions	52 (42.3)	37 (37.0)	46 (30.7)	0.136*
secondary care institutions	55 (44.7)	43 (43.0)	77 (51.3)	0.362*
tertiary care institutions	16 (13.0)	20 (20.0)	27 (18.0)	0.343*
Professional education				
specialization underway	18 (14.6)	10 (10.0)	18 (12.0)	0.571*
specialist	77 (62.6)	84 (84.0)	120 (80.0)	0.000*
general practitioner	28 (22.8)	6 (6.0)	12 (8.0)	0.000*
Additional work engagement				
medical faculty	5 (4.1)	6 (6.0)	9 (6.0)	0.738*
private sector	20 (16.3)	22 (22.0)	46 (30.7)	0.019*
other	9 (7.3)	4 (4.0)	6 (4.0)	0.391*
without	89 (72.3)	68 (68.0)	89 (59.3)	0.069*
Doctor on duty				
once a week	41 (33.3)	30 (30.0)	41 (27.3)	0.560*
twice a week	26 (21.1)	14 (14.0)	39 (26.0)	0.075*
more than twice a week	4 (3.3)	15 (15.0)	13 (8.7)	0.008*
without this work obligation	52 (42.3)	41 (41.0)	57 (38.0)	0.760*
The main workload at work†				
large number of patients	39 (31.7)	49 (49.0)	91 (60.7)	0.000*
huge administrative work	54 (43.9)	41 (41.0)	81 (54.0)	0.088*
demanding diagnostic and therapeutic procedures	3 (2.4)	8 (8.0)	14 (9.3)	0.064*
unresolved interpersonal relationships	18 (14.6)	11 (11.0)	30 (20.0)	0.146*
other burdens	8 (6.5)	9 (9.0)	11 (7.3)	0.777*
no load	16 (13.0)	5 (5.0)	1 (0.7)	0.000*

Values are presented as median (interquartile range) and numbers (percentages).

†The possibility of multiple answers is offered; *Chi-square test; **Analysis of variance.

scores, it was 53 years, with a statistically significant difference ($p < 0.001$). The median length of work experience for respondents with low EE subscale scores was 13 years, compared to 25 years for those with high EE subscale scores, also statistically significant ($p < 0.001$). The median length of specialist work experience for respondents with low EE subscale was 4 years, while for those with high EE subscale scores, it was 14.5 years, with a statistically significant difference ($p < 0.001$).

It was determined that significantly higher EE subscale scores were found among specialist physicians ($p < 0.001$), physicians with managerial status ($p = 0.031$), and those with additional work in private practice ($p = 0.019$). Statistically significantly higher values on the EE subscale were found among physicians burdened with more than two on-call duties a week ($p = 0.008$) and those who reported that their greatest work burden was the high number of patients they needed to see during the workday ($p < 0.001$). Conversely, significantly lower scores on the EE subscale were observed in physicians without specialization ($p < 0.001$) and those who reported no factors constituting significant work-related stress ($p < 0.001$).

The sociodemographic and professional characteristics of the respondents in relation to the DP subscale values (the ANOVA analysis) are shown in Table 3.

The median age for respondents with low DP subscale values was 47 years, while for those with high DP subscale values, it was 53.5 years, with a statistically significant difference ($p = 0.029$). A statistically significant increase in the values of the DP subscale was associated with the age of the respondents. However, there was no statistically significant increase in the DP subscale for respondents with more work experience or longer specialist training.

Statistically significantly higher values on the DP subscale were found among physicians with additional workload from private practice ($p = 0.015$), physicians working in shifts ($p = 0.049$), and those who reported that their greatest work burden was the high number of patients they needed to see during the workday ($p < 0.001$).

The sociodemographic and professional characteristics of the respondents in relation to the PA subscale values (the ANOVA analysis) are shown in Table 4.

The median age for respondents with low PA subscale values was 50 years. In contrast, for those with high PA subscale values, the median age was lower, 48 years, with no

Table 3

Sociodemographic and professional characteristics of respondents in relation to the level of depersonalization subscale values

Characteristics	Low (n = 253)	Moderate (n = 68)	High (n = 52)	*p*
Age (years)	47 (38–57)	50 (41–57)	53.5 (43.7–58.2)	0.029**
Work experience (years)	19 (6–29)	24 (10–30)	24 (14.5–28.5)	0.102**
Length of specialist residency (years)	10 (0–20)	14 (1–21.2)	15 (3.7–21)	0.230**
Manager position, yes	40 (15.8)	17 (25.0)	9 (17.3)	0.211*
Shift work, yes	169 (66.8)	51 (75.0)	43 (82.7)	0.049*
Level of healthcare				
primary care institutions	96 (37.9)	18 (26.5)	21 (40.4)	0.172*
secondary care institutions	121 (47.8)	29 (42.6)	25 (48.1)	0.737*
tertiary care institutions	36 (14.3)	21 (30.9)	6 (11.5)	0.063*
Professional education				
specialization underway	34 (13.4)	8 (11.8)	4 (7.7)	0.511*
specialist	186 (73.6)	52 (76.4)	43 (82.7)	0.366*
general practitioners	33 (13.0)	8 (11.8)	5 (9.6)	0.781*
Additional work engagement				
medical faculty	14 (5.5)	3 (4.4)	3 (5.8)	0.927*
private sector	49 (19.4)	24 (35.3)	15 (28.8)	0.015*
other	14 (5.5)	1 (1.5)	4 (7.7)	0.263*
without	176 (69.6)	40 (58.8)	30 (57.7)	0.101*
Doctor on duty				
once a week	74 (29.2)	23 (33.8)	15 (28.8)	0.751*
twice a week	51 (20.2)	13 (19.1)	15 (28.8)	0.339*
more than twice a week	20 (7.9)	7 (10.3)	5 (9.6)	0.789*
without this work obligation	108 (42.7)	25 (36.8)	17 (32.8)	0.332*
The main workload at work†				
large number of patients	108 (42.7)	33 (48.5)	38 (73.1)	0.000*
huge administrative work	111 (43.9)	36 (52.9)	29 (55.8)	0.169*
demanding diagnostic and therapeutic procedures	14 (5.5)	4 (5.9)	7 (13.5)	0.109*
unresolved interpersonal relationships	33 (13.0)	15 (22.1)	11 (21.2)	0.102*
other burdens	17 (6.7)	5 (7.4)	6 (11.5)	0.485*
no load	19 (7.5)	2 (2.9)	1 (1.9)	0.154*

Values are presented as median (interquartile range) and numbers (percentages).

†The possibility of multiple answers is offered; *Chi-square test; **Analysis of variance.

Table 4

Sociodemographic and professional characteristics of respondents in relation to the level of personal achievement subscale values

Characteristics	Low (n = 64)	Moderate (n = 81)	High (n = 228)	*p*
Age (years)	50 (40–56.2)	51 (40–56)	48 (39–57)	0.768**
Work experience (years)	20 (10–28.5)	23 (10–28)	20 (7–30)	0.935**
Length of specialist residency (years)	14.5 (2–20)	11 (2–21)	12 (0–21)	0.830**
Manager position, yes	2 (3.1)	17 (21.0)	47 (20.6)	0.004*
Shift work, yes	43 (67.2)	58 (71.6)	162 (71.1)	0.811*
Level of healthcare				
primary care institutions	20 (31.3)	28 (34.6)	87 (38.2)	0.562*
secondary care institutions	31 (48.4)	36 (44.4)	108 (47.3)	0.871*
tertiary care institutions	13 (20.3)	17 (21.0)	33 (14.5)	0.294*
Professional education				
specialization underway	5 (7.8)	6 (7.4)	35 (15.4)	0.084*
specialist	52 (81.3)	64 (79.0)	165 (72.3)	0.238*
general practitioners	7 (10.9)	11 (13.6)	28 (12.3)	0.890*
Additional work engagement				
medical faculty	4 (6.3)	7 (8.6)	9 (3.9)	0.257*
private sector	12 (18.7)	24 (29.6)	52 (22.8)	0.280*
other	4 (6,3)	1 (1.2)	14 (6.1)	0.203*
without	44 (68.7)	49 (60.6)	153 (67.2)	0.489*

Table 4 (continued)

Characteristics	Low (n = 64)	Moderate (n = 81)	High (n = 228)	*p*
Doctor on duty				
once a week	17 (26.6)	25 (30.9)	70 (30.7)	0.802*
twice a week	18 (28.1)	18 (22.2)	43 (18.9)	0.268*
more than twice a week	9 (14.1)	5 (6.2)	18 (7.9)	0.203*
without this work obligation	20 (31.2)	33 (40.7)	97 (42.5)	0.264*
The main workload at work†				
large number of patients	38 (54.9)	40 (18.4)	101 (44.3)	0.099*
huge administrative work	22 (34.4)	31 (17.6)	123 (53.9)	0.069*
demanding diagnostic and therapeutic procedures	6 (9.4)	4 (16.0)	15 (6.6)	0.566*
unresolved interpersonal relationships	9 (14.1)	16 (27.1)	34 (14.9)	0.540*
other burdens	9 (14.1)	6 (21.4)	13 (5.7)	0.081*
no load	4 (6.3)	7 (31.8)	11 (4.8)	0.452*

Values are presented as median (interquartile range) and numbers (percentages).
†The possibility of multiple answers is offered; *Chi-square test; **Analysis of variance).

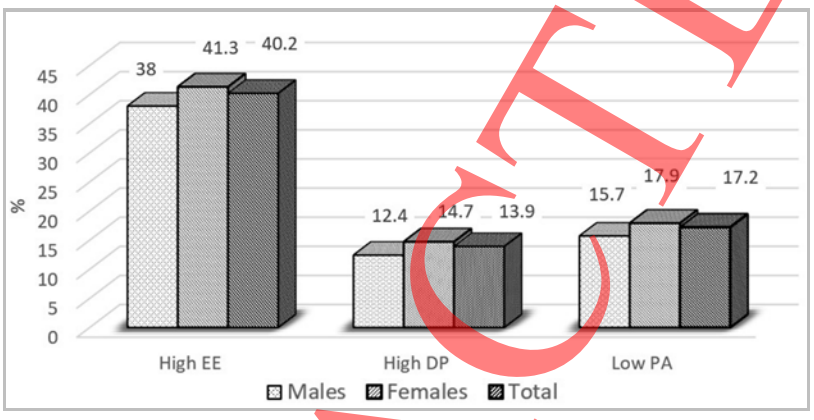

Fig. 1 – The percentage of physicians with high values on the emotional exhaustion (EE) and depersonalization (DP) subscales and low values on the personal achievement (PA) subscale.

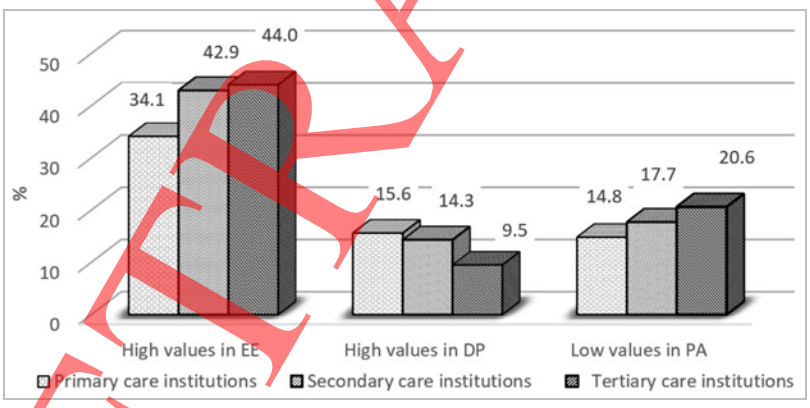

Fig. 2 – The percentage of physicians at the primary, secondary, and tertiary levels of healthcare with high values in the emotional exhaustion (EE) and depersonalization (DP) subscales and low values in the personal achievement (PA) subscale.

statistically significant difference ($p = 0.768$). Furthermore, there was no statistically significant difference among respondents with increased years of service or longer specialist experience in the DP subscale.

In relation to the level of PA subscale values, statistically higher values on the PA subscale were recorded among respondents with managerial status ($p = 0.004$).

Based on the graphical presentation in Figure 1, 40.2% of respondents had high values of the EE subscale, and therefore, were at risk for developing BS.

However, when considering high values of the DP subscale, the number of "at-risk" physicians was significantly lower (13.9%), and when considering physicians with low PA subscale values, it was 17.2%. According to the displayed percentages, women appeared to be somewhat more at risk, although this difference was not statistically significant.

As shown in Figure 2, the highest percentage of physicians in TC had high scores in the EE subscale (44.0% of TC physicians, compared to 42.9% of SC and 34.1% of PC physicians) and low values in the PA subscale (20.6% of TC

physicians, compared to 17.7% of SC and 14.8% of PC physicians). On the other hand, the highest percentage of physicians in PC had high score values in the DP subscale (15.6% of PC physicians, compared to 14.3% of SC and 9.5% of TC physicians).

Discussion

Based on the presented results and the scores of the burnout subscales obtained in our study, physicians had moderate levels of BS. The highest percentage (40.2%) of physicians had high scores on the EE subscale, while on the DP subscale, high scores were observed in 13.9% of physicians, and on the PA subscale, low scores were seen in 17.2% of physicians. The highest percentage of physicians from TC had high values on the EE subscale and low values on the PA subscale. In contrast, the highest percentage of physicians from PC had high values on the DP subscale, but the lowest percentage had low values on the PA subscale.

In our study, there was no significant difference between the sexes. Based on the percentages shown in Figure 1, women were somewhat more affected, but it was not statistically significant. Literary data on the gender role in the occurrence of this syndrome are numerous but without uniform attitudes [16–18].

Statistically significantly higher scores of the EE subscale were found among physicians ≥ 53 years of age, specialist physicians, those in leadership positions, those with additional workload, physicians with more than two on-call duties *per* week, long professional work and specialist experience, and those for whom the greatest burden was seeing a high volume of patients during the workday. Significantly high levels of DP subscale in physicians were observed among those above 53.5 years of age, those with additional workload from private practice, physicians working in shifts, and those who reported that their greatest burden was handling a high volume of patients during the workday. A statistically significant increase in the PA subscale was noted among doctors with managerial status.

Milenović [19] found higher levels of EE subscale among anesthesiologists in TC institutions in Serbia, explaining this by the competitive atmosphere among high-ranking anesthesiologists.

Age is an important and contradictory factor associated with BS. An increase in age is related to a decrease in reported burnout [20]. In a study conducted by Del Carmen et al. [21], younger doctors of various specialties reported higher levels of burnout than their older peers. Findings similar to these are presented by other authors [22, 23]. Based on our results, the EE subscale was significantly higher among physicians with more than 25 years of work experience and those with specialist work experience over 14.5 years. However, the literature shows different findings. Earlier studies have found that the level of distress decreases with increasing experience or age of physicians [24, 25]. The organization of work at the workplace is also essential in creating conditions for the development of BS [26]. In our study, higher scores on the EE and DP subscales were observed in physicians with an additional workload due to a large number of patients that need to be examined during working hours, and in SC and TC institutions this additional workload related to being on on-call duty more than twice a week. Factors such as a lower degree of responsibility, learning process, absence of on-call duty and consultation, and reduced autonomy also played a role [26].

In general, various job conditions are more strongly related to the level of the EE subscale than the other two subscales, DP and PA. BS is associated with a sense of low job satisfaction and reduced work productivity, which can lead to a lower quality of healthcare services provided by professionals – physicians affected by this syndrome. According to the results of a study on the distribution of stressors conducted with 1,755 doctors in the emergency sector in Switzerland, the most significant stressors were general overwork, stress related to health insurance, difficulties in balancing professional and private life, changes in the healthcare system, and uncertainty (reliability) of medical services. Additionally, it was found that the risk of developing the BS was higher in men than in women [27].

On the other hand, young physicians who are in specialization or have not yet completed their specialization had an overall lower level of job satisfaction and less autonomy in their work but were more satisfied with the total number of working hours. Lower scores of EE and DP subscales were found, indicating a reduced risk of burnout. Similar results are found in the literature. The authors point to the need for job reorganization and staff allocation according to appropriate characteristics including age to reduce stress and increase productivity at certain positions. It is possible that specialists, as well as young family doctors who could gain greater autonomy in their work (but also greater responsibility), could benefit from reducing work overload. This could reduce psychological distress in both groups, though it might increase it for some. Similar data is reported by Hoff and Lee [16], Vićentić et al. [24], Vićentić [25], concerning general practitioners.

In a cohort of PC physicians on the front line of patient contact, the results show there is often an inability to meet all patient demands and frequent frustration regarding professional advancement (due to inability or at least uncertainty). The greatest stress arises from the large number of patients who cannot be examined within a single day, meaning that, according to Vićentić [25], physicians cannot devote enough time to each patient. Additionally, general practitioners have little autonomy in their work and face constant pressure due to expectations for continued professional development. A cohort of TC anesthesiologists found that one-third had additional responsibilities such as working at the Faculty of Medicine in Belgrade or holding leadership positions in university or hospital departments. This increased their educational, organizational, and professional responsibilities, adding an extra layer of stress [28].

It is well-known that long-term and exhausting investments in professional and academic careers, overtime work, involvement in teaching, continuous education of mid-level medical staff, medical students, residents, and younger

doctors, clinical work in all invasive diagnostic procedures, operating rooms, intensive care units, acute and chronic pain therapy, organization of in-hospital and out-of-hospital transport, and patient safety are all part of the daily job description of anesthesiologists. All of this is recognized as part of the psycho-physical burden faced by anesthesiologists in academic healthcare institutions [19, 28, 29].

The results of a study conducted by domestic authors among psychiatrists confirmed a high degree of BS – a high level of EE and DP subscales was found in 29.1% and 12.2% of doctors, respectively, while a low level of PA subscale was found in 22.4% of doctors [24]. Similarly, in the population of anesthesiologists [19, 28–30], the levels of EE, as well as in previous comparisons, significantly exceed the results found in other specialties. This trend can be explained by the significant shortage of specialist anesthesiologists, predominantly team-based work, night shifts, and the predominantly female population of anesthesiologists. The study by West et al. [31] focused on individual measures of EE and DP, which are useful for assessing and providing information about BS among medical professionals. High levels of EE or DP are essentially indicative of burnout among high-ranking medical professionals studied in this research. A low level of PA may be less significant and, therefore, often contributes less to confirming the syndrome in these types of studies [31].

Individual measurement of EE and DP subscales is vital in assessing and displaying information about BS because high levels of EE or DP subscales essentially indicate the presence of BS among the medical professionals studied in our study. A low level of the PA subscale may be less significant. An interesting result from another study showed that public hospital physicians have higher levels of burnout and lower job satisfaction compared to private hospital physicians [32–34]. In our study, we also confirmed the link between high workload and burnout. The association between burnout – particularly the EE subscale – and workload is both strong and consistent. Excessive work demands on an individual deplete his or her energy reserves and affect the increase in EE [35]. It is not just the sheer volume of work that heightens workload; perceived additional tasks, such as administrative duties, seen as extra to a physician's primary role and/or a lack of necessary skills to perform the tasks, can also exacerbate the workload.

Conclusion

Based on the presented results, Southeast Serbia physicians had moderate burnout syndrome levels. The highest number of physicians exhibited high scores of emotional exhaustion subscale. The most significant factors associated with burnout were age above 50, specialization, leadership positions, longer both work and specialist work experience, workload, shift work, more than two on-call duties *per* week, and a large number of patients during the workday. The highest percentage of physicians from tertiary care had high values on the emotional exhaustion subscale and low values on the personal achievement subscale, while the highest percentage of physicians from primary care had high values on the depersonalization subscale. It is necessary to take appropriate preventive and corrective measures at each of the three levels of health care to preserve and improve the health of doctors. Further research on burnout syndrome and the factors contributing to its development is crucial.

