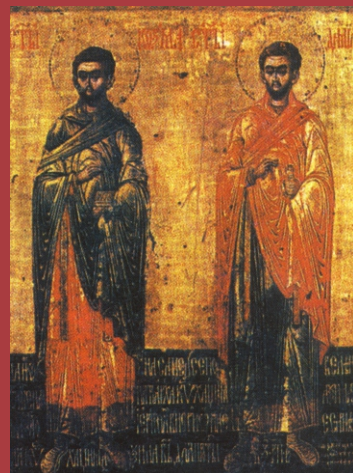
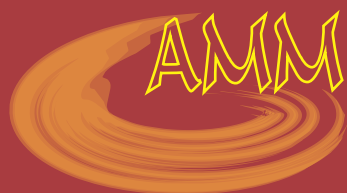


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ANALYSIS OF TRENDS IN THE PERFORMANCE OF URINARY DIVERSIONS AFTER TOTAL CYSTECTOMY: A 15-YEAR EXPERIENCE

Dragoslav Bašić^{1,2}, Ivan Ignjatović^{1,2}, Ljubinka Janković-Veličković^{1,3}, Aleksandra Ignjatović^{1,4}, Marija Mihajlović², Aleksandar Pejić², Bratislav Pejić², Aleksandar Skakić^{1,2}, Milan Potić^{1,2}, Tomislav Stamenić²

The aim of our study was to analyse trends in urinary diversion (UD) in patients who underwent radical cystectomy (RC) from 2008 to 2023, with special reference to the period of the COVID-19 pandemic. A total of 283 study patients (pts) (243 men and 40 women, mean age 65.07 ± 7.73 years) were divided by the type of UD into the following groups: Mainz-pouch II (MP II) – 85 pts, ileal conduit (IC) – 89 pts, ureterocutaneostomy (UCS) – 105 pts, percutaneous nephrostomy (PCN) – 4 pts. The relative share of incontinent and continent UD was 70% and 30%, respectively. MP II was the most represented UD from 2008 to 2013, IC from 2014 to 2019, and UCS from 2019 to 2023. The most common UD was UCS (37.1%), followed by IC (31.4%), MP II (30.0%) and PCN (1.4%). Temporal UD trends were variable, with relatively stable annual shares of MP II and IC and the lowest annual share of UCS until the period of the COVID-19 pandemic, when it significantly increased, with a peak in 2022. Trend analysis shows a significant increase of UCSs in the period 2008–2023 ($p < 0.001$), without significant change of trends in the other groups ($p > 0.05$). The trends of continent and incontinent UD after RC, shown in our study, are consistent with the data reported in the current literature.

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Key words: urinary diversions, trends, Mainz-pouch II, ileal conduit, ureterocutaneostomy

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Introduction

Recent epidemiological data show that bladder cancer (BC) is the 2nd most common genitourinary malignancy and 10th among all cancers in the general population, with the incidence rates of 9.5 in men and 2.4 in women (per 100,000), mortality rates of 3.3 in men and 0.9 in women, and a total of 573,278 new cases in

2020 worldwide. Overall incidence rates range from 3.1 in low/medium human development index (HDI) countries to 11.7 per 100,000 in high/very high HDI countries (1). Reported age-standardized incidence rates are 20 and 4.6 for the male and female populations in Europe (2). About 70–75% of BCs are detected at $\leq T1$ stages, as non-muscle-invasive bladder cancer (NMIBC), which are amenable to treatment and associated with longer survival, while the remaining 25–30% are detected at later, $\geq T2$ stages, as muscle-invasive bladder cancer (MIBC) (2, 3). Among all histopathological types of BC, the largest share belongs to urothelial carcinoma (UC) ($> 90\%$), while the total share of other types, including squamous cell carcinoma, adenocarcinoma and neuroendocrine carcinoma of the urinary bladder is less than 10% (4, 5). The primary treatment of choice for MIBC includes radical cystectomy (RC) with pelvic lymphadenectomy and urinary diversion (UD) (6, 7). So far, numerous UD techniques have been described, from simple ureterocutaneostomy (UCS) to orthotopic neobladder (ONB). There is still debate as to which UD is best in terms of feasibility, long-term outcomes, and patient quality of life. However, the more complex the

operative technique for creating UD, the higher the criteria for patient selection.