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Importance of ultrasound measurement of the liver and spleen size in the diagnosis of comorbidity with malaria and COVID-19

Značaj ultrazvučnog merenja veličine jetre i slezine u dijagnostici komorbiditeta malarije i COVID-19

[1]Dušan Krstić*, [1]Boban Krstić[†], Milica Djokić[‡], Nemanja Rančić*[†§]

***University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; Military Medical Academy, [†]Institute of Radiology, [§]Center for Clinical Pharmacology, Belgrade, Serbia; [‡]Clinical Hospital Center Zemun, Clinic for Internal Medicine, Belgrade, Serbia**

[1]equal contribution of both authors who share the first authorship

Abstract

Background/Aim. With the outbreak of the coronavirus disease 2019 (COVID-19) pandemic, patients who had malaria and COVID-19 simultaneously were also noticed. Hepatosplenomegaly is characteristic and almost always present in patients with malaria (without associated COVID-19). A significant increase in the size of the liver and spleen clearly indicates the possible development of severe malaria. The aim of this study was to determine the effect of COVID-19 on liver and spleen size in patients with malaria. **Methods.** This study was conducted while the authors were working in the Serbian Military Hospital of the United Nations Multidimensional Integrated Stabilization Mission (MINUSCA-UN) in Bangui, Central African Republic. Data from 1,710 patients who underwent ultrasound examination of the abdomen, where the diameters of the liver and spleen were monitored, were analyzed. The total number of examined patients was divided into four groups: group with 816 control patients without malaria (C group), 480 with active malaria (M^+), 353 patients who had malaria in the past 2–6 months (M^{past}), and 61 patients with active malaria and active COVID-19 (M^+Cov^+). **Results.** The liver size was significantly greater in the M^+, M^{past}, and M^+Cov^+ groups of respondents compared with the C group. Statistically significant spleen enlargement was observed only in the M^+ group but not in the M^+Cov^+ group compared to the C group. **Conclusion.** There is no enlargement of the spleen in patients with a coinfection with the severe acute respiratory syndrome coronavirus 2 and *Plasmodium malariae*. Therefore, in the presence of clear splenomegaly, we can indicate, with a high probability, the absence of COVID-19 while waiting for the results of the polymerase chain reaction test, even in the first hours of hospitalization.

Key words:
comorbidity; covid-19; diagnosis; liver; malaria; spleen; ultrasonography.

Apstrakt

Uvod/Cilj. Sa izbijanjem pandemije bolesti korona virusa 2019 [*coronavirus disease 2019* – (COVID-19)], zapaženi su i bolesnici koji su imali malariju i COVID-19 istovremeno. Hepatosplenomegalija je karakteristična i skoro uvek prisutna kod bolesnika sa malarijom (bez pridruženog COVID-19). Značajno povećanje veličine jetre i slezine jasno ukazuje na mogući razvoj teškog oblika malarije. Cilj rada bio je da se utvrdi uticaj COVID-19 na veličinu jetre i slezine kod obolelih od malarije. **Metode.** Istraživanje je sprovedeno dok su autori radili u srpskoj Vojnoj bolnici misije *United Nations Multidimensional Integrated Stabilization Mission* (MINUSCA-UN) u Bangiju, Centralnoafrička Republika. Analizirani su podaci 1 710 pacijenata, koji su podvrgnuti ultrazvučnom pregledu abdomena, kojim su mereni prečnici jetre i slezine. Ukupan broj pregledanih pacijenata podeljen je u četiri grupe: 816 kontrolnih pacijenata bez malarije (K grupa), 480 bolesnika sa aktivnom malarijom (M^+), 353 onih koji su imali malariju u poslednjih 2-6 meseci (M^{past}) i 61 bolesnik sa aktivnom malarijom i manifestacijama COVID-19 (M^+Cov^+). **Rezultati.** Jetra je bila značajno veća u M^+, M^{past} i M^+Cov^+ grupama, u poređenju sa K grupom. Statistički značajno povećanje slezine primećeno je samo u M^+ grupi ali ne i u M^+Cov^+ grupi, u poređenju sa K grupom. **Zaključak.** Kod bolesnika sa koinfekcijom korona 2 virusom izazivačem teškog akutnog respiratornog sindroma i *Plasmodium malariae* nema uvećanja slezine. To znači da se kod bolesnika sa jasnom

Correspondence to: Dušan Krstić, University of Defence, Faculty of Medicine of the Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: dusankrstic94@live.com

splenomegalijom može već u prvim satima hospitalizacije, u fazi čekanja na rezultate testa lančane reakcije polimeraze, sa velikom verovatnoćom ukazati na odsustvo COVID-19.

Ključne reči:
komorbiditet; COVID-19; dijagnoza; jetra; malarija; slezina; ultrasonografija.

Introduction

Malaria is a disease caused by protozoa of the genus Plasmodium. It is transmitted by the bite of an infected female mosquito of the genus *Anopheles*. There are about 400 species of *Anopheles mosquitoes*, but about 40 species are significant for the transmission of *Plasmodium* [1]. After hatching and going through several stages of development, young female mosquitoes feed on plant nectar until they mature. In adulthood, in order to lay eggs, females need blood meals, which supply them with the protein needed for developing eggs. At this stage of development, the mosquito, after it bites a person suffering from malaria, becomes a carrier of *Plasmodium*, causing malaria [1].

The disease goes through several stages after the *Plasmodium* enters the host organism. The initial phase of the *Plasmodium* infection is necessary, followed by a phase of asymptomatic disease. Then a phase of uncomplicated disease develops, in which the infection can be cured after the provided therapy. Otherwise, the disease passes to the next stage of severe manifestation of malaria, which often leads to the patient dying [2].

Malaria is a disease that affects and kills the largest number of people. According to the World Health Organization (WHO), in 2019, about 229 million people worldwide fell ill, mostly in 87 endemic countries [3]. Among these countries, 29 are a part of sub-Saharan Africa, including the Central African Republic (CAR), where 94% (2,708,497) of all reported malaria cases are from [3]. Mortality from malaria is high, and in 2019, there was a total of 409,000 deaths. Out of this, 95% falls in 32 countries of sub-Saharan Africa (CAR – 2,017 malaria deaths) [4].

According to the WHO recommendations, the rapid diagnostic test (RDT) is used for rapid orientation in the diagnosis of malaria. In addition, as the main diagnostic method in all suspected patients with malaria, microscopy of thick blood smear (TBS) is used. In addition to these procedures, other diagnostic procedures must be performed such as laboratory analyses, ultrasound examination of the abdomen and small pelvis, X-rays of the heart and lungs, and others [5–9].

Most patients with malaria do not have specific physical findings, but some of them may come with splenomegaly. Symptoms may include a flu-like illness with fever, headache, malaise, fatigue, myalgia, diarrhea, and anemia. Severe malaria manifests as cerebral malaria, severe anemia, respiratory symptoms, and renal failure [9].

As the outbreak of the coronavirus disease 2019 (COVID-19) pandemic occurred at the end of 2019, so did the occurrence of patients with simultaneous infection of *Plasmodium* (the malaria causative agent) and infection with the severe acute respiratory syndrome coronavirus 2 – SARS-CoV-2 (the causative agent of COVID-19 disease). This is important both because of the diagnosis and the therapy, so in such patients, there was a need to modify the treatment protocol. As many authors have described, there is a great similarity between the symptoms of malaria and COVID-19, such as fever, headache, fatigue, shortness of breath, muscle aches, sweating, and feeling cold. All this indicates that it is difficult to distinguish between these two diseases without specific tests based only on the clinical picture and the low reliability of rapid tests [10–15].

The aim of this study was to determine the effect of COVID-19 on liver and spleen size in patients with malaria.

Methods

This study was created during the work of the authors in the Serbian military hospital level II and II+ within the three rotations of the United Nations Multidimensional Integrated Stabilization Mission in the Central African Republic (MINUSCA) in Bangui, the capital of the Central African Republic (CAR). The rotations were in 2017, 2018/2019, and 2021, for over 850 days. The hospital is in charge of treating members of the MINUSCA [military contingents, police forces, civilian personnel, employees of contractors, members of organizations under the auspices of the United Nations (UN), members of the European Union (EU) Military Assistance Mission (EUMAM) in the CAR, members of embassies in the CAR, members of the CAR government, and local population employed in the mission]. One of the most common and serious diseases we encountered in this region is malaria, and during the last rotation, COVID-19.

A prospective cohort study was conducted during three rotations of MINUSCA. Data from all 1,710 patients who underwent ultrasound examination of the abdomen and small pelvis, where the diameters of the liver and spleen were monitored, were processed. Patients were divided into four groups. The first group, which was also the control group (C), consisted of patients who had never suffered from malaria or had not had malaria in more than six months, and their ultrasound examination was performed due to other diseases or symptoms. The second group consisted of patients with active malaria and malaria in the past month (M^+) who did not have COVID-19. The third group consisted of patients who had malaria in the last two to six months (M^{past}) but not COVID-19. The fourth group consisted of patients with active malaria and active or past COVID-19 (M^+Cov^+). The exclusion criteria included patients with some form of chronic or active hepatitis (six of them), which affects the size of the liver.

Patients with hepatic steatosis were monitored in all groups. In these patients, enlargement of the liver caused by

steatosis was certainly diagnosed. It turned out that in patients with hepatomegaly of steatotic origin, during malaria disease, there was an additional increase in the size of the liver in the same percentage as in patients without steatosis. Patients were examined on Mindray DC-N6 ultrasound devices until 2020, and after that, on Mindray DC-8 Exp ultrasound devices, with convex probes in the 1–5 megahertz frequency range. Within all groups, in addition to other parameters and pathological changes, the diameters of the liver and spleen were especially monitored.

There are numerous ways of measuring the liver in the literature [16-19]. Yet, for this paper, we used the measurement of the oblique line from the most caudal anterior part of the liver on the medioclavicular section to the most distant cranial-posterior point on the liver capsule. In order to standardize the measurements, they were performed on sections where the right kidney is at least partially visible in a completely lying position. The patient was required to take a deep breath, hold the air, and tense the abdomen in order to push the abdominal organs out of the costal arch artifacts.

Measurement of the spleen is much more standardized, and almost all authors agree to measure the longest longitudinal and transverse sections of the spleen in a supine or oblique position in a deep breath [20, 21].

Variations in the size of the liver and spleen concerning age, race, height, and weight were not taken into account in the data processing since, in each group of subjects, all these variations were present in a similar percentage. During the survey, respondents from 116 countries from all continents were examined. Most patients were of medium to short stature and without signs of obesity.

Statistical data analysis was performed in the statistical program IBM SPSS version 26.0. Attribute variables were presented in the form of frequencies, and statistical significance was tested by the Chi-square test. Continuous variables were presented in the form of mean value and standard deviation. The normality of the distribution was tested using the Kolmogorov-Smirnov test. The significance of the difference of continuous variables was tested by one-way analysis of variance followed by Tukey B *post hoc* test because more than two groups were compared. The correlation was tested using Pearson correlation. Analyses were estimated at the level of statistical significance of $p < 0.05$.

Results

The total number of examined patients (1,710) was divided into four groups: 816 patients in the C group, 480 in the M^+ group, 353 in the M^{past}, and 61 patients in the M^+Cov^+ group.

There were significantly more male patients in all groups but slightly more women in the M^+Cov^+ group than in the other two groups (Table 1). No significant difference was found between the groups regarding the age of the respondents. However, it could be noticed that patients in the M^+Cov^+ group were about two years older on average compared to other groups. The age range of the patients was generally 30 to 60 years.

The diameter of the liver and spleen differed significantly between these groups (Table 2). The liver size was significantly greater in the M^+ group, M^{past}, and M^+Cov^+

Table 1

Sociodemographic characteristics of examined patients

Characteristics	Groups				*p*-value
	C	M^+	M^+Cov^+	M^{past}	
Sex					
male	660 (80.9)	419 (87.3)	40 (65.6)	266 (75.4)	< 0.001#
female	156 (19.1)	61 (12.7)	21 (34.4)	87 (24.6)	
Age, years	40.45 ± 10.03	39.16 ± 9.40	42.08 ± 9.69	40.17 ± 10.18	0.051*
< 20	2 (0.2)	1 (0.2)	0	2 (0.6)	
20–29	114 (14.0)	67 (14.0)	7 (11.5)	48 (13.6)	
30–39	294 (36.0)	207 (43.1)	18 (29.5)	134 (38.0)	0.032#
40–49	236 (28.9)	131 (27.3)	18 (29.5)	98 (27.8)	
50–59	144 (17.6)	66 (13.8)	18 (29.5)	53 (15.0)	
> 60	26 (3.2)	8 (1.7)	0	18 (5.1)	

Values are given as numbers (percentages) or mean ± standard deviation.
***– One-way analysis of variance; # – Chi-square test.**
Description of the groups is given in the paragraph Methods.

Table 2

Liver and spleen diameter measured by ultrasound examination of the abdomen

Diameter	Groups				*p*-value
	C	M^+	M^+Cov^+	M^{past}	
Liver	137.62 ± 12.91	168.97 ± 16.91	172.26 ± 13.28	157.30 ± 11.85	0.001*
Spleen AP	99.03 ± 14.96	131.93 ± 23.79	103.77 ± 17.04	104.50 ± 14.71	0.001*
Spleen CC	46.11 ± 9.51	59.67 ± 13.51	49.52 ± 13.09	50.94 ± 30.92	0.001*

AP – anterior-posterior; CC – craniocaudal.
Description of the groups is given in the paragraph Methods.
The ultrasound measurement unit is a millimeter (mm). Values are given as mean ± standard deviation.
***– One-way analysis of variance.**

compared to the C group. The liver diameter was increased by 22.78% on average in the M^+ group compared to the C group, while in the M^+Cov^+ group, there was an even bigger increase by 25.17% on average compared to the C group, and this increase was monitored for the next six months after the onset of malaria (Figure 1).

On the other hand, statistically significant spleen enlargement was observed only in the M^+ group compared to the C group but not in the M^+Cov^+ group compared to the C group and M^{past} group (Table 2). Anterior-posterior (AP) spleen diameter was increased by 33.22% on average in the M^+ group compared to the C group, while in the M^+Cov^+ group, there was an increase by only 4.79% compared to the C group (Figure 2). Similar data was observed for craniocaudal (CC) spleen diameter, when there was an increase in the M^+ group by 29.41% on average compared to the C group, while in the M^+Cov^+ group, there was an increase on average by 7.39% compared to the C group (Figure 3).

Correlation analysis showed that the size of the liver and spleen was statistically significantly related to the categories of subjects (Table 3). A strong positive correlation of all three measurements with the category of subjects was obtained. The strongest correlation was between liver size and groups of patients, which means that with the addition of a number of different infections, first only malaria, then

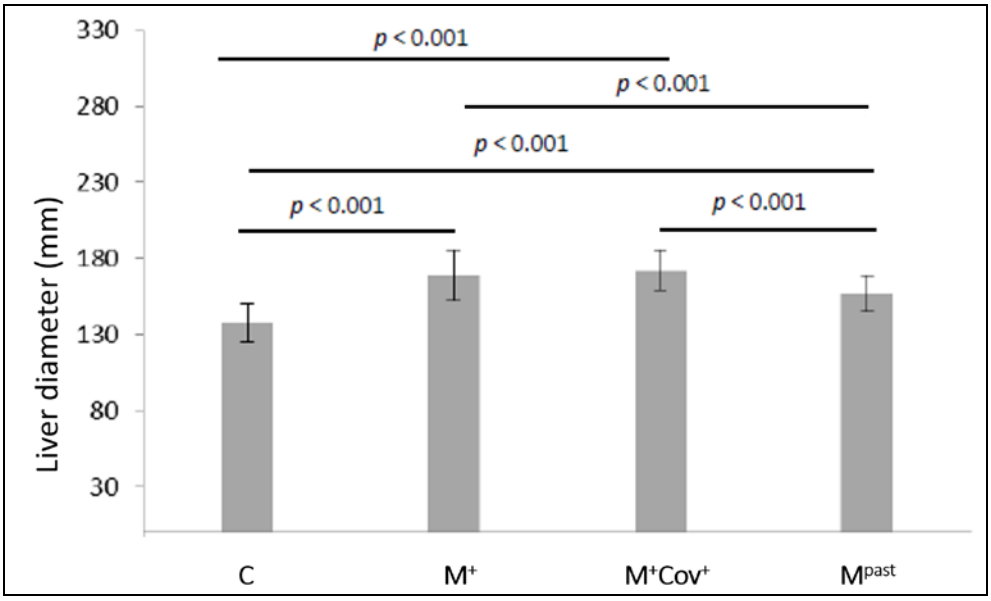

Fig. 1 – Comparison of the liver diameter among examined groups.
Description of the groups is given in the paragraph Methods.
One-way analysis of variance, *post hoc* Tukey B test.

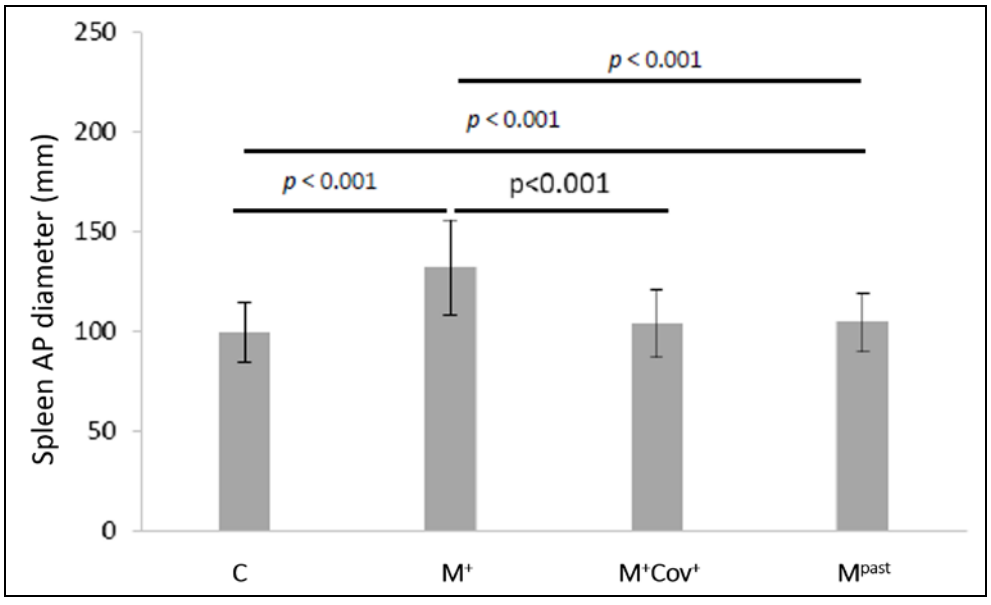

Fig. 2 – Comparison of the spleen anterior-posterior (AP) diameter among examined groups.
Description of the groups is given in the paragraph Methods.
One-way analysis of variance, *post hoc* Tukey B test.

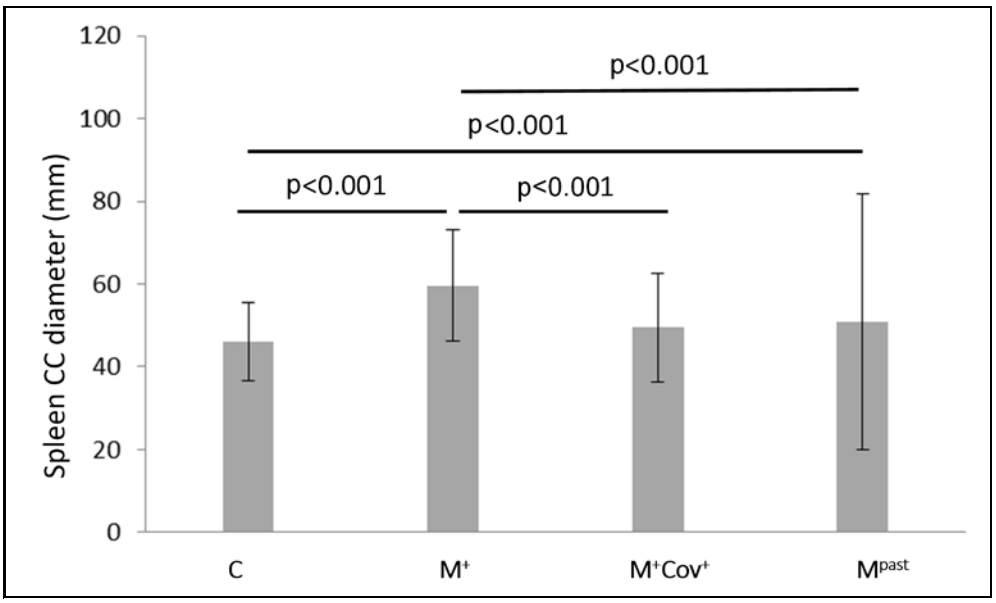

Fig. 3 – Comparison of the spleen craniocaudal (CC) diameter among examined groups. Description of the groups is given in the paragraph Methods. One-way analysis of variance, *post hoc* Tukey B test.

Table 3

Correlation of ultrasound findings with analyzed groups of respondents

Parameter	Liver diameter	Spleen AP diameter	Spleen CC diameter
Spleen AP diameter			
r	0.439		
p	< 0.001		
Spleen CC diameter			
r	0.248	0.418	
p	< 0.001	< 0.001	
Groups			
C			
M^+			
M^+Cov^+			
r	0.425	0.103	0.107
p	< 0.001	< 0.001	< 0.001

AP – anterior-posterior; CC – craniocaudal.
Description of the groups is given in the paragraph Methods. *r* – Pearson correlation.
Note: M^{past} group was not included in this correlation analysis since the results acquired did not match the results of other subgroups due to patient recovery.

malaria and COVID-19, the size of the liver also increased. A similar finding was shown for the spleen in malaria, but in the combined malaria and COVID-19, this expected increase in the spleen was conspicuously absent.

Discussion

A total of 480 patients with malaria (87.3% male and 12.7% female) were examined by the end of this study. The average age of the patients was 39.16 years. Already in the first phase of the examination, a significant increase in the size of the liver and spleen was noticed, which later turned out to be correct in a large number of patients. Control examinations of malaria patients showed that the enlargement of the spleen was maintained for up to a month from the day of the disease.

We observed a significant increase in the liver size, especially the spleen size, even in patients with a negative RDT test and incomplete TBS microscopy. Based on the experience gained, after noticing this regularity, we were able to tell our colleagues who treated these patients that it was a case of malaria and that it could even be predicted, based on the size of the liver and spleen, that a severe form of malaria would develop. Based on that, they could apply appropriate therapy promptly.

Control examinations of a group of patients with malaria after the first month from the day of the disease showed that the enlargement of the liver was maintained for up to six months from the day of the disease, unlike the spleen, which returns to normal after the first month of malaria.

Out of the total of 202 patients with COVID-19 examined in our ward, 66 were suspected of having co-

infection with the SARS-CoV-2 virus and *Plasmodium malariae*. In addition to the prescribed protection measures, we performed radiographic examinations of the lungs and heart and ultrasound examinations of the abdomen and pelvis. Out of these 66 patients, the coexistence of *Plasmodium malariae* infection and SARS-CoV-2 infection was confirmed in 61 patients based on polymerase chain reaction (PCR) SARS-CoV-2 test and TBS microscopy.

As there were a large number of patients with COVID-19 in the first 15 days of March and April 2021 and sporadic cases of malaria, we, like our colleagues across Africa and the world, were afraid, both because of the already tense hospital capacities and because of the expectation of a "super COVID-19/malaria disease", whether we would be able to care for all patients [22–29].

The first cases heightened fears that the combined COVID-19/malaria comorbidity would be severe for patients, as two of the first three patients had died. However, it turned out that the real reasons for the fatal outcome were the late reporting of the patient to the doctor and the delayed air transport to the hospital due to bad weather in the first case and combat operations in the second case. Fortunately, almost all patients with associated diseases had milder forms of the clinical picture of both COVID-19 and malaria. This coincided with the research of other authors [14, 30].

Over time, by inserting liver and spleen size measurement data into the already existing database, to our surprise, it was noticed that there was a deviation from the already, as a rule of large numbers, confirmed regularities in patients with malaria. Deviations were observed only in patients with active or previous SARS-COV-2 infection, so we were forced to single out a new group of patients with co-infection with SARS-CoV-2 and *Plasmodium malariae*, which included 61 patients.

The absence of splenic enlargement in malaria patients with concomitant disease or a history of COVID-19 has been shown to be a significant feature. The absence of splenic enlargement proved to be a pattern in patients with COVID-19 and malaria. This finding allowed us to indicate in three cases, with a high probability, that the patient does not have SARS-CoV-2 infection but a severe malaria picture based on marked enlargement of the spleen and enable rapid and adequate treatment of the patients.

Interestingly, the curve of the number of malaria and COVID-19 patients examined in our hospital almost coincides with the curve published by WHO in the World Malaria Report 2020 [31]. It has been observed that there is a reverse reciprocity of patients with COVID-19 and malaria. All this is accompanied by the seasonal schedule of the dry and rainy period, which directly determines the number of patients with malaria because their number in the rainy season (May to October) is incomparably higher than in the dry season (November to April) [32].

Conclusion

This study proved in a large number of patients that ultrasound examination of the abdomen and pelvis is not only useful but also necessary in patients with suspected malaria. Based on these examinations, the following conclusions can be drawn: the existence of hepato-splenomegaly, which is very clearly and simply determined by ultrasound examination of the abdomen, is characteristic and almost always present in patients with malaria (without associated COVID-19); a significant increase in the size of the liver and spleen clearly indicates the possible development of severe malaria; in a patient with a coinfection with the SARS-CoV-2 and *Plasmodium malariae*, there is an absence of spleen enlargement. Therefore, in the presence of clear splenomegaly, we can indicate, with a high probability, the absence of COVID-19 while waiting for the results of the polymerase chain reaction SARS-CoV-2 test in the first hours of hospitalization.

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Evaluation of clinical application effect of traditional Chinese medicine fumigation technology on postoperative patients with anorectal diseases

Procena efekta kliničke primene tehnologije fumigacije tradicionalne kineske medicine na obolele od anorektalnih oboljenja u postoperatvnom periodu

Tianyang Xie*, Zichen Zhang†, Liu Liu‡

Anhui Provincial Hospital of Traditional Chinese Medicine, *Department of Anorectal, †Department of Dermatology, Hefei City, Anhui Province, China; ‡Anhui Chest Hospital, Operating Room, Hefei City, Anhui Province, China

Abstract

Background/Aim. Treatment after anorectal surgery plays a key role in wound healing and reducing complications. The aim of this study was to assess the clinical efficacy of traditional Chinese medicine (TCM) fumigation in the postoperative management of anorectal diseases, comparing TCM fumigation with conventional treatments. **Methods.** This study included 100 anorectal disease patients who underwent surgery at Anhui Chest Hospital, China, from October 2021 to October 2023. The patients were randomly split into two groups – observation and control (50 patients each). TCM fumigation was administered to the observation group, while the control group received potassium permanganate *sitz baths*. Both groups received 14 days of treatment. The research assessed visual analog scale (VAS), symptom eradication, wound healing, serum substance P (SP) and interferon-gamma (IFN)-γ levels, and therapy efficacy before and after treatment. Side effects were also examined in both groups. **Results.** The overall effective rate for the observation group was 96%, and for the control group, it was 86%. The observation group's clinical efficacy exceeded the control group's clinical efficacy. VAS for pain in both groups dropped after treatment, after which the observation group had lower scores than the control group. The observation group had less anal distension, wound edema, wound fluid, and recovery time than the control group. Within both groups, higher levels of SP and lower levels of IFN-γ in serum were recorded after treatment compared to their pre-treatment values. After treatment, higher levels of SP and lower levels of IFN-γ were recorded in the observed group compared to the control group. The observation group had fewer side effects than the control group (6% vs. 28%). **Conclusion.** TCM fumigation techniques may increase clinical efficacy, mitigate clinical symptoms, surgical adverse effects, discomfort, and accelerate wound healing following anorectal disorders.

Key words:
fumigation; medicine, chinese traditional; postoperative period; rectal diseases; treatment outcome.

Apstrakt

Uvod/Cilj. Lečenje nakon anorektalne hirurgije igra ključnu ulogu u zarastanju rane i smanjenju komplikacija. Cilj ove studije bio je da se proceni klinička efikasnost fumigacije primenom tradicionalne kineske medicine (TKM) u postoperativnom lečenju anorektalnih bolesti, u poređenju sa konvencionalnim tretmanima. **Metode.** Ovom studijom obuhvaćeno je 100 bolesnika obolelih od anorektalnih bolesti operisanih u Bolnici za grudne bolesti Anhui, Kina, od oktobra 2021. do oktobra 2023. godine. Bolesnici su nasumično podeljeni u dve grupe – posmatranu i kontrolnu (50 bolesnika u svakoj). Fumigacija TKM primenjena je kod bolesnika u posmatranoj grupi, dok su u kontrolnoj grupi primenjivane kupke sa kalijum permanganatom. Tretman obe grupe je primenjivan 14 dana. Procenjivani su vizuelna analogna skala (VAS), eradikacija simptoma, zarastanje rane, nivoi supstance P (SP) i interferona (IFN)-γ u serumu i efikasnost terapije pre i nakon tretmana. Takođe, ispitivane su neželjee reakcije u obe grupe. **Rezultati.** Ukupna efektivna stopa za posmatranu grupu iznosila je 96%, a za kontrolnu grupu 86%. Klinička efikasnost u posmatranoj grupi premašila je kliničku efikasnost u kontrolnoj grupi. VAS za bol u obe grupe opao je nakon tretmana, nakon čega je posmatrana grupa imala niže skorove od kontrolne grupe. Posmatrana grupa imala je manju analnu distenziju, edem rane, sekreciju iz rane i vreme oporavka u poređenju sa

Correspondence to: Liu Liu, Anhui Chest Hospital, Operating Room, 397 Jixi Road, Shushan District, Hefei City, Anhui Province, 230 031, China. Email: l27871113@gmail.com; liuliu5399@163.com

kontrolnom grupom. Unutar obe grupe zabeleženi su viši nivoi SP i niži nivoi IFN-γ u serumu nakon tretmana u poređenju sa njihovim vrednostima pre tretmana. Nakon tretmana, u posmatranoj grupi u poređenju sa kontrolnom grupom, zabeleženi su viši nivoi SP i niži nivi IFN-γ. Posmatrana grupa je imala manje neželjenih efekata od kontrolne grupe (6% vs. 28%). **Zaključak.** Tehnike fumigacije TKM mogu povećati kliničku efikasnost, ublažiti kliničke simptome, hirurške neželjene efekte, nelagodnost i ubrzati zarastanje rane nakon anorektalnih poremećaja.

Ključne reči:
fumigacija; medicina, kineska, tradicionalna; postoperativni period; rektum, bolesti; lečenje, ishod.

Introduction

Anorectal diseases (ADs) are a general term for anal and intestinal diseases. There are many causes for ADs, primarily separated into internal and external factors [1]. Internal sources are genetic factors, anatomical structure, and congenital abnormalities of embryonic development, while the external causes are poor lifestyle habits [2]. There are more than 100 kinds of common ADs in clinical practice, many of which are often accompanied by ADs, bringing great harm to the health of patients [3]. Many ADs need to be treated surgically. Continuous treatment after anorectal surgery plays a crucial role in wound healing and reducing complications [4, 5]. Under the guidance of ancient Chinese medicine theory, the old-fashioned Chinese medicine fumigation is an external treatment method that selects certain traditional Chinese medicine (TCM) for syndrome differentiation. The medicine is dissolved in water or processed into Chinese medicine liquid or even made into powder. Then, it is mixed with hot water to carry out local fumigation and aesthetic-bath on anorectal patients [6]. It directly acts on the body through the body surface using warmth and drugs to dredge meridians, dispel wind and remove dampness, lessen edema and ease discomfort, encourage blood stasis, etc. [7]. From this point on, several researchers have concentrated on the process of fumigation. On the other hand, researchers have not comprehensively investigated the efficacy of the fumigation application [8].

TCM fumigation therapy has been used for centuries as an external treatment method to promote healing and alleviate symptoms in various diseases, including anorectal conditions. Rooted in the principles of TCM, fumigation involves using herbal steam to deliver therapeutic compounds directly to the affected area [9]. This method combines the benefits of heat therapy with the pharmacological actions of medicinal herbs, aiming to expel toxins, reduce inflammation, alleviate pain, and promote tissue repair [10]. Potassium permanganate sitz baths are commonly used in postoperative care for their antiseptic properties. However, they primarily offer antimicrobial action without additional therapeutic effects such as anti-inflammatory or analgesic benefits [11]. In contrast, TCM fumigation therapy utilizes a combination of herbs with multiple pharmacological actions, potentially offering a more holistic approach to postoperative care [12]. Therefore, further study is required to have a complete understanding of this facet. TCM, particularly fumigation therapy, has been used for its anti-inflammatory, analgesic, and wound-healing effects [12].

The aim of this study was to investigate whether TCM fumigation can complement standard postoperative care, improving outcomes in pain reduction, wound healing, and adverse reaction rates. By comparing TCM fumigation with conventional sitz baths, the study assesses its potential integration into care protocols to enhance patient outcomes.

Methods

General data

This study included 100 patients diagnosed with ADs who underwent standardized surgical procedures at Anhui Chest Hospital, China, from October 2021 to October 2023. This study was approved by the Ethics Committee of the Anhui Chest Hospital (No. 2021/TCM/FT/76456/1122). The research complies with the ethical guidelines and requirements of the Declaration of Helsinki. Surgeries were performed by the same surgical team using consistent techniques to ensure uniformity in the extent of intervention. Patients were randomly assigned into two groups (50 patients each).

Inclusion and exclusion criteria

Inclusion criteria were as follows: patients 18–80 years old diagnosed with ADs requiring surgical intervention according to the "Modern Anorectal Diagnosis and Treatment Standards" [13]; patients who underwent standardized surgical procedures with similar extents of intervention; patients with mild to moderate inflammation levels, as assessed by preoperative clinical evaluations, to minimize variability in postoperative outcomes; patients with no other cardiovascular and cerebrovascular diseases; patients with complete clinical case data and follow-up data; patients willing to participate in the study.

Exclusion criteria were as follows: patients with malignant tumors; patients with severe cardiopulmonary damage; patients with mental illnesses; patients with severe inflammation or infection that could significantly affect wound healing; patients lacking sufficient clinical data to determine efficacy.

Therapeutic method

The control group used potassium permanganate sitz bath treatment after the operation. Two pieces of potassium permanganate external tablets (Shandong Mingren Freda Pharmaceutical Co., Ltd., National Medicine Approval H20063384, specification: 0.1g) were used with 1,000 mL of water.

The water temperature of 40º C is appropriate for the potassium permanganate to completely dissolve in water. It is then stirred until it becomes pink to the naked eye, and, as such, it can be used. Patients sit in the bath for 5–10 minutes each time, once in the morning and once in the evening. This treatment is widely recognized and routinely used in clinical settings for managing postoperative symptoms in AD patients.

In the observation group, fumigation with TCM was used after the operation. Patients received TCM fumigation therapy instead of the potassium permanganate sitz bath. The fumigation treatment involved a specific herbal formula designed to promote wound healing and reduce inflammation and pain. The fumigation drug was composed of the following herbs: *Sophora flavescens* 9 g, *Houttuynia* 15 g, *Ulmus sinensis* 9 g, *Dandelion* 15 g, *Mirabilis* 3 g, *Ilex* 9 g, *Phellodendron* 9 g, *Honeysuckle* 18 g, *Schisandra chinensis* 6 g, and *Angelica* 6 g. About 2,500 mL of water is added to the herbs. The herbs are first brought to a boil and then cooked over a low flame for 20 min. The slag that is created is removed and the solution is taken while hot. Wait until the temperature is suitable for a sitz bath, and use the solution each time for 30 min, *bis in die*.

For 14 days, both groups received constant treatment.

Observational index

(1) Clinical efficacy: the therapeutic efficacy rating scale designed by our hospital was utilized to assess the clinical efficacy of the patients, comprising four sections of incision infection, pain degree, edema degree, and wound healing, with five questions in each section. There were 20 questions in total. Every question scored five points, and the total score was 100. A score of 90 or above indicates obvious effectiveness, a score of 70–89 indicates effectiveness, and a score below 70 indicates ineffectiveness. Total effective rate = (number of significantly effective cases + number of effective cases)/total number of cases × 100%. (2) Postoperative pain degree: the pain degree of patients in the two groups was assessed before the treatment and at 3, 7, and 14 days after treatment, and the degree of pain in patients was evaluated using a visual analog scale (VAS). Pain is measured on a 0 to 10 scale, with 0 indicating no pain and 10 indicating very intense pain. (3) Symptom improvement and wound healing time: the disappearance of anal distension, wound edema, wound seepage, and time of wound healing of the two groups were correlated. (4) Laboratory indicators: before and after medication, both groups had their venous blood drawn in the early morning on an empty stomach. Serum levels of substance P (SP) and interferon-gamma (IFN)-γ were measured using enzyme-linked immunosorbent assay (ELISA) (Shanghai Enzyme-linked Biotechnology Co., Ltd). (5) Compare the incidence of adverse drug reactions among the two groups: observe adverse reactions between the two groups of patients, including infection, rash, blistering, and abnormal feeling.

Statistical analysis

All statistical analyses were conducted using SPSS version 26.0 to ensure rigorous data processing and interpretation. Continuous variables were expressed as mean ± standard deviation, with between-group comparisons carried out using independent *t*-tests and within-group comparisons conducted using paired *t*-tests to evaluate changes over time within each group. Categorical data were analyzed using Chi-square tests or Fisher's exact tests, depending on the sample size and the distributional characteristics of the data. Wilcoxon rank-sum tests were utilized for ordinal variables, particularly where the normality assumption was not satisfied, thereby ensuring the appropriate handling of non-parametric data. The significance level was set at $\alpha = 0.05$ to determine statistical significance. Sample size calculations were conducted using G*Power software, incorporating a significance level of $\alpha = 0.05$, a statistical power of 0.8, and effect sizes informed by previous studies. Although a minimum of 30 participants *per* group was estimated to achieve sufficient power, we ultimately recruited 50 participants *per* group to enhance the reliability of the results and account for potential attrition.

Results

The assessment of general data among two groups is shown in Table 1.

The observation group's overall effective rate was 96% (48/50), whereas the control group's total effective

Table 1

Assessment of general data among the two groups

Parameters	Observation group (n = 50)	Control group (n = 50)	$\chi 2/t$ value	*p*-value
Age (years)	53.27 ± 12.48	54.13 ± 11.87	0.346	0.765
Gender			0.124	0.856
male	35 (70)	33 (66)		
female	15 (30)	17 (34)		
Course of disease (years)	4.87 ± 3.56	4.91 ± 3.48	0.287	0.821
Type of disease (case)			0.825	0.173
hemorrhoids	34 (68)	35 (70)		
anal fistulas	5 (10)	4 (8)		
anal fissures	4 (8)	5 (10)		
perianal abscesses	5 (10)	3 (6)		
other	2 (4)	3 (6)		

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

rate was 86% (43/50). The observation group exhibited superior clinical effectiveness compared to the control group, with a statistically significant difference ($p < 0.05$) (Table 2).

Pain VAS of the two groups at 3, 7, and 14 days after treatment was progressively decreased ($p < 0.05$), and the pain VAS score of the observation group was lower than that of the control group during the same period. The difference was statistically noteworthy ($p < 0.05$) (Table 3).

The diminishing time of anal distension, wound edema, and wound seepage, and the wound healing time in the observation group was shorter compared to the control group. The difference was statistically significant ($p < 0.05$) (Table 4).

Following therapy, both groups had increases in serum SP ($p < 0.05$) and decreases in IFN-γ ($p < 0.05$). Following the treatment, the observation group's serum SP and IFN-γ levels were shown to be greater ($p < 0.05$) and lower, respectively, compared to the control group's (Table 5).

The observation group experienced 6% (3/50) of adverse reactions, whereas the control group experienced 28% (14/50). In the observation group, there was a reduced incidence of adverse reactions compared to the control group ($p < 0.05$) (Table 6).

Table 2

Assessment of clinical efficacy among the two groups

Clinical efficacy	Observation group (n = 50)	Control group (n = 50)	χ^2-value	*p*-value
Obvious	26 (52)	16 (32)		
Effective	22 (44)	27 (54)	5.421	0.018*
Ineffective	2 (4)	7 (14)		
Total effective rate	48 (96)	43 (86)		

n – number. All values are given as numbers (percentages).
***p < 0.05 compared with the control group.**

Table 3

Assessment of postoperative pain visual analog scale scores among the two groups

Parameters	Observation group (n = 50)	Control group (n = 50)	*t*-value	*p*-value
Before treatment	9.34 ± 2.45	9.28 ± 2.38	0.256	0.764
After treatment (days)				
3	6.21 ± 1.45	8.17 ± 1.48	9.257	< 0.001
7	4.42 ± 2.56	6.89 ± 2.41	8.326	< 0.001
14	3.01 ± 1.87	5.38 ± 2.14	7.458	< 0.001

n – number. All values are given as mean ± standard deviation.
A statistically significant difference ($p < 0.05$) was found before and after treatment in both groups, as well as after treatment between the two groups on all tested days.