Aim

The aim of the present study was to analyse trends in UD in patients who underwent RC and UD in our clinic over a period of 15 years, with special reference to the period of the COVID-19 pandemic.

Materials and Methods

For the present retrospective study, we identified a total of 283 consecutive patients (243 men and 40 women, mean age 65.07 ± 7.73 years) who underwent RC and UD during a 15-year period, from 2008 to 2023. Based on the patients' medical records, the following data were analysed: patient demographics, indication for RC, the date of the operation, type of UD and histopathological report of the RC specimen (type of cancer, pathological grade and stage). In order to analyse temporal variations in UD trends, patients were examined both in relation to the type of UD and the time period, with special reference to the period of the COVID-19 pandemic. This study was conducted according to the Declaration of Helsinki ethical principles, with guaranteed discretion of personal data, and had been approved by the Ethics Committee of the University Clinical Center Niš, protocol number 28305.

According to the type of UD, patients were divided into the following groups:

1. Group MP II consisting of 85 patients with sigma-rectum pouch (Mainz-pouch II) UD
2. Group IC consisting of 89 patients with ileal conduit UD
3. Group UCS consisting of 105 patients with ureterocutaneostomy UD
4. Group PCN consisting of 4 patients with percutaneous nephrostomy UD.

Statistical data are presented in the form of arithmetic mean, standard deviation, minimum and maximum values. The comparison of categorical features was performed using the Chi-square test. For the trend analysis of the number of UD in the monitoring period, joinpoint regression analysis was used. The analysis was done in the program package Joinpoint regression Analysis Joinpoint Regression Program, Version 4.8.0.1. April 2020; Statistical Research and Applications Branch, National Cancer Institute). Other statistical analyses were performed in the OpenEPI Info software package.

Results

Over the study period, a total of 283 patients underwent RC with UD. Of these, incontinent diversion (ID) was performed in 198 patients (70%), and continent diversion (CD) in

85 patients (30%). The annual number of cystectomies in the study period shows significant variations, ranging from 4 to 46 per year, without the existence of a linear trend of increase or decrease (Table 1). The lowest annual number of operations was performed in 2017 ($n = 4$) and the highest in 2022 ($n = 46$). Figure 1 shows the time trends and relative share of CD and ID after cystectomy.

In 2008, 2009, 2017 and 2023, the annual number of operations was low (< 10 per year), while 2010, 2011, 2013, 2014 and 2015 had been characterized by a slightly higher number of operations (10–20 per year). During 2012 and 2018–2022, there were more than 20 operations per year, with the largest annual number in 2022 ($n = 46$). The annual number of operations is shown in Table 1.

Table 2 shows the demographic and clinical characteristics of the studied population. The average age of the entire examined population was 65.07 ± 7.73 years (37 years, 82 years). In relation to gender distribution, 83.5% of patients were male and 16.5% female. The most common UD was UCS (37.1%), followed by IC (31.5%), MP II (30.0%) and PCN (1.4%). In terms of histological grade (HG), the most common was HG 3 (75.6%), followed by HG 2 (21.8%), HG 4 (1.8%) and HG 1 (0.8%) (Table 2). The highest relative share of pT4 stage was in the UCS group (35.2%), followed by MP II group (10.6%), IC group (13.5%) and PCN group (25%). There is no statistically significant difference among the groups in terms of gender ($p = 0.452$), age ($p = 0.525$) and HG ($p = 0.504$). In all groups, the most represented age category was 60–69 years (Table 2).

According to the histopathological report of the RC specimen, the most common indication for RC was UC of the bladder, in 91.5%. Other indications were less prevalent, as follows: UC with squamous differentiation (3.6%), pure neuroendocrine carcinoma (1.1%), colonic adenocarcinoma (1.1%), undifferentiated UC (0.7%), urothelial adenocarcinoma (0.4%), UC of prostatic urethra (0.4%), prostate adenocarcinoma (0.4%), vesico-intestinal fistula (0.4%) and nonresectable inflammatory conditions of the bladder (0.4%).