Table 4

Assessment of symptom improvement and wound healing time among the two groups

Parameters	Observation group (n = 50)	Control group (n = 50)	*t*-value	*p*-value
Diminishing time (weeks)				
anal distension	5.48 ± 0.76	7.82 ± 1.121	6.784	< 0.001
wound edema	5.17 ± 0.62	7.31 ± 0.82	8.125	< 0.001
wound seepage	3.98 ± 0.51	5.76 ± 0.63	10.142	< 0.001
Wound healing time (weeks)	8.74 ± 1.85	11.02 ± 2.15	12.765	< 0.001

n – number. All values are given as mean ± standard deviation.

Table 5

Assessment of serum SP and IFN-γ before and after treatment among the two groups

Parameters	Observation group (n = 50)		Control group (n = 50)	
	before treatment	after treatment	before treatment	after treatment
Substance P (pg/mL)	132.45 ± 12.86	207.84 ± 22.47	133.75 ± 12.63	172.81 ± 19.65
IFN-γ (pg/mL)	45.81 ± 7.56	20.91 ± 5.18	45.36 ± 7.24	32.85 ± 6.23

n – number; IFN – interferon. All values are given as mean ± standard deviation.
A statistically significant difference ($p < 0.05$) was found before and after treatment in both groups, as well as after treatment between the two groups in both tested parameters.

Table 6

Assessment of adverse reactions among the two groups

Adverse reactions	Observation group (n = 50)	Control group (n = 50)	χ^2-value	*p*-value
Infections	1 (2)	3 (6)		
Rash	1 (2)	4 (8)		
Blistering	0 (0)	5 (10)	6.524	0.009
Feeling strange	1 (2)	2 (4)		
Total incidence	3 (6)	14 (28)		

n – number. All values are given as numbers (percentages).
A statistically significant difference ($p < 0.05$) was found between the two groups in all manifested adverse reactions.

Discussion

In the current investigation, after the anorectal surgery, the patients were administered TCM fumigation. Fumigating matrine and dandelion given on prescription, are used to clear heat and dry dampness. Houttuynia species (sp.) is a traditional herb used for clearing heat and eliminating toxins and has also been used for treating severe acute respiratory disease [12]. Honeysuckle is effective in clearing heat, detoxifying the body, eliminating carbuncles, and facilitating the discharge of pus [13]. Ulmus sp. serves to cool the blood, stop the bleeding, and facilitate detoxification to collect sore [14]. Schisandra sp. exhibits astringent properties [15]. Angelica sp. demonstrates a beneficial impact on alleviating trapped wind pain, reducing swelling, and promoting drainage [16]. It effectively moisturizes dryness, smooths the intestinal lining, promotes blood circulation, and alleviates pain [17, 18]. The study's findings demonstrated that the observation group's overall effective rate was greater than the control's, indicating that the application of TCM fumigation technology after the operation of ADs improved the treatment efficiency of patients. At the same time, the occurrence of postoperative adverse reactions in the observation group was suggestively lesser than in the control group, indicating that the application of TCM fumigation technology after the operation of ADs improved the safety of the follow-up treatment and greatly reduced the probability of postoperative adverse reactions such as infection, rash, blistering, and abnormal feeling in patients.

The study's findings demonstrated that 3, 7, and 14 days following therapy, the observation group's pain VAS was lower than the control group's. This suggests that applying TCM fumigation technology can efficiently alleviate the degree of pain in postoperative ADs.

In this study, diminishing time of anal distension, wound edema, and wound seepage, and the time of wound healing was shorter in the observation than in the control group. After treatment, serum SP increased and IFN-γ decreased in the observation group compared to the control. This suggests that the application of TCM fumigation technology after ADs can improve clinical symptoms and promote wound healing.

These results are consistent with previous research supporting the efficacy of TCM fumigation in postoperative care for ADs [1]. For instance, a study by Wang et al. [12] demonstrated that TCM fumigation significantly reduced postoperative pain and promoted wound healing in patients undergoing hemorrhoidectomy. Similarly, Kang and Yu [19] reported that the combined application of TCM fumigation and acupuncture significantly decreased edema, alleviated pain, and accelerated the healing process in patients undergoing anorectal surgery. These findings align with our study, where the observation group showed lower pain VAS scores and faster wound healing times compared to the control group. Regarding adverse reactions, our study found a significantly lower incidence in the observation group. This is in line with the Hospital Authority's report indicating that potassium permanganate sitz baths can cause skin irritation and allergic reactions in some patients [20]. In contrast, TCM fumigation therapy may have fewer adverse effects due to its gentle pharmacological properties and localized application. In summary, our study corroborates existing literature, further validating the effectiveness and safety of TCM fumigation therapy in postoperative rehabilitation for ADs. However, larger-scale, multicenter randomized controlled trials are necessary to confirm these findings and better inform clinical practice.

ADs have become common in our country, which seriously troubles people's health. Due to the great changes in the working and living style of Chinese residents, the incidence of ADs is getting higher and higher [21, 22]. In reality, people do not have a deep understanding of the harm of ADs. Therefore, ADs often do not get scientific and effective diagnosis and treatment, which can easily lead to constipation, blood stool, swelling, and unbearable pain, and in severe cases, it may transform into rectal tumors [23, 24]. ADs often require surgical treatment, but the postoperative pain and long course of the disease discourage the patients from undergoing the surgery. For that reason, finding a way to reduce pain and shorten the course of treatment is important for solving a series of clinical problems [25, 26]. With the help of thermal action, these drugs stimulate perianal and local skin, expand subcutaneous blood vessels, accelerate lymphatic and blood flow, improve local tissue nutrition, accelerate metabolism, and enable drugs to enter the body through skin absorption in order to improve the therapeutic effect [27, 28]. By exploring the reasons, we can see that TCM fumigation through the skin or the orifice directly affects the lesion and the stimulation of other organs of the human body is small. Furthermore, the effect of TCM is mild, even if it directly acts on the human skin, the chance of redness,

swelling, and blistering is very small [29, 30]. That being said, it is safe to use TCM fumigation following anorectal surgical treatment. Analyzing the reasons, not only can TCM fumigation prescription promote blood and relieve pain but also inhibit the growth of a variety of pathogenic bacteria, play the role of clearing heat and dampness, reducing swelling and releasing pain, cleaning wounds, antibacterial and anti-inflammatory, can make local Qi and blood channels run smoothly, and effectively relieve patients' postoperative pain [27, 28]. Analysis of the reason is that the direct contact of TCM liquid to the wound and blood vessels in the fumigation expansion promotes the local absorption of drugs, inhibits the proliferation of wound bacteria, and promotes the absorption of local inflammatory exudates [31, 32]. After wound formation, serum SP can promote the growth of wound fibroblasts and repair the synthesis of deoxyribonucleic acid to heal the wound. TCM fumigation can improve wound blood circulation, hasten the fiber proliferation rate and epithelial coverage, and shorten the wound recovery time [33, 34]. IFN-γ is a pro-inflammatory factor, and the increase in its level can increase the permeability of blood vessels and aggravate swelling, pain, and other symptoms [35, 36]. TCM fumigation can efficiently constrain the stimulation of inflammatory response to the body and relieve symptoms [37]. Recognizing the potential impact of surgical variability and inflammation on postoperative outcomes [38], we standardized surgical procedures for all patients and included only those with mild to moderate inflammation. This approach minimized confounding factors and allowed us to attribute differences in postoperative recovery primarily to the treatment interventions (TCM fumigation vs. potassium permanganate sitz bath).

This study has several limitations. The relatively small sample size (50 patients per group) limits the statistical power to detect rare adverse events and affects generalizability. The low incidence of adverse reactions (6%) may not adequately reflect the true risk profile, necessitating larger-scale studies for validation. Additionally, the single-center nature of the study limits external validity, as variability in practitioner expertise, institutional protocols, and patient demographics could affect outcomes in different settings. The follow-up period was restricted to 14 days, assessing only short-term outcomes like pain relief and wound healing, while long-term effects such as recurrence and late adverse reactions remain unknown. Confounding factors, such as variability in patient adherence to postoperative care, may also have influenced outcomes despite efforts to standardize treatment protocols. Future multi-center studies with larger sample sizes and extended follow-up are needed to confirm the efficacy and safety of TCM fumigation.

Conclusion

Our study demonstrates that traditional Chinese medicine fumigation therapy significantly improves postoperative outcomes for anorectal disease patients compared to potassium permanganate sitz baths. Specifically, traditional Chinese medicine fumigation was associated with greater reductions in postoperative pain, faster wound healing, enhanced symptom alleviation, and a lower incidence of adverse reactions. These findings suggest that traditional Chinese medicine fumigation may offer a viable alternative to conventional sitz bath methods for postoperative care in anorectal diseases, providing targeted therapeutic benefits with fewer side effects.

Conflicts of Interest

The authors declare no conflict of interest.

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Anti-inflammatory properties of an A3 adenosine receptor agonist, piclidenoson, in a model of human peripheral blood mononuclear cell culture

Anti-inflamacijska svojstva agoniste A3 receptora adenozina, piklidenozona, na modelu kultivisanih humanih mononuklearnih ćelija periferne krvi

Ljiljana Kozić*, Vanja Mališ*, Marija Drakul*, Sara Rakočević*, Dejan Bokonjić*, Dušan Mihajlović*†

***University of East Sarajevo, Faculty of Medicine Foča, Foča, Republic of Srpska, Bosnia and Herzegovina; †Military Medical Academy, Clinic for Dermatovenerology, Belgrade, Serbia**

Abstract

Background/Aim. Piclidenoson (CF101, IB-MECA), a selective agonist of the A3 adenosine receptor (A3AR), is used in clinical trials for the treatment of psoriasis. Emerging data from *in vitro* and *in vivo* studies suggest that piclidenoson possesses anti-inflammatory and immunomodulatory properties, but its action on human peripheral blood mononuclear cells (PBMCs) remains unknown. The aim of this study was to examine the anti-inflammatory effects of piclidenoson in a model of phytohaemagglutinin (PHA)-stimulated human PBMCs culture. **Methods.** Human PBMCs were isolated from the venous blood of healthy donors (n = 4) and treated with different concentrations of piclidenoson. Flow cytometry and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test were used to determine cell viability, while the MTT method and the carboxyfluorescein succinimidyl ester (CFSE) staining method were used to analyze the effect of piclidenoson on cell proliferation. Levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, IL-23, IL-36, IL-5, interferon (IFN)-γ, IL-17, and IL-10 were measured using a specific sandwich enzyme-linked immunosorbent assay (ELISA). **Results.** The results of cytotoxicity tests showed that the highest applied concentration of piclidenoson (1,500 nM) reduced the metabolic activity of PBMCs ($p < 0.05$) and increased the percentage of late apoptotic ($p < 0.05$) and necrotic cells ($p < 0.01$). Non-toxic concentrations (250, 500, and 1,000 nM) decreased the proliferation of PBMCs ($p < 0.05$) compared to the control cells. These concentrations also decreased the production of TNF-α ($p < 0.001$). Piclidenoson at concentrations of 250 and 1,000 nM reduced the production of IL-23 ($p < 0.05$) while the concentrations of 500 and 1,000 nM reduced the production of IL-36 ($p < 0.05$). Piclidenoson at 1,000 nM increased IL-1β production, while other concentrations decreased its production ($p < 0.01$). The highest concentration (1,000 nM) inhibited the production of IL-5 ($p < 0.05$) and IFN-γ ($p < 0.01$) while all applied concentrations inhibited the production of IL-17 ($p < 0.001$). Furthermore, piclidenoson increased the production of IL-10 in all applied concentrations ($p < 0.01$). **Conclusion.** At non-toxic concentrations, piclidenoson exerts anti-inflammatory properties associated with the inhibition of proliferation and modulation of cytokine production in PHA-stimulated PBMCs culture.

Key words:
blood; cytokines; flow cytometry; inflammation; in vitro techniques; leukocytes, mononuclear; phytohemagglutinins; receptors, purinergic.

Apstrakt

Uvod/Cilj. Piklidenozon (CF101, IB-MECA), selektivni agonist A3 adenozinskog receptora (A3AR), koristi se u kliničkim ispitivanjima za lečenje psorijaze. Brojni podaci *in vitro* i *in vivo* studija ukazuju da piklidenozon poseduje anti-inflamacijska i imunomodulacijska svojstva, ali njegovo dejstvo na humane mononuklearne ćelije periferne krvi (*peripheral blood mononuclear cells* – PBMCs) nije u potpunosti istraženo. Cilj ovog istaživanja bio je da se ispitaju anti-inflamacijski efekti piklidenozona na modelu kultivisanih, fitohemaglutininom (*phytohaemagglutinin* – PHA)-stimulisanih, humanih PBMCs. **Metode.** Humane PBMCs izolovane su iz

Correspondence to: Dušan Mihajlović, Military Medical Academy, Clinic for Dermatovenerology, Crnotavska 17, 11 000 Belgrade, Serbia. E-mail: dusan.a.mihajlovic@gmail.com

venske krvi zdravih donora (n = 4) i tretirane različitim koncentracijama piklidenozona. Protočna citometrija i *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide* – MTT test korišćeni su za određivanje vijabilnosti ćelija, dok su MTT metoda i metoda bojenja *carboxyfluorescein succinimidyl ester* (CFSE) korišćeni za analizu efekta piklidenozona na proliferaciju ćelija. Nivoi faktora nekroze tumora (*tumor necrosis factor* – TNF)-α, interleukina (IL)-6, IL-1β, IL-23, IL-36, IL-5, interferona (IFN)-γ, IL-17 i IL-10 određeni su specifičnim „sendvič" *enzyme-linked immunosorbent assay* – ELISA testom. **Rezultati.** Rezultati testova citotoksičnosti pokazali su da je najveća primenjena koncentracija piklidenozona (1 500 nM) smanjila metaboličku aktivnost PBMCs ($p < 0,05$) i povećala procenat ćelija u kasnoj apoptozi ($p < 0,05$) i nekrotičnih ćelija ($p < 0,01$). Netoksične koncentracije (250, 500, i 1 000 nM) smanjile su proliferaciju PBMCs ($p < 0,05$) u poređenju sa kontrolnim ćelijama. Ove koncentracije su takođe smanjile produkciju TNF-α ($p < 0,001$). Piklidenozon u koncentracijama od 250 i 1 000 nM smanjio je produkciju IL-23 ($p < 0,05$), dok su koncentracije od 500 i 1 000 nM smanjile produkcju IL-36 ($p < 0,05$). Piklidenozon je u koncentraciji od 1 000 nM povećao produkciju IL-1β, dok su ostale koncentracije smanjile njegovu produkciju ($p < 0,01$). Najveća koncentracija (1 000 nM) inhibirala je produkciju IL-5 ($p < 0,05$) i IFN-γ ($p < 0,01$), dok su sve primenjene koncentracije inhibirale produkciju IL-17 ($p < 0,001$). Takođe, piklidenozon je u svim primenjenim koncentracijama povećao produkciju IL-10 ($p < 0,01$). **Zaključak.** U netoksičnim koncentracijama, piklidenozon ispoljava anti-inflamacijske efekte povezane sa inhibicijom proliferacije i modulacijom produkcije citokina u kulturi PHA-stimulisanih PBMC.

Ključne reči:
krv; citokini; citometrija, protočna; zapaljenje; in vitro; leukociti, mononuklearni; fitohemaglutinini; receptori, purinergički.

Introduction

Elevated levels of A3 adenosine receptor (A3AR) expression have been identified in mononuclear and tumor cells from patients diagnosed with breast, colon, lung, pancreatic, and melanoma cancers [1–5]. In addition to being a therapeutic target, the A3AR is recognized as a biological marker of disease due to its overexpression in immune cells (neutrophils, monocytes, eosinophils, macrophages, dendritic cells) and tumor cells compared to healthy cells [1, 6–8]. In recent years, various agonists, antagonists, and modulators of the A3AR have been explored for potential therapeutic applications [9–13].

Piclidenoson (IB-MECA, CF101), a selective A3AR agonist, has undergone testing in preclinical models of colitis, uveitis, rheumatoid arthritis (RA), and osteoarthritis [14–16]. This A3AR agonist, demonstrating promising results as an anti-inflammatory and anti-cancer agent, is currently in phase III clinical trials for the treatment of psoriasis [17–20].

Emerging data from *in vivo* and *in vitro* experiments suggest that piclidenoson possesses anti-inflammatory and immunomodulatory properties. In murine models of endotoxemia, administration of piclidenoson (dose range, 0.2–0.5 mg/kg) reduced lipopolysaccharide (LPS)-induced plasma levels of interleukin (IL)-12 and interferon (IFN)-γ [21]. In a collagen-induced RA model, piclidenoson (0.5 mg/kg) significantly decreased tumor necrosis factor (TNF)-α expression levels in the lymph nodes, spleen, and synovial tissue [22]. Furthermore, piclidenoson (dose range, 10–100 μM) demonstrates the ability to inhibit the respiratory burst of human monocytes by inhibiting the activity of nicotinamide adenine dinucleotide phosphate oxidase *in vitro* [23]. Madi et al. [24] showed that the A3AR is overexpressed in peripheral blood mononuclear cells (PBMCs) from patients with RA compared to healthy subjects, and this overexpression is associated with an increase in nuclear factor (NF)-κB protein expression in PBMCs. In the same study, piclidenoson (10 nM) decreased A3AR expression in phytohaemagglutinin (PHA) and LPS-stimulated PBMCs, as well as reduced TNF-α production in LPS-stimulated PBMCs *in vitro*. PBMCs play a significant role in defending the body against infections, cancer, and other external threats.

The aim of this study was to examine the effects of piclidenoson on the proliferation and cytokine production of PBMCs *in vitro*.

Methods

Human PBMCs were collected from a group of healthy volunteers (n = 4) who signed an informed consent. The study was approved by the Ethics Committee of the Faculty of Medicine Foča, University of East Sarajevo, Bosnia and Herzegovina, (No. 01-2-32, from June 5, 2023).

Peripheral blood mononuclear cells

PBMCs were isolated from the blood of healthy volunteers using Lymphoprep density gradient centrifugation, as previously described [25, 26]. After layering on the Histopaque – 1077 gradient (Sigma-Aldrich, Darmstadt, Germany; density 1,077 g/mL), the blood was centrifuged at 2,200 rpm for 20 minutes at room temperature. The PBMC layer was washed three times with 0.02% sodium-ethylenediaminetetraacetic acid (NaEDTA) in phosphate-buffered saline. Cells were resuspended in a complete medium comprising of RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 μM 2-mercaptoethanol (all from Sigma-Aldrich, Darmstadt, Germany), 2 mM l-glutamine, and antibiotics: 20 μg/ mL of gentamicin, 100 U/mL of penicillin, and 100 μg/mL of streptomycin (all from Galenika, Belgrade, Serbia), for

further use. Cell viability was determined using the trypan blue staining.

Piclidenoson

A3AR agonist with the chemical name 1-deoxy-1-[6-[[(iodophenyl)methyl]amino]-9H-purine-9-yl]-N-methyl-(-D-ribofuranuronamide), piclidenoson (IB-MECA), was produced by Can-Fite BioPharma Ltd., Petah Tikva, Israel. A stock solution of 10 mM was prepared in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Darmstadt, Germany).

MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used as the first method to assess the cytotoxicity of piclidenoson on PBMCs. The PBMCs (3×10^5 cells *per* well) were seeded in 96-well plates (Sarstedt, Nümbrecht, Germany) containing a complete medium. The cells were then treated with increasing concentrations of piclidenoson (250 nM – 1,000 nM) and incubated for 24 hrs at 37 °C with 5% CO_2 and 90% humidity. Cells without piclidenoson were used as a control.

For the proliferation assay, PBMCs were stimulated with 10 μg/mL PHA (Sigma-Aldrich, Darmstadt, Germany) and cultured alone or with piclidenoson (250 nM – 1,500 nM) for 72 hrs. After the incubation period, a solution of MTT (Sigma-Aldrich, Darmstadt, Germany) with a final concentration of 500 μg/mL was added to the wells and incubated for an additional 4 hrs. Subsequently, 10% sodium dodecyl sulfate (Merck KGaA, Darmstadt, Germany) was added to each well, and the results were analyzed the next day using a multimode reader (Synergy HTX, BioTek, Winooski, Vermont, USA) at wavelengths of 670 nm and 570 nm. The values were presented as metabolic activity (in percentages) relative to the analogous negative controls, which were used as 100%.

Flow cytometry

Flow cytometry was the second method for evaluating the cytotoxicity of piclidenoson and its effects on the proliferation of PBMCs.

Apoptosis and necrosis were assessed using an Annexin V/Propidium Iodide (PI) staining kit (BioLegend, San Diego, California), following the manufacturer's protocol. Flow cytometric analysis was conducted using a flow cytometer (Attune, Thermo Fisher Scientific). Data obtained were analyzed offline using the FlowJo X software. Necrotic cells were identified by exclusive PI staining, while cells stained only with Annexin V-fluorescein isothiocyanate were considered to be in the initial apoptotic phase. Cells positive for both markers were recognized as being in the late phase of apoptosis. Results were reported as percentages.

For the analysis of proliferation, cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) staining dye (Thermo Fisher Scientific, Dreieich, Germany), treated with piclidenoson (250 nM–1,000 nM) and then stimulated with PHA (10 μg/mL) for the next 72 hrs. After incubation, cells were collected and stained with PI (50 μg/mL, Sigma-Aldrich, Darmstadt, Germany).

Cytokine production

TNF-α, IL-6, IL-23, IL-36, IL-lβ, IL-10, IL-5, IL-17, and IFN-γ production were determined using the enzyme-linked immunosorbent assay (ELISA) method, according to manufactory instructions (all from R&D Systems, Minneapolis, USA). After isolation, PBMCs (2×10^5 *per* sample) were treated with different concentrations of piclidenoson for 1 hr and then stimulated with PHA (30 μg/mL). After 72 hrs of incubation (37 °C, 5% CO_2), cells were centrifuged, and supernatants were collected to analyze cytokine levels. Unstimulated cells were used as a control.

Statistical analysis

The results were assessed using analysis of variance (ANOVA) test and the Student's *t*-test. Values of $p < 0.05$ were regarded as statistically significant. Mean values from different experiments were compared, and all data is presented as mean ± standard deviation. The data was analyzed using GraphPad Prism software (GraphPad, La Jolla, CA).

Results

Metabolic activity of PBMCs was significantly decreased at a piclidenoson concentration of 1,500 nM ($p < 0.05$) (Figure 1A). As shown in Figure 1B and C, flow cytometry confirmed the results obtained by MTT. Piclidenoson at a concentration of 1,500 nM decreased the viability of the cells and increased the percentage of late apoptotic and necrotic cells ($p < 0.01$, $p < 0.05$, $p < 0.01$, respectively).

As shown in Figure 2A, piclidenoson at concentrations of 250, 500, and 1,000 nM significantly inhibited PBMC proliferation, as measured by the MTT assay, compared to the corresponding control ($p < 0.05$). CFSE labeling assay confirmed these results (Figure 2B and C). No dose-dependent manner was observed in either assay.

Levels of five pro-inflammatory cytokines (TNF-α, IL-6, IL-23, IL-36, and IL-1β) and four key cytokines of T helper (Th) response, IFN-γ, IL-5, IL-17, and IL-10, were determined from PHA-stimulated PBMC supernatants. Cytokine production was undetectable from PHA-unstimulated cells. TNF-α production was downregulated at all piclidenoson concentrations applied ($p < 0.001$).

There is no modulation of IL-6 production. Piclidenoson at concentrations of 250 nM and 1,000 nM reduced IL-23 production, while there was no statistically significant difference at a concentration of 500 nM ($p < 0.05$). Furthermore, piclidenoson at concentrations of 500 nM and 1,000 nM reduced IL-36 production, with no statistically significant difference at a concentration of 250 nM ($p < 0.05$). Interestingly, an A3AR agonist administered at a concentration of 1,000 nM elevated the production of IL-1β, whereas the other two concentrations (250 nM and 500 nM) resulted in a statistically significant decrease of IL-1β production ($p < 0.01$). Th1 cytokine (IFN-γ) and Th2 cytokine (IL-5) were significantly downregulated at the highest concentration of piclidenoson (1,000 nM) ($p < 0.01$, $p < 0.05$, respectively). Moreover, piclidenoson in all applied concentrations decreased Th17 cytokine (IL-17) production ($p < 0.01$) and increased IL-10 production ($p < 0.01$) (Figure 3).

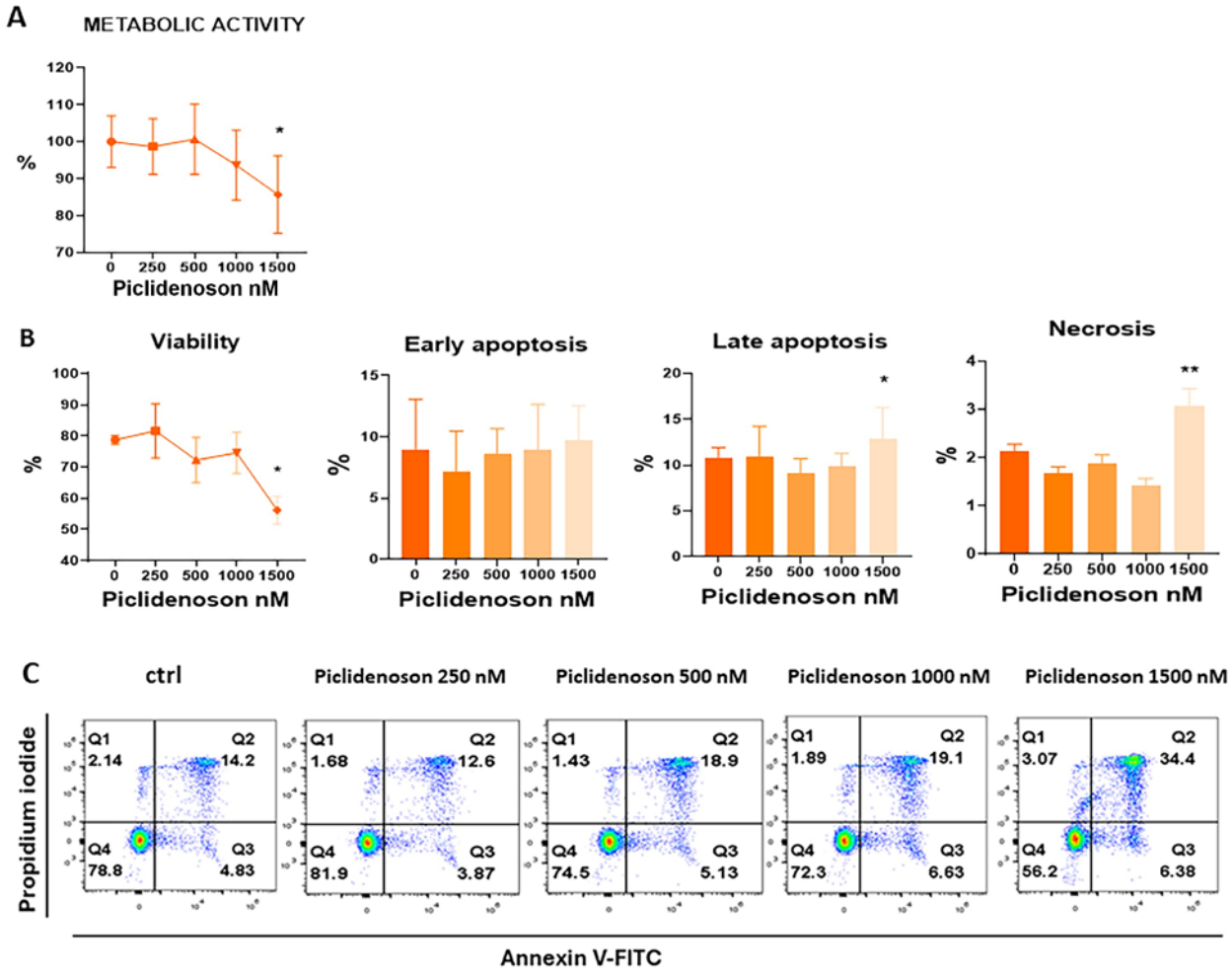

Fig. 1 – Effect of piclidenoson on the metabolic activity, apoptosis, and necrosis of human PBMCs. Cells were treated with increasing concentrations of piclidenoson (250 nM – 1,500 nM) for 24 hrs. Cytotoxicity was determined using the MTT assay and the results are presented as the percent of metabolic activity, relative to the control (100%) (A); the percentage of viable cells, apoptotic cells (Annexin-V$^+$ PI$^-$ for early apoptosis; Annexin-V$^+$ PI$^+$ for late apoptosis), and necrotic cells (Annexin-V$^-$ PI$^+$) is presented, as determined by Annexin-V/PI staining and flow cytometry analysis (B), with a representative example shown in panel C. Values are presented as mean ± standard deviation from 4 independent experiments (A, B).
*** $p < 0.05$, ** $p < 0.01$ compared with corresponding control (piclidenoson-non-treated PBMCs).**
MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; FITC – fluorescein isothiocyanate; PBMCs – peripheral blood mononuclear cells; Ctrl – control cells.

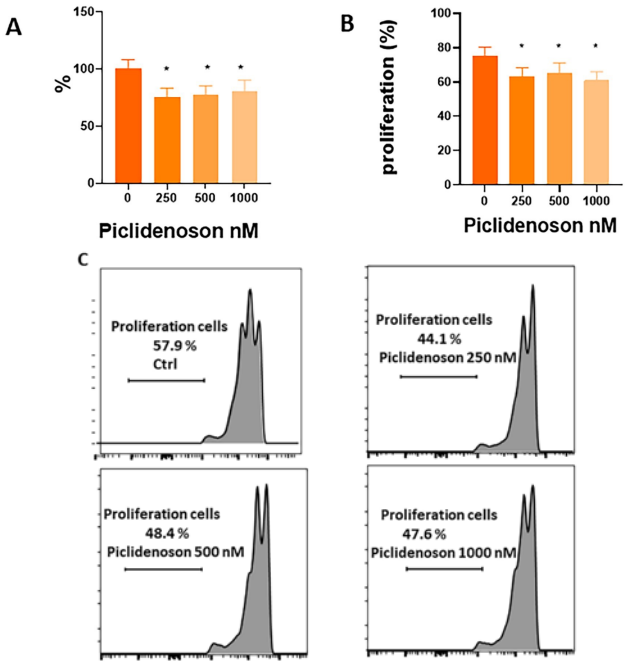

Fig. 2 – The effects of piclidenoson on PHA-stimulated proliferation of PBMCs. Cells were cultivated with PHA alone or PHA with different concentrations of piclidenoson for 3 days. Proliferation was evaluated using MTT assay (the results are presented as the percent relative to the control – 100%) (A) and CFSE staining dye followed by flow cytometry analysis (B, C). Data are expressed as mean ± standard deviation from 4 independent experiments (A, B); the analysis of CFSE dilution is shown on histograms from one representative experiments (C). *$p < 0.05$.
PHA – phytohaemagglutinin; CFSE – carboxyfluorescein succinimidyl ester.
For other abbreviations, see Figure 1.

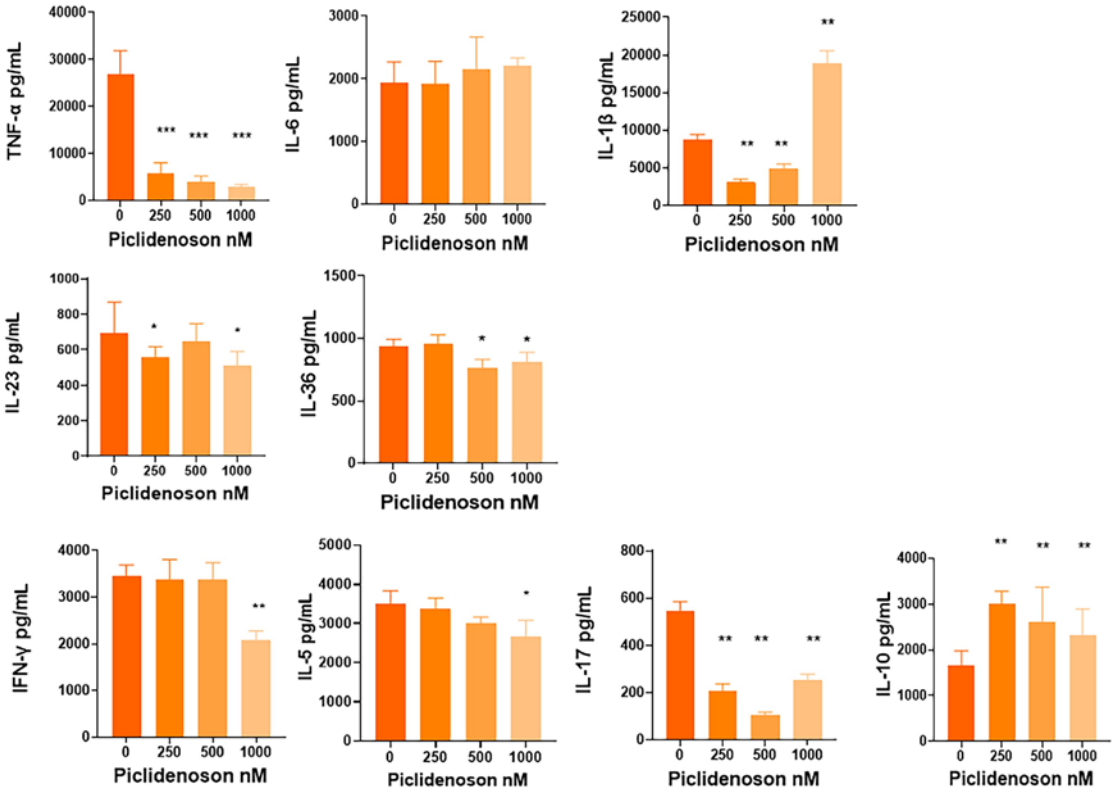

Fig. 3 – Effects of piclidenoson on pro-inflammatory and T-helper cytokine production by PHA-stimulated PBMCs. Cells were cultivated with PHA alone or PHA with different concentrations of piclidenoson for 3 days. Cytokine production was measured in the supernatants of PBMC cultures using ELISA kits. Cytokine levels are expressed as mean ± standard deviation (n = 4). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the control.
TNF-α – tumor necrosis factor-alpha; IL – interleukin; n – number; ELISA – Enzyme-Linked Immunosorbent Assay.
For other abbreviations, see Figures 1 and 2.

Discussion

This study investigated the anti-inflammatory properties of piclidenoson in a model of PHA-stimulated PBMCs. PBMCs are invaluable for *in vitro* immunological research. Predominantly consisting of T lymphocytes, PBMCs offer a practical model for evaluating the effects of various treatments on cell division and cytokine production [27]. PHA interacts with certain carbohydrates on T-cell surfaces and is frequently applied in *in vitro* experiments to stimulate the activation of lymphocytes, transforming them into lymphoblasts that divide, proliferate, and release cytokines [28]. The previous report has shown that PHA stimulation leads to a swift increase in the expression of A3AR in T cells [29].

Piclidenoson is being investigated as a therapeutic option for autoimmune inflammatory disorders such as psoriasis and RA. The results of clinical trials affirm its safety, good tolerability, and significant anti-inflammatory properties [19, 30]. Previous studies have reported increased A3AR expression in the PBMCs of patients with autoimmune inflammatory conditions like Crohn's disease, RA, and psoriasis in contrast to healthy subjects [31]. The precise mechanism of piclidenoson's action remains uncertain. Earlier findings suggest that it involves the modulation of critical signaling proteins like phosphatidylinositol-3 kinase (PI3K), protein kinase A (PKA), protein kinase B (PKB/AKT), and IkappaB kinase. This modulation appears to lead to the disruption of the NF-κB and Wnt/β-catenin pathway and the suppression of inflammatory cytokine production [22]. In this investigation, we found that piclidenoson, applied at non-toxic concentrations, suppressed proliferation and modulated cytokine production of pro-inflammatory cytokines IL-1β, TNF-α, IL-23, and IL-36, as well as Th cytokine profile such as IFN-γ, IL-5, IL-17, and IL-10, in human PBMCs *in vitro*.

Our results demonstrated that piclidenoson, at a concentration of 1,500 nM, increased the number of late apoptotic and necrotic cells and reduced the metabolic activity of PBMCs *in vitro*. Earlier studies showed that A3AR agonists affect cell survival in different ways [32]. At low nanomolar concentrations, they protect human promyelocytic leukemia cell line (HL-60) and pro-monocytic cell line (U937) cells from apoptosis and preserve chick cardiac ventricular myocytes from hypoxic heart damage [33, 34]. However, at high micromolar concentrations (20–40 μM), A3AR agonists induce the death of rat astroglial cells, cerebellar granule neurons, and HL-60 cells [35–37]. According to some studies, A3AR activation leads to apoptotic cell death [33, 36], while other authors report necrotic cell death [37]. Cytotoxicity tests showed that piclidenoson exerts cytotoxic properties at the highest concentration of 1,500 nM, so in further research, we used lower concentrations of 250, 500, and 1,000 nM.

For the first time, our study suggests that piclidenoson inhibits PBMC proliferation in the nanomolar range (250 nM–1,000 nM). According to the results of the MTT test and apoptosis/necrosis assay, inhibited proliferation was not attributed to drug cytotoxicity. Jeffe et al. [38] demonstrated that piclidenoson, at a concentration of 20 μM, can inhibit the proliferation of PHA-stimulated human PBMCs *in vitro*. Similarly, other authors reported that piclidenoson inhibits PHA-stimulated human lymphocyte proliferation at concentrations of 10 μM, 100 μM, and 250 μM [39]. However, they did not employ any cytotoxicity assay method in their research, so the relationship between these effects and cellular apoptosis remains unclear. As reported by Cohen et al. [40], piclidenoson at a nanomolar concentration (10 nM) demonstrated the ability to inhibit the proliferation of immortalized human keratinocyte line cells (HaCaT), which is in accordance with our results.

Based on their cytokine secretion profiles, there are at least four types of Th cells: Th1, Th2, Th17, and T regulatory (T_{reg}). Th1 cells are characterized by the production of IL-2 and IFN-γ, while Th2 cells produce IL-4, IL-5, IL-10, and IL-13. Th17 cells are primarily defined by the production of IL-17A and IL-22 [41]. Lastly, T_{reg} cells, known for their immunosuppressive role, generate IL-10 and transforming growth factor (TGF)-β [42]. Lymphocytes produce pro-inflammatory cytokines, which play crucial roles in both physiological and pathological conditions, modulating immune responses and disease progression. The anti-inflammatory effects of A3AR agonists have been demonstrated in various experimental models and *in vitro* studies. Importantly, our study is the first to examine the effects of piclidenoson on IL-23 and IL-36 production in a model of PHA-stimulated PBMCs. In a model using cultured PBMCs from RA patients, A3AR agonist N^6-(3-iodo-benzyl)-2-chloro-adenosine-5'-N-methyluronamide (CI-IB-MECA) (100 nM) was found to decrease the production of TNF-α, IL-6, and IL-1β, along with reducing NF-κB activation. These effects were observed in PBMCs stimulated with phorbol myristate acetate for 24 hrs [43]. In the model of LPS-stimulated RAW 264.7 mouse macrophage cells, Lee et al. [44] demonstrated that CI-IB-MECA suppressed TNF-α production after 6 hrs of incubation. Additionally, in the same study, CI-IB-MECA reduced IL-1β messenger ribonucleic acid (mRNA) expression after 4 hrs and IL-1β protein expression after 8 hrs. Similarly, in LPS-treated BV-2 microglial cells, adenosine (100 μM) and Cl-IB-MECA (1 μM) suppressed LPS-induced TNF-α protein and mRNA levels by inhibiting PI3K/AKT and NF-κB activation after 4 hrs of incubation [45]. As reported by Cohen et al. [40], piclidenoson (10 nM) inhibits the proliferation of HaCaT cells through the dysregulation of the NF-κB signaling pathway and also suppresses IL-17 and IL-23 expression levels. Our results confirmed that piclidenoson, across all applied concentrations, reduced TNF-α production but did not modify IL-6 production. However, in contrast to previous investigations on A3AR agonists [43, 44, 46], the highest concentration of piclidenoson led to an increase in IL-1β production, while other concentrations decreased its production. The drug may influence the processing and secretion of IL-1β by modulating the activity of inflammasomes or proteases, which are essential for the release of IL-1β from its precursor. The inflammasome nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) activates

Caspase-1, responsible for IL-1β release. Research in a diabetic rat kidney model has shown that A3AR antagonism blocks the increase in Caspase-1 and reduces NF-κB expression in the renal tubular epithelium, leading to a decrease in IL-1β and IL-18 production, which was confirmed by measuring the urinary secretion of these cytokines. The effect of A3AR antagonism with MRS 1220 (0.1 mg/kg) resulted in reduced levels of the profibrotic marker alpha-smooth muscle actin (α-SMA) at the histological level and restoration of proteinuria in diabetic rats, significantly reducing kidney damage. The authors concluded that A3AR antagonism may serve as a potential therapeutic target through selective blockade of the NLRP3 inflammasome [47]. Furthermore, in a mouse macrophage model, the non-selective adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (10 μM), acting through A2AR, increased IL-1β secretion by maintaining inflammasome activity *via* the cAMP/PKA/CREB/HIF-1α pathway [48]. A potential explanation for our results may be that piclidenoson, at micromolar concentrations, stimulates IL-1β secretion, maintaining inflammasome activity through A3 or A2 adenosine receptors or another signaling pathway, while at nanomolar concentrations, it inhibits secretion. The molecular mechanisms underlying this process require further investigation.