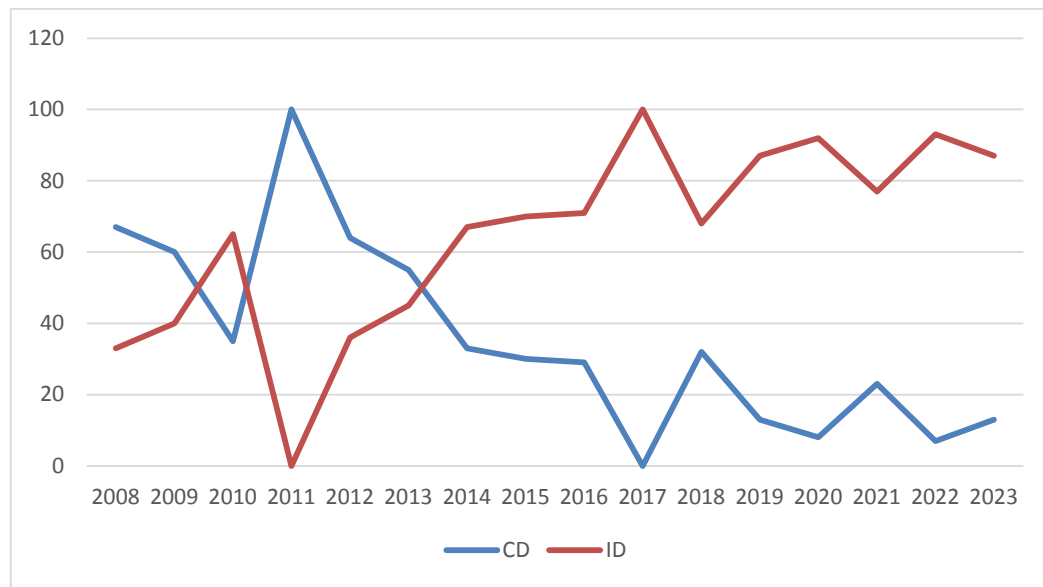


Figure 1. Time trends of CD and ID

Table 1. Number of cystectomies with UD by type

Year	Continent diversion (CD)		Incontinent diversion (ID)					CD + ID	
	MP II		IC	UCS	PCN	ID	TOTAL	TOTAL	
	No	%	No	No	No	No	%	No	%
2008	6	67	1	-	2	3	33	9	100
2009	3	60	1	-	1	2	40	5	100
2010	5	35	9	-	-	9	65	14	100
2011	11	100	-	-	-	-	-	11	100
2012	16	64	8	-	1	9	36	25	100
2013	6	55	5	-	-	5	45	11	100
2014	5	33	7	3	-	10	67	15	100
2015	3	30	5	2	-	7	70	10	100
2016	6	29	12	3	-	15	71	21	100
2017	-	0	3	1	-	4	100	4	100
2018	8	32	9	8	-	17	68	25	100
2019	3	13	10	10	-	20	87	23	100
2020	2	8	4	19	-	23	92	25	100
2021	7	23	9	15	-	24	77	31	100
2022	3	7	6	37	-	-	-	46	100
2023	1	13	-	7	-	-	-	8	100
TOTAL	85	100	89	105	4	198	100	283	100

Table 2. Demographic and clinical characteristics of the studied population

		MP II		IC		PCN		UCS		p
Characteristic		No	%	No	%	No	%	No	%	
Gender	Male	73		73	82.0	3	75.0	94	89.5	0.452
	Female	12	14.1	16	18.0	1	25.0	11	10.5	
	Total	85	100	89	100	4	100	105	100	
Age	< 50	3	3.5	2	2.2	1	25.0	5	4.8	0.525
	50–59	23	27.1	21	23.6	0	0.0	18	17.1	
	60–69	45	52.9	45	50.6	2	50.0	54	51.4	
	70–79	13	15.3	20	22.5	1	25.0	26	24.8	
	80 +	1	1.2	1	1.1	0	0.0	2	1.9	
Histological grade (HG) *	I	1	1.2	1	1.2	0	0.0	0	0.0	0.504
	II	22	27.2	17	20.2	0	0.0	18	19.1	
	III	58	71.6	65	77.4	3	100.0	72	76.6	
	IV	0	0.0	1	1.2	0	0.0	4	4.3	
Pathological tumor stage (pT) **	NEM	0	0.0	1	1.1	0	0.0	0.0	0.0	
	pTa	5	5.9	3	3.4	0	0.0	6	5.7	
	pT1	6	7.41	8	9.0	0	0.0	4	3.8	
	pT2	25	29.4	24	27.0	1	25.0	20	19.0	
	pT3	37	43.5	41	46.1	2	50.0	34	32.4	
	pT4	9	10.6	12	13.5	1	25.0	37	35.2	

*Available for 275 patients; **available for 262 patients.