In our study, piclidenoson decreased IL-23 production in concentrations of 250 nM and 1,000 nM, but there is no statistically significant difference for concentrations of 500 nM. Furthermore, piclidenoson at concentrations of 500 nM and 1,000 nM has also decreased the production of IL-36 from PHA-stimulated PBMCs. As a pro-inflammatory cytokine, IL-23 is generated by activated monocytes, as well as activated antigen-presenting cells such as dendritic cells (DCs) and macrophages, T cells, B cells, and endothelial cells. It plays a crucial role in the pathogenesis of some diseases by modulating the activities of Th17 cells. These cells, in response to IL-23, produce IL-17 [49]. Elevated IL-17 levels have been observed in various conditions such as psoriasis, multiple sclerosis, Behcet's disease, uveitis, and the synovial fluid of RA patients [50]. The identification of the IL-23/IL-17 pathway has significantly improved the understanding of the pathogenesis of inflammatory diseases and expanded therapeutic possibilities [51, 52]. Within the IL-36 cytokine family, there are three agonists (IL-36α, IL-36β, and IL-36γ) and one antagonist (IL-36 receptor antagonist). Although primarily synthesized by keratinocytes, other cell types, including mononuclear cells, inflammatory macrophages, and DCs, can also produce members of the IL-36 cytokine family [53]. New findings suggest that IL-36 disorder has an important role in some autoimmune disorders, including psoriasis, atopic dermatitis, RA, and allergic contact dermatitis [54]. To our knowledge, there have been no similar studies investigating the impact of A3AR agonist on IL-36 production so far, and it will be very important to conduct similar experiments on other cell lines *in vitro*.

Our findings indicate that piclidenoson, at its highest concentration, reduced the production of IFN-γ and IL-5 and Th1 and Th2 cytokine subsets, respectively. Moreover, all applied concentrations of piclidenoson decreased IL-17 production, a cytokine characteristic of Th17 cells. From these results, we can speculate that piclidenoson at concentrations of 1,000 nM exerts a suppressive effect on the Th1/Th2 response and that all applied concentrations can suppress the Th17 response. Additionally, we observed an increase in the secretion of IL-10 at all tested concentrations of piclidenoson. IL-10, generated by various immune cells, including T and B cells, is very important in regulating excessive inflammation by suppressing the production of pro-inflammatory cytokines [55]. IL-10 can decrease the production of IL-12 by macrophages and DCs, a key cytokine for Th1 differentiation [56]. Likewise, IL-10 can inhibit the proliferation and production of IL-2, IFN-γ, IL-4, IL-5, and TNF-α by CD4 T cells and thus regulate innate and adaptive Th1 and Th2 responses [57]. Our research results on PBMCs and previous *in vitro* studies [58, 59] on LPS-activated RAW 264.7 macrophages and mouse CD4 T lymphocytes indicate that piclidenoson increases the production of IL-10. In this context, we can suggest that lower Th1/Th2 response can be connected with increased IL-10 production. In addition, early study [60] demonstrated that lower IL-17 production is associated with decreased IL-23 production, which is in accordance with our findings. IL-23, released by monocytes/macrophages or DCs, stimulates the generation of IL-17-producing T cells [49, 52].

Conclusion

We showed that piclidenoson exerts anti-inflammatory properties through a model of PHA-stimulated PBMCs. Piclidenoson can modulate PBMC proliferation in nanomolar concentrations and inhibit TNF-α, IL-23, and IL-36 production. Moreover, we showed that the highest concentration of piclidenoson can suppress the production of Th1 and Th2 cytokines, IFN-γ and IL-5. All applied concentrations of piclidenoson can suppress IL-17 production, a cytokine specific to Th17 cells. This knowledge could provide a foundation for further research to support the potential therapeutic applications of piclidenoson. Additional investigations are needed to understand the molecular processes underlying these effects. Furthermore, research on other cell lines will be essential to validate these findings and explore the broader potential of piclidenoson's immunomodulatory effects.

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Differences in the response of various urogynecology synthetic grafts to infection by *Staphylococcus epidermidis*: an experimental animal study

Razlike u reakciji uroginekoloških sintetskih materijala na infekciju bakterijom *Staphylococcus epidermidis*: studija na eksperimentalnim životinjama

Milan Potić*†, Ivan Ignjatović*†, Zoran Damnjanović*‡, Petar Djekić§, Nebojša Jovanović‖

University of Niš, *Faculty of Medicine, §Faculty of Mechanical Sciences, Niš, Serbia; University Clinical Center Niš, †Urology Clinic, ‡Vascular Surgery Clinic, Niš, Serbia; ‖Military Medical Academy, Center for Gynecology and Human Reproduction, Belgrade, Serbia

Abstract

Background/Aim. Polypropylene synthetic meshes are used in urogynecology for the primary treatment of stress urinary incontinence. Infection of the graft could influence the outcome of the surgery as well as the adequate tissue reinforcement. The aim of the study was to compare responses to infection in six different synthetic grafts. **Methods.** The study included six different grafts, with polypropylene as their major component, used for the primary repair of full-thickness abdominal wall defects in male Wistar rats. From a total of 144 Wistar rats, six groups of 24 animals each were created. Each group consisted of 12 animals for noninfected and 12 animals for infected graft testing. In the subgroups for infected graft testing, grafts were inoculated with isolates of *Staphylococcus epidermidis*. After six weeks, the animals were sacrificed, and groups were compared for inflammatory response, collagen quantification, and abdominal wall reinforcement. The inflammatory response was calculated as the total number of inflammatory cells under a magnification of ×200, including polymorphonuclears, foreign body giant cells, and macrophages. Collagen quantification was determined by colorimetric measurement of hydroxyproline for alkaline hydrolysates. Abdominal wall reinforcement was determined as minimal disintegration load on a standardized shredding device. In order to detect bacterial biofilm and characterize collagen fibers, scanning electron microscopy (SEM) of fresh samples was performed. **Results.** Reinforcement of the abdominal wall with a titanium-coated polypropylene graft was most significantly degraded by the infection ($p < 0.001$). The inflammatory response was the most prominent in the infected multifilament polypropylene graft compared to the low-weight polypropylene graft, titanium-coated graft, and multifilament polypropylene graft with polyglactin ($p < 0.01$). In terms of collagen deposition, the greatest differences of all grafts were noted between noninfected and infected low- and high-weight monofilament polypropylene grafts ($p < 0.01$). Using SEM, biofilm formation was detected, and collagen fibers were described as immature. **Conclusion.** The results of this experimental animal study suggest that infection of synthetic urogynecology grafts results in a significant reduction in tissue reinforcement. In addition, the negative effects of the infection are the most pronounced in multifilament and semi-absorbable multifilament grafts.

Key words:
biofilms; infection; rats; surgical mesh; urinary incontinence, stress.

Apstrakt

Uvod/Cilj. Polipropilenski sintetski graftovi se koriste u uroginekologiji za primarni tretman stres urinarne inkontinencije. Infekcija grafta može uticati na ishod operativnog lečenja kao i na odgovarajuću potporu tkiva. Cilj rada bio je da se uporedi odgovor na infekciju kod šest različitih sintetskih graftova. **Metode.** U studiju je uključeno šest različitih graftova, sa polipropilenom kao glavnom komponentom, koji su korišćeni za primarnu reparaciju defekata prednjeg trbušnog zida mužjaka Wistar pacova. Od ukupno 144 Wistar pacova formirano je šest grupa od po 24 životinja. U svakoj grupi bilo je po 12 životinja za testiranje neinficiranih i 12 životinja za testiranje inficiranih graftova. U podgrupama za testiranje inficiranih graftova, graftovi su inokulirani izolatom *Staphylococcus epidermidis*. Posle šest nedelja, eksperimentalne životinje su žrtvovane i unutar grupa je upoređivan stepen inflamacijskog odgovora, količina kolagena i ojačanje abdominalnog zida. Inflamacijski odgovor je kvantifikovan

Correspondence to: Milan Potić, University of Niš, Faculty of Medicine, Blvd. Dr Zorana Djindjica 81, 18 108 Niš, Serbia. E-mail: uropota@gmail.com

izračunavanjem ukupnog broja ćelija zapaljenja pod uvećanjem ×200, uključujući polimorfonukleare, gigantske ćelije stranog tela i makrofage. Kvantifikacija kolagena je određivana kolorimetrijskim merenjem hidroksiprolina za alkalne hidrolizate. Ojačanje abdominalnog zida određivano je kao minimalna dezintegraciona sila na standardizovanom tenzinom meraču. Za detekciju bakterijskog biofilma i karakterizaciju sazrevanja kolagenih vlakana primenjena je skening elektronska mikroskopija (SEM) svežih preparata. **Rezultati.** Ojačanje abdominalnog zida polipropilenskim graftom obloženog titanijumom najznačajnije je degradirano infekcijom ($p < 0,001$). Inflamacijski odgovor bio je najizraženiji kod inficiranog multifilamentnog polipropilenskog grafta u poređenju sa polipropilenskim graftom niske težine, graftom obloženog titanijumom i multifilamentnog polipropilenskog grafta sa poliglaktinom ($p < 0,01$). U pogledu deponovanja kolagena, od svih graftova, najveće razlike su zabeležene između neinficiranih i inficiranih monofilamentnih polipropilenskih graftova niske i visoke težine ($p < 0,01$). Korišćenjem SEM otkriveno je formiranje biofilma, a kolagena vlakna su opisana kao nezrela. **Zaključak.** Rezultati ove studije na eksperimentalnim životinjama sugerišu da infekcija uroginekoloških sintetskih graftova dovodi do značajnog smanjenja potpore tkiva. Takođe, negativni efekti infekcije su najizraženiji kod multifilamentnih i poluresorptivnih multifilamentnih graftova.

Ključne reči:
biofilmovi; zapaljenje; pacovi; hirurška mrežica; inkontinencija, urinarna, stres.

Introduction

Pelvic organ prolapse and stress urinary incontinence (SUI) are common urological pathologies, and surgery is frequently required. The recurrence rate resulting from inadequate tissue support remains a key issue in this area. Polypropylene synthetic meshes are commonly used to treat SUI (tension-free slings) and, in certain instances, pelvic organ prolapse (i.e., sacrocolpopexy and hysteropexy). Although stability and biocompatibility have been substantially proven, there are unresolved concerns and unpredictable consequences in the case of graft infection [1, 2]. The current standard in case of graft infection is to remove the graft completely [3]. On the other hand, when extrusion of the graft occurs, resection of the extruded segment seems to be the most obvious choice [4, 5]. In clinical terms, when resection of the extruded segment is performed, there is an unanswered question of what happens with the intended tissue reinforcement, keeping in mind the inevitable contamination of the residual graft [4]. The steps for preventing unintentional graft inoculation are to give antibiotic prophylaxis. The importance of antibiotic prophylaxis is to secure sterile conditions for graft stabilization and tissue integration [6]. To the best of our knowledge, research on the graft type with the most chance to withstand the infection has not been done. However, cellular changes and fluctuations in tensile strength during graft infection are rarely discussed [6, 7].

The aim of the study was to compare six different grafts in terms of their responses to infection.

Methods

The methodology for this animal experiment was adopted from our previous study, where we successfully analyzed the influence of oxidative stress on abdominal wall (AW) reinforcement [8].

Urogynecology synthetic grafts used in the study

Six graft types were used in the experimental study: two monofilaments Ethicon 76 g/m^2 as high-weight polypropylene (HWPP) and Gynecare Gynemesh® Ethicon 43 g/m^2 as low-weight polypropylene (LWPP); two multifilament grafts Surgipro™ multifilament polypropylene (MPP) Tyco 97 g/m^2 and Vypro mesh Ethicon 25 g/m^2 multifilament polypropylene with polyglactine (MPPG); two coated grafts Sepra meshTM Genzyme® 96 g/m^2 as collagen coated polypropylene (CPP) and Titanium coated polypropyleneTM Gesellschaft für elektrometallurgie® 16 g/m^2 as titanium polypropylene (TPP).

Design of the experimental study

Six groups of 24 animals for each graft group (with 12 animals for noninfected and 12 for infected graft testing) were created from a total of 144 male Wistar rats. Each group was then allocated to use one of the grafts. Experimental animals used in the study were acquired from the Biomedical Research Institute of the Faculty of Medicine of the University of Niš, Serbia. The experimental investigation was approved by the Ethics Committee of the Faculty of Medicine, University of Niš (No. 01-2066-9, from April 1, 2010). Anesthesia for the experimental animals was provided by administering 10% ketamine (Richter Austria) injected subcutaneously. AW defect 20 × 25 mm was made with respect to the peritoneum and repaired with different grafts. When repairing the AW defect, the overlay technique was used with graft dimensions 25 × 30 mm. Nonabsorbable sutures (Surgipro™ II; 4/0) were used for the fixation of the grafts with continuous sutures. In the subgroup of 12 animals, the grafts were inoculated (infected) with isolates of *Staphylococcus (S.) epidermidis* 10^8 colony-forming unit/mL (standardized isolates acquired from the microbiology laboratory of the Faculty of Medicine of the University of Niš) *via* swabs three times in a row for 15 sec on the entire surface of the graft. The subcutis (subcutaneous layer) and skin were sutured with absorbable 3/0 polyglactin suture in all groups and subgroups. Prophylactic antibiotic treatment with gentamycin 60 mg/mL in the dosage of 0.2 mL/day was administered for three days.

Six weeks after AW repair, animals were sacrificed by ketamine overdose. The entire AW was dissected with a graft in the middle and at least 30 mm of neighboring tissue. Explants were dissected into 50 × 10 mm samples with graft in the middle and stored in saline solution for tensiometry. Load-displacement minimal disintegration limits were determined for both infected and noninfected groups.

Mechanical sample testing for minimal disintegration load

As *per* Afonso et al. [9], specimens standardized to 50 × 10 mm were obtained from each experimental animal, and the specimens were tested for minimal disintegration load (MDL). An HBM Spider 8 (HBM, Darmstadt, Germany), a digitalized acquisition system with an HBM catman, was used for the mechanical testing of the MDLs. The samples were tested until they were completely disrupted, at which point the ratio of applied force (given in Newton – N) to material stretching (mm) was determined. Mechanical testing was performed at the Faculty of Mechanical Sciences of the University of Niš.

Histological sample preparation and inflammatory cell quantification

Tissue samples for histology analysis were prepared in a standard manner, and hematoxylin and eosin staining was then performed. Inflammatory cells were quantified as described by Konstantinovic et al. [10]. Two different observers quantified the number of inflammatory cells under x200 magnifications (in matrices) on ten high-power fields in the near proximity of the grafts. The middle value of ten fields for inflammatory cell numbers in each graft group was further included in the calculations. The total number of inflammatory cells, including polymorphonuclears, foreign body cells, and macrophages, was calculated.

Quantification of the collagen deposits

Collagen quantification was performed as described by da Silva et al. [11] from tissue stripped directly from the surface of the grafts. Fresh collagen samples were subjected to alkaline hydrolysis in accordance with the specified procedure to produce a sensitive hydroxyproline assay of hydroxylates. Hydroxyproline for alkaline hydrolysates was measured colorimetrically *via* a spectrophotometer (SP-22, Bio Spectro, Brazil) with 1 cm optical glass cuvettes at a 1/100 dilution. The samples were prepared using a 50% solution of sodium hydroxide (Vetec Brazil). After each sample was hydrolyzed for 40 min, it underwent an identical pH correction procedure *via* a pH meter (model HI3222, Hanna, Instruments, USA).

Electron microscopy of the explanted samples

Explanted samples were analyzed by scanning electron microscopy (SEM) for polypropylene fibers related to inflammatory cells and collagen deposits as well as for bacterial biofilm analysis. The freshly explanted samples were covered with gold *via* the "sputter" method for 10 min and then analyzed *via* SEM.

Statistical analysis

For pairwise analysis, ANOVA Kruskal Wallis variance followed by the Mann-Whitney *U* test was performed. Comparisons of inflammatory cell numbers, collagen amounts, and MDLs were performed for infected vs. noninfected grafts. For paired comparison, corrections were made according to Bonferroni. Values of $p < 0.05$ were considered significant. SPSS 16 Chicago USA statistical package was used in the calculations.

Results

All 144 animals survived the entire six-week study period. During the study period, infections occurred in all groups inoculated with *S. epidermidis.* In the infected MPP graft group, one mesh extrusion was detected and excluded from further studies. In 2 out of 12 animals in the MPP group, pus was detected on the surface of the graft when it was explanted.

The results of the AW reinforcement tests of the noninfected explants are presented in Figure 1. The noninfected group presented mostly comparable MDL results with no statistical differences among tested grafts. The strongest AW reinforcement was measured for the TPP, which reached a 14.2 N MDL. HWPP graft groups presented better tolerance for distention compared to other grafts.

The results of the AW reinforcement tests of the infected explants are presented in Figure 2. Compared with noninfected samples, all infected samples presented significantly weaker AW reinforcement ($p < 0.05$). When infected, the deterioration level of the strongest TPP in the noninfected samples barely reached the 4.1 N limit for the MDL ($p < 0.001$). Infection was the most significant parameter in the semiabsorbable MPPG group, where the weakest degree of AW reinforcement was measured (3.8 N). When the HWPP and the MPP were infected as the heaviest grafts (g/m^3), the MDL was 6.6 N despite their difference in construction. Displacement tolerance was comparable between the HWPP groups in both the infected and noninfected circumstances ($p = 0.23$). All other samples presented slightly lower displacement tolerances when infected ($p = 0.79$).

Inflammatory cell quantification results (noninfected and infected grafts) are presented in Figure 3. Compared with the noninfected grafts, all the infected grafts presented with greater numbers of inflammatory cells. The most significant difference between infected vs. noninfected grafts was in the TPP group ($p < 0.001$) compared to the CPP and LWPP ($p < 0.01$) and the HWPP, MPP, and MPPG groups ($p < 0.5$). Significant differences were also detected among the infected grafts. Compared with the LWPP-, TPP-, and MPPG-infected ($p < 0.01$) and HWPP- and CPP-infected ($p < 0.05$) grafts, the infected MPP groups presented more

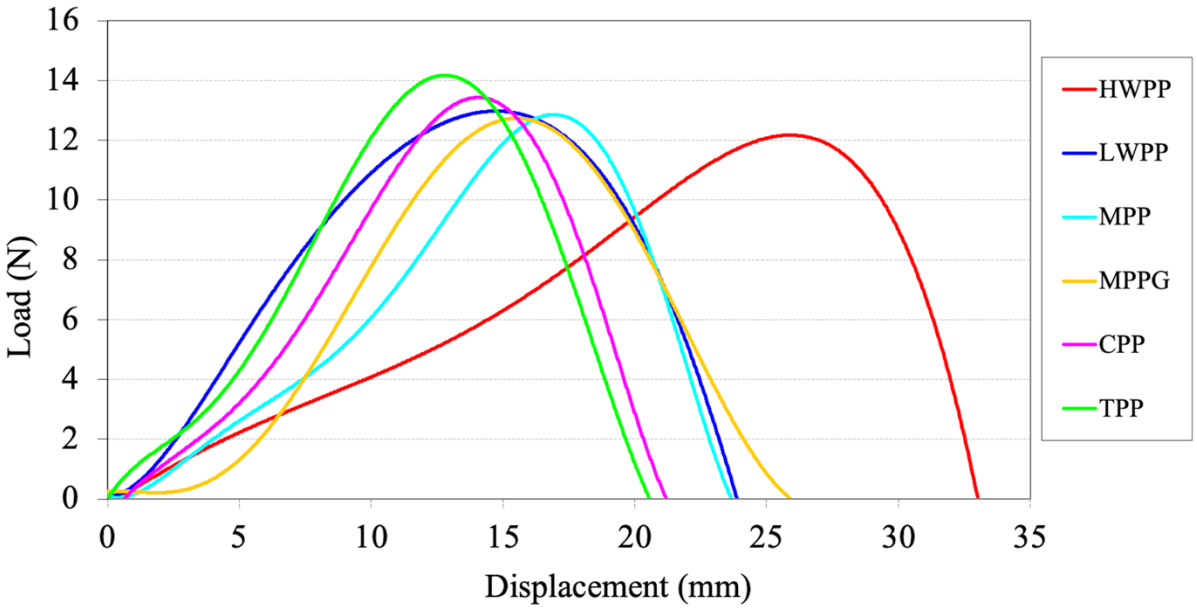

Fig. 1 – Uniaxial tension test results for minimal disintegration load of the abdominal wall explants with noninfected grafts after six weeks. HWPP – high-weight polypropylene; LWPP – low-weight polypropylene; MPP – multifilament polypropylene; MPPG – multifilament polypropylene with polyglactin; CPP – collagen-coated polypropylene; TPP – titanium-coated polypropylene; N – Newton.

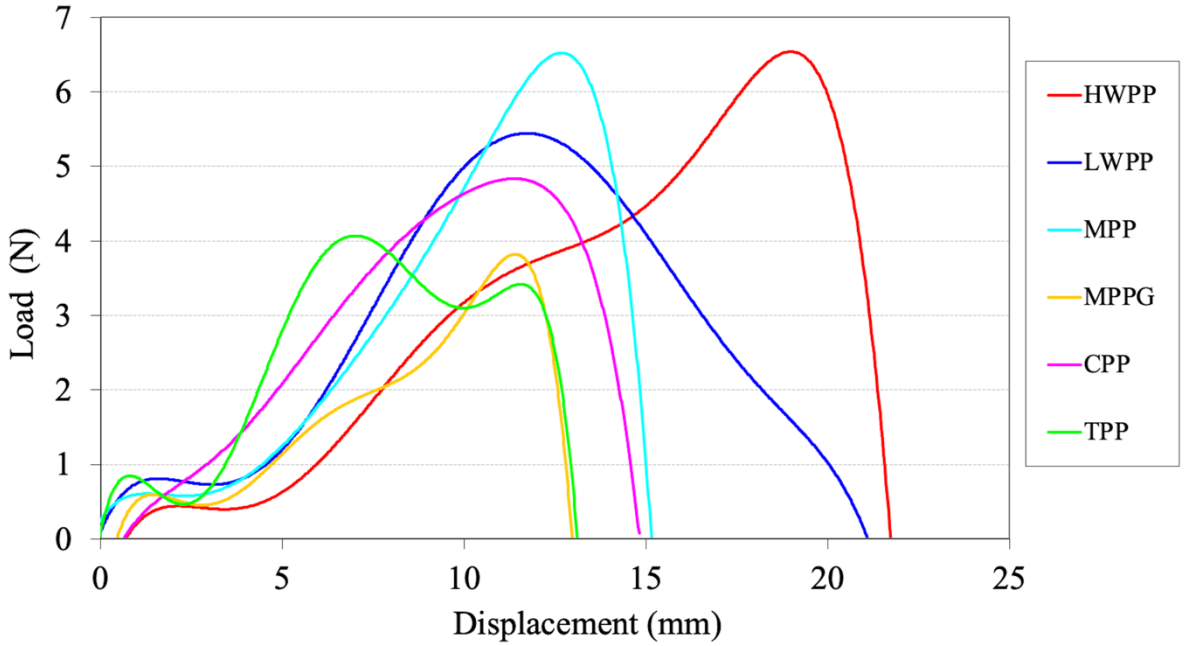

Fig. 2 – Uniaxial tension test results for minimal disintegration load of the abdominal wall explants with infected grafts after six weeks. For abbreviations, see Figure 1.

pronounced inflammatory reactions. Compared with those in the MPPG ($p < 0.05$) and MPP ($p < 0.001$) groups, the number of inflammatory cells in the noninfected groups was lower.

Collagen quantification from samples stripped from the grafts is presented in Figure 4. In all infected samples, collagen deposits presented greater amounts of collagen. The monofilament grafts (HWPP and LWPP) unexpectedly presented the greatest difference in all samples between the infected and noninfected groups ($p < 0.01$). Collagen- and titanium-coated graft collagen deposits between infected and noninfected grafts were slightly more pronounced than those in the multifilament groups ($p < 0.05$). The least significant differences in collagen deposition among the infected and noninfected groups were recorded for the semi-absorbable

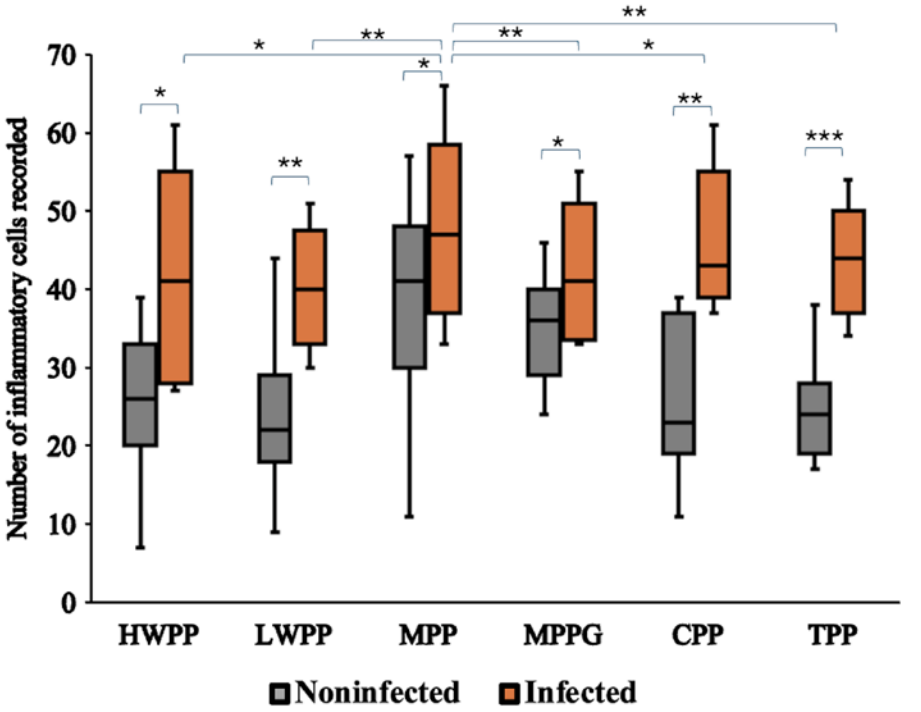

Fig. 3 – Number of inflammatory cells recorded (at magnification ×200) in the near proximity of the grafts (*per* high power field) after six weeks (median/quartile range).
***p* < 0.05; ****p* < 0.01; *****p* < 0.001. For abbreviations, see Figure 1.**

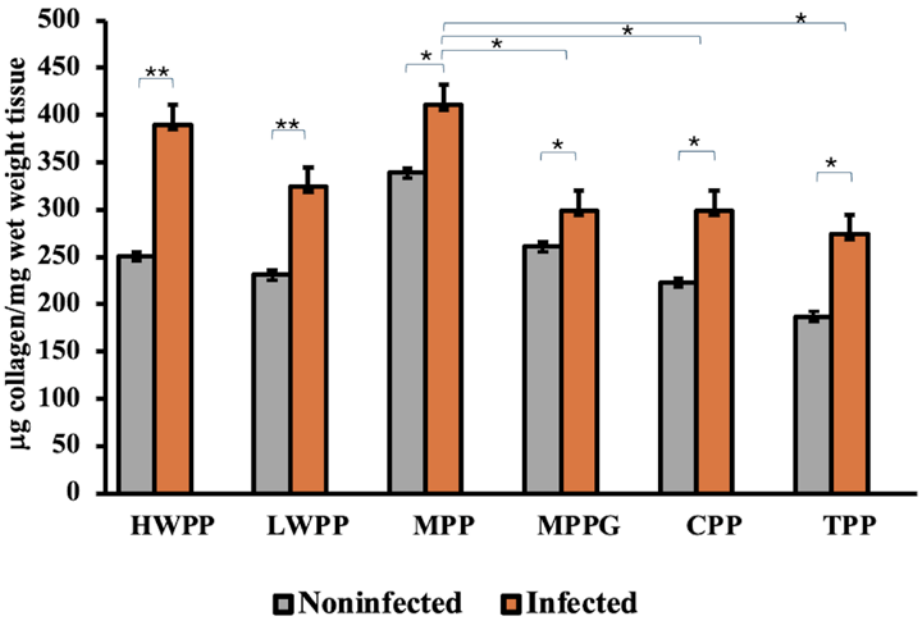

Fig. 4 – Collagen quantification from explanted tissue samples stripped down from the grafts after six weeks (mean + standard error).
***p* < 0.05; ****p* < 0.01. For abbreviations, see Figure 1.**

MPPG in all the samples ($p < 0.05$). When infected grafts are compared, the MPP presented significantly higher collagen deposition compared to the MPPG, CPP, and TPP ($p < 0.05$).

SEM studies of the fresh samples revealed biofilm formation in all the infected groups. Fibroblasts were detected on the surface of the grafts in the noninfected samples, whereas in the infected samples, fibroblasts were detected on the surface of the biofilm. The characterization of collagen fibers differed among the groups. In the noninfected samples, the collagen fibers were characterized as mature, organized, and in contact with the filaments filling the pore spaces of the grafts. In infected samples, collagen fibers were characterized as predominantly immature, in abundance, and not penetrating the interfilamentous spaces (Figure 5). In the infected grafts, inflammatory cells were recorded in abundance on the biofilm covering the fibers.

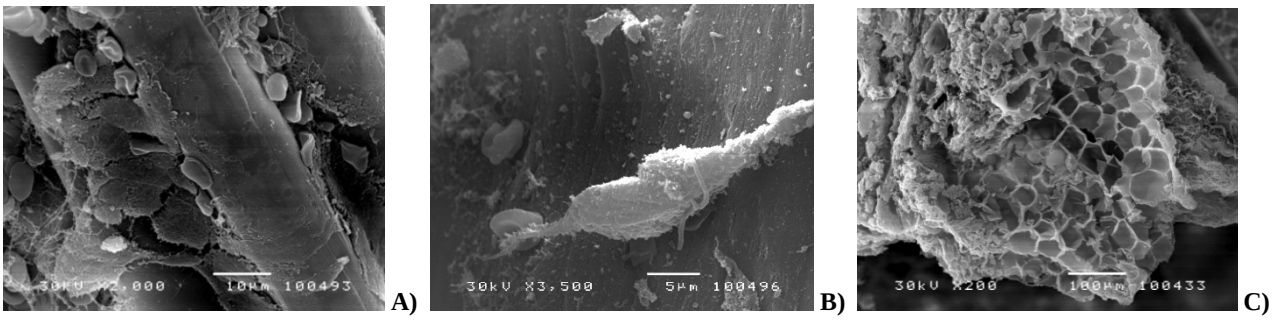

Fig. 5 – Scanning electron microscopy characteristics of the samples: A) biofilm identification; B) fibrocyte detected on the biofilm; C) exposed multifilament graft with no interfilamentous infiltrate.

Discussion

Polypropylene meshes are now commonly used to treat SUI in women. Nevertheless, these meshes are still linked to certain problems, such as mesh shrinkage, mesh migration, and the possibility of infection [12]. The incidence of mesh infection in the human population ranges from 0.3% to 8%. However, the exact incidence is not well established because of the lack of a consistent definition [13].

Our investigation revealed a 100% infection rate when *S. epidermidis* was inoculated according to the described method. We focused our work on *S. epidermidis* because these bacteria are frequently associated with biofilm infections of surgical implants [14, 15]. Several investigations have suggested that infection can be prevented when antibiotic prophylaxis is given [16, 17]. In our study, antibiotic prophylaxis proved not to be successful. One of the reasons could be the time of only three days of administration. In our opinion, the biofilm formation on the graft filaments protected the bacteria from antibiotics and thus enabled the infection to persist. Furthermore, the intentional inoculation, as described with large quantities of bacteria, might be the reason for prophylaxis to be unsuccessful. The presence of bacterial biofilms has been observed in both inguinal and ventral hernia repair, suggesting that they are important causative mechanisms of non-septic failure in most surgical implants investigated to date [13, 15, 18].

The inflammatory response was severe, as anticipated in the infected groups, with noticeable variations observed among the different mesh groups. The TPP and LWPP were the most significantly different between the noninfected and infected groups in all the samples ($p < 0.001$). Based on prior studies, the presence of many inflammatory cells in the MPP- and HWPP-infected graft groups indicates that the strength of the inflammatory reaction may be determined by the sheer quantity of the graft, namely its weight [2]. When infected, the TPP and CPP coatings designed to enhance biocompatibility presented a greater presence of inflammatory cells than the monofilament alone. The presence of a pronounced inflammatory reaction in all infected graft groups indicates a protracted infection, whereas the noninfected graft group had already completed its acute inflammatory reaction. Zheng et al. [19] reported that acute infection reached its highest point between 7 and 14 days and then became insignificant by day 90, which is like our noninfected control groups. The acute inflammatory response is replaced by a persistent reaction that facilitates healing and produces low-intensity granulomas, which subsequently leads to collagen deposition [20]. The persistence of infection and the elevated presence of inflammatory cells in our infected sample group resulted in a longer healing phase that lasted for six weeks. Several studies have suggested that *S. epidermidis* adhesion is greater in multifilament and composite (semi-absorbable) grafts than in monofilament grafts, which supports the findings of our study [21]. This may explain the most significant disparity in inflammatory cell counts between noninfected and infected MPPG grafts in our study. Furthermore, compared with the other samples, the infection had the most significant effect on reducing the strength (MDL) of the MPPG.

Fibroblasts play a significant role in the inflammatory response by producing collagen in response to activation by macrophages [22]. Under typical conditions, collagen is deposited outside of the cells in the matrix and pore spaces, where it covers the mesh fibers [23]. Our investigation revealed that collagen deposits in the infected subgroups covered the pore gaps rather than filled them. Unlike previous studies suggesting that steroid soaking is a means of decreasing collagen deposition [21, 24], our investigation revealed that infection of the graft significantly increased the inflammatory response and promoted collagen deposition. The collagen deposits exhibited a lack of organization and were present in large quantities. Yet, they did not have any effect on the structural support of the tissue, as evidenced by previous research [8].

Using SEM, we successfully identified the fibroblasts. However, variations were observed in the spatial distribution of the fibroblasts. In noninfected grafts, fibroblasts were observed in direct contact with the filaments in all experimental groups. Nevertheless, in the infected graft groups, fibroblasts were discovered specifically on the biofilm that covered the graft or graft bundles, as illustrated in Figure 5. Fresh sample testing, as recommended by Patiniott et al. [15], successfully confirmed the accurate identification of the biofilm. Discrepancies were observed in the maturation of collagen. Infected grafts had a high presence of immature collagen, with collagen fibers visibly not in contact with the graft filaments, indicating excessive scarring. In the noninfected groups, the collagen fibers were predominantly mature, in direct contact with the filaments, and covered the pore spaces. The presence of a bacterial biofilm in infected samples hinders direct contact between collagen fibers and polypropylene filaments,

thus affecting their integration and proper support. The fibers appear to be more encapsulated rather than incorporated into the AW. The work of Klinge and Klosterhalfen [25] defined simple mesh porosity in contrast to effective mesh porosity, with bacterial biofilms being responsible for reducing effective porosity due to mesh constriction.

The results of tensile strength testing of the infected graft groups revealed that increased collagen deposition did not result in enhanced abdominal support, contrary to what other studies have suggested [26]. In fact, all infected samples presented significantly weaker abdominal support and lower tolerance to elongation than the noninfected samples. Our findings indicate that reinforcement in the event of graft infection is insufficient despite the high levels of collagen observed. The SEM images suggest that the collagen fibers are disordered, which may result in inadequate support and excessive scarring. The persistence of a high number of inflammatory cells may contribute to the insufficient bonding of collagen with the graft filaments. The quantity of collagen did not correlate with studies on strengthening the abdominal wall, even in the groups without any infection in the graft.

This study has several limitations. The experimental animal study is performed on Wistar rats to gain insight into the cellular and tensile changes. Although conclusions can be drawn, there may be differences in the human population. Bacterial biofilms are not readily characterized in standard histopathological and SEM techniques due to their challenging composition assessment. The causative organisms require a broader evaluation to identify all bacteria implicated in the biofilm. We used SEM biofilm identification only as an orientation to confirm the presence of biofilm.

Conclusion

The findings of the presented animal study have indicated that infection of the urogynecology synthetic graft leads to a substantial decrease in tissue reinforcement in all grafts. The presence of an infection causes an increase in the inflammatory response, affecting the titanium-coated grafts the most and changing the way collagen is deposited. This results in a situation where, despite the presence of a large amount of collagen, it does not correlate with tissue reinforcement. Our investigation revealed that the overall unfavorable consequences of infection are more evident in multifilament and semi-absorbable multifilament grafts. According to our study results, low-weight polypropylene is the most resilient to infection and, therefore, can be preferred for use. Antibiotic prophylaxis has not been efficient in our study, probably because of the bacterial biofilm formation on the polypropylene fibers.

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CASE REPORT

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Crack lung – a case report

Kokainska pluća

Mira Vasiljević*, Sanja Šarac*†, Rade Milić*†, Biljana Lazović-Popović‡

***Military Medical Academy, Clinic for Pulmonology, Belgrade, Serbia; ‡University Clinical Hospital Center "Zemun", Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia**

Abstract

Introduction. Crack cocaine (CC), a potent form of cocaine, is well-known for its rapid and intense effects on the central nervous system. Its detrimental impact on the respiratory system is often disregarded. This type of cocaine originates from cocaine hydrochloride, a compound extracted from the leaves of the coca plant (*Erythroxylum coca*). Through various pathophysiological mechanisms, its pharmacokinetics and interaction with the respiratory system contribute to acute and chronic lung damage. **Case report.** We present a 35-year-old male with progressive dyspnea and chronic lung damage caused by long-term abuse of CC. Morphological changes in the lungs along with abnormalities in pulmonary function tests were also observed. The patient was treated with a combination of medication therapy and enrolled in a detoxification support program. The applied therapeutic measures led to a gradual reduction in symptoms and significant improvement in pulmonary function tests. **Conclusion.** CC-induced lung damage represents a clinical challenge with profound implications for patient health and well-being. Substance abuse counseling, relapse prevention strategies, and social support services are key components of comprehensive treatment to support patient recovery and prevent relapse.

Key words:
cocaine; diagnosis; lung diseases; substance-related disorders; therapeutics.