The annual number of UD across all studied groups is shown in Figure 2. During the period 2008–2013, not a single UCS was performed. UCS has been implemented since 2014 (3 cases), and after that, only a few cases were done per year, ending in 2017. From 2018, an increasing trend in the annual number of UCS was observed, peaking in 2022, with as many as 37 cases. There was no statistically significant trend of change in the number of cases in other studied groups ($p > 0.05$).

Figure 3 shows the annual number of UCS from 2008 to 2023. Trend analysis shows that there was a statistically significant trend of increasing the number of UCS in the period 2008–2023, with an annual percentage change (36.0, 95% CI 24.4–48.7, $p < 0.001$).

Figure 4 shows the variability of the number of UCS UD performed over 4 time intervals during the entire period: 2008–2010, 2011–2014, 2015–2018, and 2019–2023. The frequency of UCS changed statistically significantly during the study period ($p < 0.001$). In relation to the total number of UCS ($n = 105$), the relative share of the time interval 2019–2023 was 66.2%, while in other time intervals it was less: 23.3% in 2015–2018, 4.8% in 2011–2014, and 0% in 2008–2010.

There were a total of 59 patients (20.8%) with pT4 stage. The relative share of pT4 stage was the highest in the UCS group (35.2%), and this difference compared to the other groups is statistically significant (MP II-10.6%, IC-13.5%, PCN-25%) ($p < 0.001$) (Figure 5).

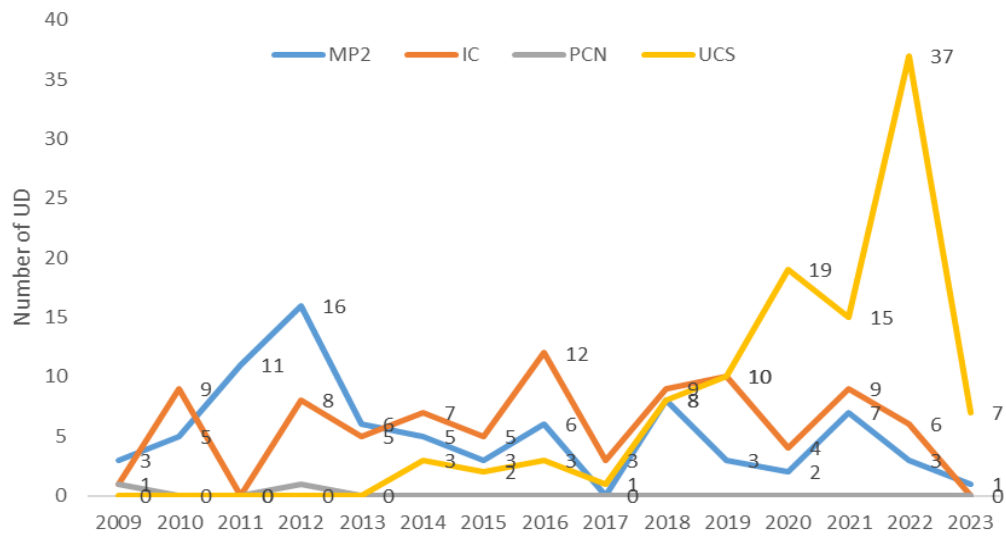


Figure 2. Number of urinary diversion by type

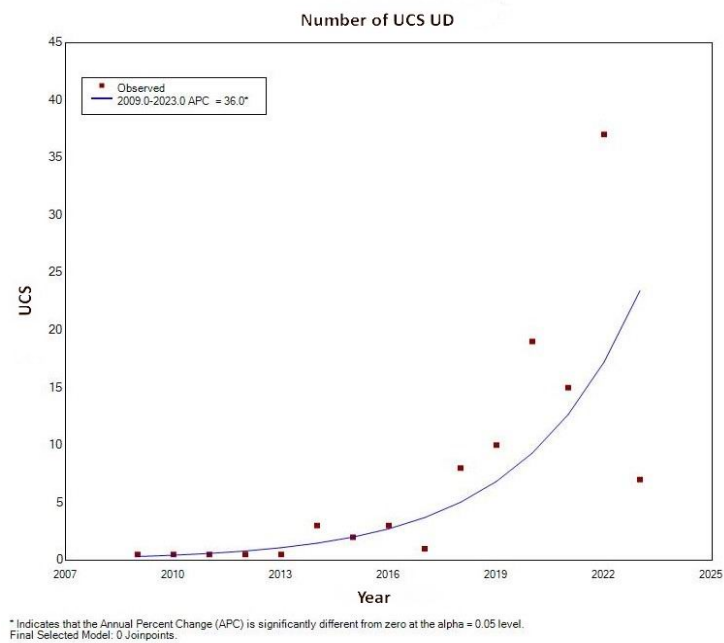


Figure 3. Number of UCS UD in the study period

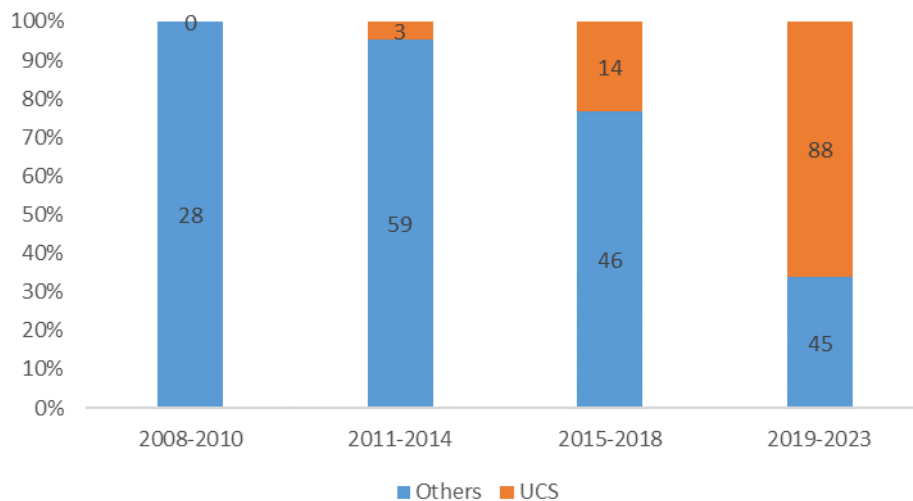


Figure 4. Proportion of UCS UD in the study period

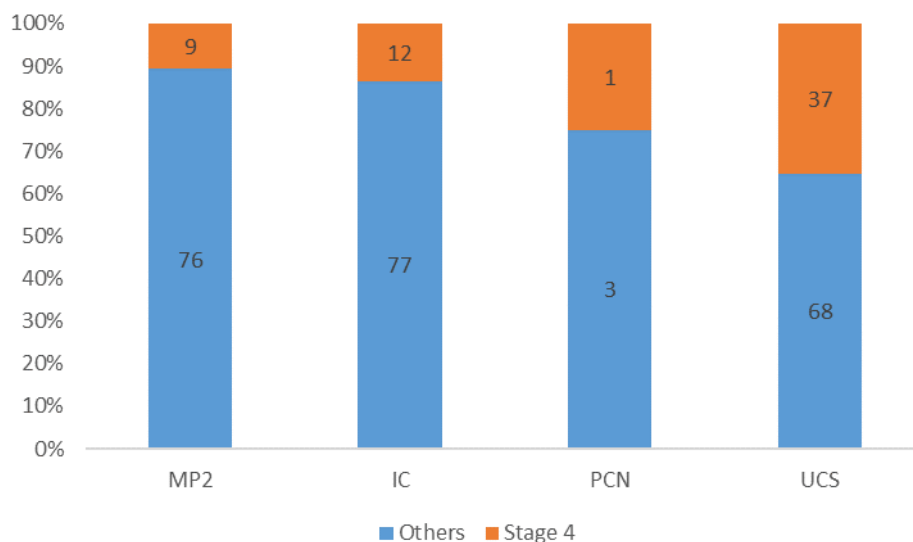


Figure 5. Proportion of T4 stage in the study period

Discussion

A recent report by Farber et al. on the disparity in the use of CD after RC for BC, in a sample of 76,040 patients operated in the United States of America (USA) from 2001 to 2012, showed IC and CD rates of 90.8% and 9.2%, respectively (8). The relative share of CDs had an increasing trend, with a peak in 2008 (almost 12%), with a significant decreasing trend thereafter. A higher proportion of CDs was associated with urban areas, high-volume academic hospitals and patients from the western regions of the country. Similarly, the California group published an analysis of 10-year trends (2004–2013) in the USA on the use of ID and CD after RC in 27,170 patients (6). It was shown that the relative share of ID and CD was 85.5% and 14.5%, respectively. However, the relative share

of CD showed a statistically significant decrease, from 17.2% during the first three years to 12.1% during the last 3 years (6). Comparing early operative results of IC versus CD after RC for BC in the USA, Rezaee et al. reported that of 4,755 RCs for BC in 2019 and 2020, 677 patients (14.2%) received CD (9).

In our series, the proportion of ID and CD was 70% and 30%, respectively. Temporal variations were most pronounced in 2011 (ID-0%; CD-100%) and 2017 (ID-100%; CD-0%), while in other periods the difference was more moderate. The increase in the share of ID compared to CD has been recorded since 2013/2014, with a relatively stable ratio until 2023 (Table 1, Figure 2). As in the presented series, the proportion of female patients in our series is significantly lower compared to men (6, 8). When it comes to pathohistological indications for RC, UC was

present in 91.5% of our patients. The relative share of other UC morphological subtypes and benign indications was 7.7% and 0.8%, respectively.

According to the 2022 WHO classification, as well as the data presented by Amin et al. and Comp  rat et al., urothelial carcinoma is the most common morphological subtype of UC occurring in > 90% of patients with MIBC, which is consistent with our results (4, 5, 10). Benign diseases as indications for cystectomy are very rare, including neurogenic bladder, post-irradiation conditions, interstitial cystitis and urinary incontinence (11–16).

Our results show evident temporal variability of UD during certain time periods in our series. Thus, MP II was the most represented UD from 2008 to 2013, IC from 2014 to 2019, and UCS from 2019 to 2023.