Apstrakt

Uvod. Krek kokain (KK), potentna forma kokaina, poznata je po svom brzom i intenzivnom uticaju na centralni nervni sistem. Njegov štetan uticaj na respiratorni sistem obično se zanemaruje. Ova vrsta kokaina potiče od kokain-hidrohlorida, jedinjenja koje se ekstrahuje iz listova biljke koke (*Erythroxylum coca*). Različitim patofiziološkim mehanizmima, njegova farmakokinetika i interakcija sa respiratornim sistemom doprinose akutnom i hroničnom oštećenju pluća. **Prikaz bolesnika.** Prikazan je 35-godišnji muškarac sa progresivnom dispnejom i hroničnim oštećenjem pluća izazvanim dugogodišnjom zloupotrebom KK. Uočene su i morfološke promene u plućima, kao i abnormalnosti u testovima plućne funkcije. Bolesnik je lečen kombinovanom medikamentnom terapijom i uključen je u program podrške za odvikavanje od psihoaktivnih supstanci. Primenjene terapijske mere dovele su do postepene redukcije simptoma i značajnog poboljšanja parametara testova plućne funkcije. **Zaključak.** Oštećenje pluća izazvano KK predstavlja klinički izazov sa dubokim posledicama za zdravlje i dobrobit bolesnika. Savetovanje o zloupotrebi supstanci, strategije prevencije recidiva i socijalne usluge podrške su ključne komponente sveobuhvatnog lečenja, kako bi se podržao oporavak bolesnika i sprečio recidiv.

Ključne reči:
kokain; dijagnoza; pluća, bolesti; poremećaji izazvani supstancama; lečenje.

Introduction

Crack cocaine (CC), a potent form of cocaine, is notorious for its rapid and intense effects on the central nervous system. Its detrimental impact on the respiratory system is often overlooked. CC is derived from cocaine hydrochloride (HCl), a compound extracted from the leaves of the coca plant (*Erythroxylum coca*) [1]. Unlike HCl, which is typically snorted or injected, CC is smoked, resulting in rapid absorption into the bloodstream through the lungs [2]. CC exerts its effects primarily by blocking the reuptake of neurotransmitters, such as dopamine, norepinephrine, and serotonin, leading to increased neurotransmitter levels in the brain's synapses [3]. Smoking CC produces a rapid onset of effects, with peak blood concentrations reached within minutes [4]. The short duration of action (approximately

Correspondence to: Mira Vasiljević, Military Medical Academy, Clinic for Pulmonology, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: mirasmc@gmail.com

5–10 min) contributes to its high addictive potential and frequent binge use patterns [5]. CC use has been associated with urban, low-income communities, although its use is not limited to any particular demographic group. Furthermore, this form of cocaine is not commonly consumed in our region, i.e., Southeastern Europe [6]. Many CC users also use other substances concurrently, such as alcohol, marijuana, or opioids, which can compound the risks and health consequences. CC's pharmacokinetics and interaction with the respiratory system contribute to acute and chronic pulmonary injury through various mechanisms, including direct toxicity, vasoconstriction, oxidative stress, inflammation, and impaired mucociliary clearance [7, 8].

Case report

A 35-year-old male football coach presented to the emergency department complaining of worsening dyspnea, chronic cough, and episodes of minimal hemoptysis over the past six months. He also reported a history of CC abuse for the past ten years, smoking approximately 10 rocks *per* day. He denied any recent fever, chest pain, or wheezing but admitted to progressive fatigue and exercise intolerance. His symptoms had escalated over the past few months, prompting him to seek medical attention. No significant past medical history was reported. There was no history of asthma, chronic obstructive pulmonary disease (COPD), or other respiratory conditions. The patient was a heavy smoker (about 20 pack-years). He denied alcohol or drug use other than CC. Physical examination revealed mild dyspnea. Vital signs were notable for tachypnea, with a respiratory rate of 24 breaths *per* minute. Lung auscultation revealed decreased bilateral breath sounds with diffuse expiratory wheezing. Cardiac examination was unremarkable, and there were no signs of peripheral edema or cyanosis. Arterial blood pressure was 130/80 mmHg, heart rate was 90 beats *per* minute, and oxygen saturation was 82% on room air, measured with a pulse oximeter (normal range from 95% to 100%). Laboratory tests were within normal limits, including a complete blood count and comprehensive metabolic panel. However, urine toxicology screening was positive for cocaine metabolites.

Chest X-ray demonstrated discrete bilateral interstitial changes (Figure 1).

Multi-detector computed tomography (MDCT) of the chest revealed ground-glass opacities (GGO), interstitial thickening, and mild bronchial wall thickening, suggestive of interstitial lung disease (Figure 2).

Pulmonary function tests were performed. Spirometry showed a mixed ventilation disorder with reduced forced vital capacity (FVC) – 43.8% [normal range (NR): 80–120% predicted], forced expiratory volume in 1 second (FEV1) – 37.4% (NR: 80–120%), FEV1/FVC ratio 62.85% (NR: 70–80%), along with decreased diffusion capacity of the lungs for carbon monoxide (DLCO) – 66.8% (normal above 80%) (Figure 3).

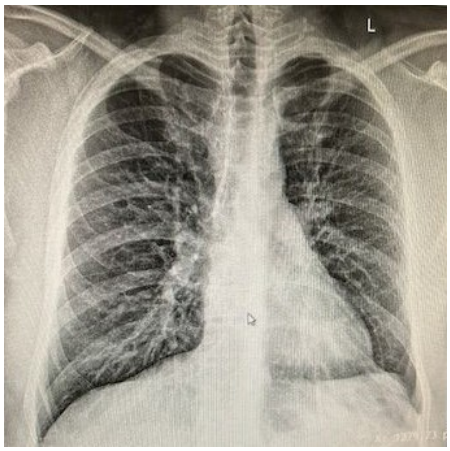
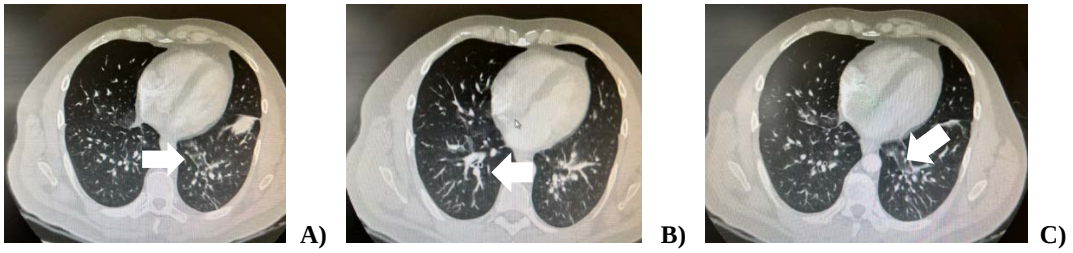


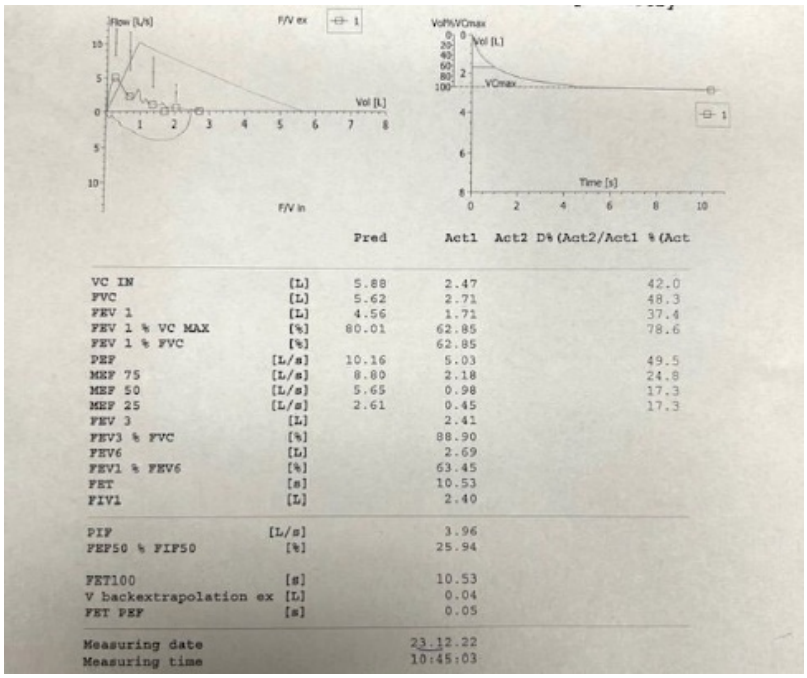
Fig. 1 – The chest radiograph shows bilateral interstitial changes.



A) B) C)

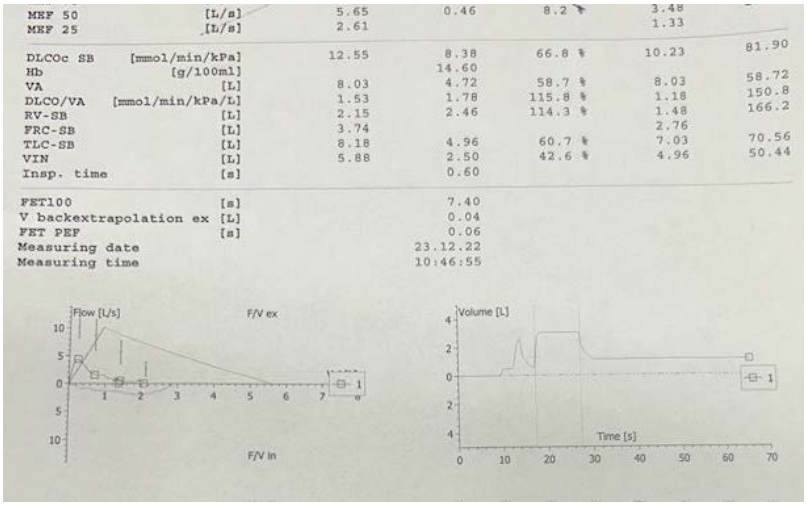
Fig. 2 – Multi-detector computed tomography shows: ground-glass opacities (A), mild bronchial wall thickening (B), and interstitial thickening (C).

A)



		Pred	Act1	Act2	D% (Act2/Act1	% (Act
VC IN	[L]	5.88	2.47			42.0
FVC	[L]	5.62	2.71			48.3
FEV 1	[L]	4.56	1.71			37.4
FEV 1 % VC MAX	[%]	80.01	62.85			78.6
FEV 1 % FVC	[%]		62.85			
PEF	[L/s]	10.16	5.03			49.5
MEF 75	[L/s]	8.80	2.18			24.8
MEF 50	[L/s]	5.65	0.98			17.3
MEF 25	[L/s]	2.61	0.45			17.3
FEV 3	[L]		2.41			
FEV3 % FVC	[%]		88.90			
FEV6	[L]		2.69			
FEV1 % FEV6	[%]		63.45			
FET	[s]		10.53			
FIV1	[L]		2.40			
PIF	[L/s]		3.96			
FEF50 % FIF50	[%]		25.94			
FET100	[s]		10.53			
V backextrapolation ex	[L]		0.04			
FET PEF	[s]		0.05			
Measuring date			23.12.22			
Measuring time			10:45:03			

B)



MEF 50	[L/s]	5.65	0.46	8.2 %	3.48	
MEF 25	[L/s]	2.61			1.33	
DLCOc SB	[mmol/min/kPa]	12.55	8.38	66.8 %	10.23	81.90
Hb	[g/100ml]		14.60			
VA	[L]	8.03	4.72	58.7 %	8.03	58.72
DLCO/VA	[mmol/min/kPa/L]	1.53	1.78	115.8 %	1.18	150.8
RV-SB	[L]	2.15	2.46	114.3 %	1.48	166.2
FRC-SB	[L]	3.74			2.76	
TLC-SB	[L]	8.18	4.96	60.7 %	7.03	70.56
VIN	[L]	5.88	2.50	42.6 %	4.96	50.44
Insp. time	[s]		0.60			
FET100	[s]		7.40			
V backextrapolation ex	[L]		0.04			
FET PEF	[s]		0.06			
Measuring date			23.12.22			
Measuring time			10:46:55			

Fig. 3 – A) Forced spirometry: a mixed ventilatory disorder with reduced forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), and FEV1/FVC ratio; B) diffusing capacity of the lungs for carbon monoxide (DLCO): decreased DLCO – mild degree.

Arterial blood gas analysis revealed hypoxemia with a partial pressure of oxygen (PaO2) of 45 mmHg (NR: 75–100 mmHg) with normal partial pressure of carbon-monoxide (PaCO2) of 38 mmHg (NR: 35–45 mmHg) and respiratory alkalosis with a pH of 7.48 (NR: 7.35–7.45).

The patient was admitted for hospital treatment for further evaluation and treatment. He was started on supplemental oxygen therapy to maintain oxygen saturation above 90%. Bronchodilators (short-acting beta-agonists and anticholinergics) and inhaled corticosteroids were initiated to reduce bronchospasm and inflammation. Sputum culture was negative for bacterial and fungal pathogens. Laboratory tests for viruses, such as human immunodeficiency virus, Epstein-Barr virus, hepatitis A and hepatitis B, and cytomegalovirus, were negative.

The patient received smoking cessation counseling, and nicotine replacement therapy was offered to assist with cessation. He was referred to addiction support services for comprehensive substance abuse (SA) treatment, including behavioral therapy and pharmacotherapy.

During hospitalization, the patient showed gradual improvement of symptoms with bronchodilators, corticosteroids, and supplemental oxygen therapy. However, he remained tachypneic with persistent wheezing on auscultation. Arterial blood gases and oxygen saturation were normalized. After two weeks of hospitalization, the patient was discharged from the hospital and referred to outpatient counseling and a program for SA rehabilitation.

Due to the history of smoking and suspicion of COPD, dual bronchodilator therapy (tiotropium/olodaterol) was included.

Despite initial improvement, long-term follow-up revealed persistent respiratory symptoms, emphasizing the chronic nature of CC-induced lung injury. The patient's respiratory symptoms gradually improved with treatment, which included oral corticosteroid (prednisone) in tapering dose and inhalation bronchodilator, although he continued to experience dyspnea during physical activities. He has not reported hemoptysis since discharge from the hospital. Furthermore, he remained abstinent from CC and engaged in ongoing SA counseling. Pulmonary rehabilitation was initiated to improve his exercise tolerance and lung function.

Six months after he was discharged from the hospital, follow-up spirometry showed significant improvement – mild obstructive ventilation disorder (Figure 4).

There was no significant improvement in DLCO. The patient discontinued inhalation bronchodilator therapy after three months because he concluded that he no longer needed it.

Nine months after treatment and discontinuation of CC use, the patient reported a much-improved condition and tolerance to physical exertion. A follow-up MDCT of the chest was performed, which showed mild bronchial wall and interstitial thickening without GGO (Figure 5).

Long-term follow-up was planned due to the possibility of disease progression and to provide ongoing support for his SA recovery.

Discussion

Chronic CC abuse can lead to severe and often irreversible pulmonary damage, as exemplified in the presented case. CC-induced lung injury encompasses a complex array of pathological changes, including pulmonary edema, interstitial fibrosis, bronchiolitis obliterans, and alveolar hemorrhage [9]. The inhalation of CC exposes the lungs to various toxic compounds, resulting in acute and chronic pulmonary injury. Several mechanisms contribute to that damage. Direct

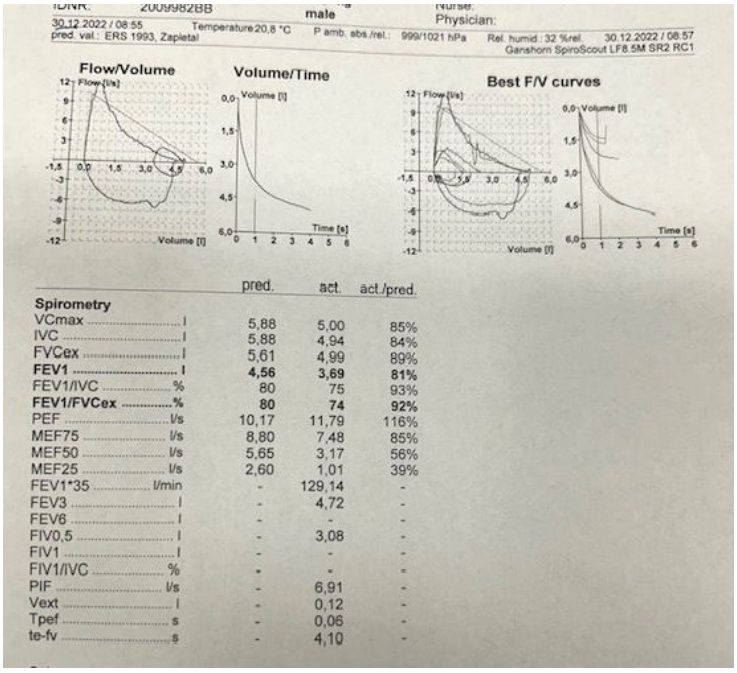


Fig. 4 – Forced spirometry after six months of discharge from hospital treatment – the maximal expiratory flow (MEF) was significantly reduced at 50% and 25% of the forced vital capacity – MEF 50 and MEF 25, respectively.

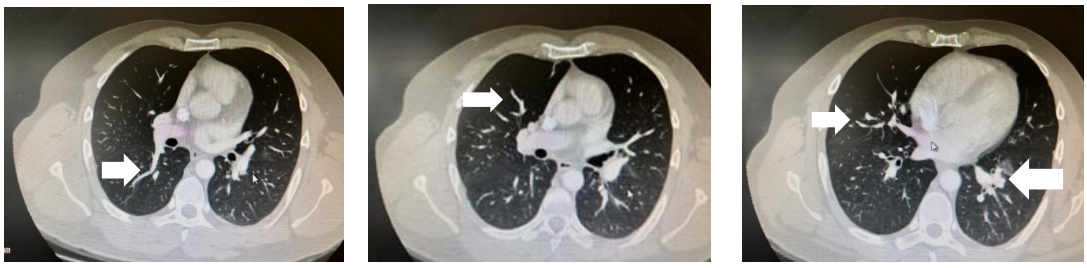


Fig. 5 – A follow-up multidetector computed tomography of the chest was performed after nine months of treatment and discontinuation of crack cocaine use – bronchial wall and interstitial thickening.

harmful effects are caused by a mixture of toxic substances, including particulate matter, volatile compounds, and pyrolysis byproducts [10]. These substances irritate the respiratory epithelium, leading to inflammation, bronchospasm, and tissue injury. The mentioned changes were present in the given patient. Vasoconstriction is also an effect of CC, achieved through its impacts on the sympathetic nervous system and endothelin release [3]. This can lead to pulmonary hypertension, increasing the workload on the right heart ventricle and predisposing to other cardiovascular complications. The metabolism of CC generates reactive oxygen species, leading to oxidative stress and cellular damage. Oxidative stress can impair pulmonary function, exacerbate inflammation, and contribute to the development of respiratory diseases. Chronic exposure to CC smoke triggers an inflammatory response in the lungs, characterized by the recruitment of inflammatory cells, the release of cytokines, and tissue remodeling [11]. That chronic inflammation can lead to airway remodeling, pulmonary fibrosis, and impaired lung function over time. CC smoke inhibits mucociliary clearance, impairing the lung's ability to remove inhaled particles and pathogens. This can increase the risk of respiratory infections and exacerbate existing lung diseases.

A multidisciplinary approach is essential in managing comprehensively CC-induced lung injury. Respiratory specialists, addiction counselors, social workers, and primary care providers collaborate to address the complex medical, psychological, and social aspects of the patient's condition. Pulmonary rehabilitation programs play a role in optimizing lung function, improving exercise tolerance, and enhancing the overall quality of life for individuals with chronic lung diseases, including those related to SA [12, 13].

The case underscores the importance of recognition and intervention in managing CC-induced pulmonary damage. Prompt diagnosis through thorough clinical evaluation, radiographic imaging, and pulmonary function testing enables healthcare providers to initiate appropriate treatment strategies tailored to the patient's needs.

Through early recognition and ongoing support, healthcare providers can diminish the adverse effects of CC abuse on the respiratory system and improve outcomes for affected individuals. However, addressing the broader societal factors contributing to SA remains essential in addressing the root causes of this public health issue.

Ongoing SA counseling, relapse prevention strategies, and social support services are essential components of comprehensive care to support the patient's recovery and prevent relapse [14].

Conclusion

Although the emphasis is on damage to the central nervous system, crack cocaine-induced pulmonary damage represents a significant clinical challenge with profound implications for patient health.

Conflicts of interest

The authors declare no conflict of interest.

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b) Full names of the authors signed as follows: ***, †, ‡, §, ||, ¶, **, ††, ...** .

c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

d) Conclusion could be a separate chapter or the last paragraph of the discussion;

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The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

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Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

References

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Examples of references:

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

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

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

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Primeri referenci:

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

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

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

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

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