Mainz-pouch II is a modification of the ureterosigmoidostomy (USS) and was originally presented by Fisch in 1993 (17). It has fewer contraindications compared to ONB and is acceptable for a large number of candidates, including those with advanced stages of BC. The operative technique is relatively simple compared to other CDs and has been promoted as their alternative, with low complication rates and good health related quality of life (HRQoL) (18–20). It has been successfully applied in our clinic since 1995, to overcome the drawbacks of UCS, USS and ONB, including insufficient support for the needs of stoma devices, postoperative care and nutrition, as well as the management of surgical and metabolic complications. Several series of our results have been published, confirming that the technique is associated with low complication rates and good HRQoL, with certain disadvantages such as malignant transformation of the ureterointestinal anastomosis and metabolic acidosis (18–21).

Since its introduction by Bricker in 1950, IC has become widely accepted due to the absence of metabolic complications and has since evolved into the gold standard for UD (22). Farber et al. reported that from 2001 to 2012 in the USA, IC was the UD of choice after RC in nearly 90% of cases (8). There are several reasons for this disparity in favour of IC, including the technical simplicity of the procedure, shorter operative time, better training of urologists for this type of procedure, fear of possible complications, the increasing use of robotic surgery and the poor financial benefit of CD (8, 23). In a five-year series of the total number of cystectomies with UD from 2001 to 2005 in the USA, Gore et al. reported that out of 27,494 patients, 83.5% underwent IC and 16.5% underwent CD (24). Analysing five-year trends in UD at a high-volume tertiary urology clinic in the USA, Lowrance et al. stated that IC was the most frequent UD, with a relative participation of 63%, with an increasing time trend, while ONB was performed in 37% of cases. The authors also point out that there is a trend of IC, especially associated with female gender, advanced age,

locally advanced disease and significant comorbidity factors (25). Furthermore, the recommendations of the American Urological Association (AUA) Guidelines include mandatory preoperative counselling with the patient regarding the choice of IC or CD as UD (26). In our series, IC was more prevalent compared to MP II in the age group 70–79 years (15.3% vs. 22.5%), as well as in pT3/T4 stages (60% vs. 56%). A meta-analysis of 18 large studies on the overall HRQoL of patients after IC or CD did not find significant differences (27).

The simple technique of UCS dates back to the 19th century, when Hayes Agnew from Philadelphia performed a ureterocutaneous stoma on the abdominal wall due to an injury to the ureter (2, 20). In a recent systematic review comparing IC and UCS, Korkes et al. concluded that UCS is more feasible than IC, especially in elderly and fragile patients, not only because of the avoidance of bowel surgery, but also due to shorter operative time, perioperative care and hospitalization, as well as lower rates of bleeding, transfusion and complications (28). Deliveliotis et al. emphasize that UCS is particularly indicated in high-risk elderly patients, as well as in patients with a solitary kidney, with certain disadvantages including higher rates of ureterocutaneous stenosis and ascending UTIs, compared to techniques with ureteral diversion through intestinal cutaneostomy (29). The occurrence of stomal stenosis can be successfully prevented by postoperative ureteral double-J stenting, and Kozacio  lu reported positive experiences in this regard (30).

Comparing the complications of three UD, including IC, UCS, and colon conduit (CC), a study by Picha et al. showed that major diversion-related complications were significantly more frequent in IC and CC and absent in UCS, while late reinterventions had occurred more frequently in IC (20%) compared to CC (5.8%) and UCS (2.4%), highlighting this as an important advantage of UCS, especially with regard to HRQoL (31). A recent meta-analysis by Nabil et al. on long-term outcomes between IC and UCS, including 10 studies with 3,689 patients, showed advantages of UCS over IC in terms of length of stay, with similar results in terms of major complications, HRQoL and survival rates (32).

As already mentioned, there were 17 UCS from 2008 to 2018, and 88 from 2019 to 2023 in our series. A statistically significant increase in the number of UCS was observed throughout all analysed time periods, with progressive peaks recorded during 2019, 2020 and 2022, making the relative share of UCS in this period as much as 66.2% of the total number of UCS in the entire series. In addition, the relative share of pT4 stage in the UCS group was 62.7% (37 out of 59 patients), significantly higher compared to the other groups. It is evident that the increase in the number of UCS coincides with the beginning of the COVID-19 pandemic and the corresponding restructuring of health capacities, in accordance with certain measures in the fight against the

pandemic. These measures included a whole series of systemic solutions, related to patient admission protocols, repurposing of spatial capacities, redirection of human resources, preservation of equipment, prevention of iatrogenic infection, while giving certain priorities to elective oncological operations in circumstances of limited resources (33). In an attempt to precisely define the criteria for establishing priorities in the triage of uro-oncological elective operations, several clinical guidelines have been published containing the same provisions regarding urgent indications, including radical cystectomy for MIBC, radical or partial nephrectomy for renal cell carcinoma (RCC) in T3 tumours and nephroureterectomy for high-grade and/or clinical T1+ tumours (34, 35).

Conclusion

In our study, a total of 283 RC with UD performed from 2008 to 2023 were analysed. Of these, ID and CD were performed in 198 (70%) and 85 (30%) patients, respectively. The annual number of operations ranged from 4 to 46, depending on the number of patients. The most represented UD was UCS, followed by IC, MP II and PCN. The highest relative share of pT4 stage was in the UCS group, followed by the MP II group, the IC group and the PCN group. The most common indication for cystectomy was urothelial carcinoma of the bladder. The annual share of MP II and IC was relatively stable, with slight variations. The annual share of UCS shows the lowest values until the period of the COVID-19 pandemic, when it increases significantly, with a peak in 2022. Due to its simplicity and feasibility, UCS has proven to be a suitable UD during the COVID-19 pandemic. The trends regarding ID and CD after RC, shown in our study, are consistent with the trends reported in the current literature.

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ANALIZA TRENDOVA U IZVOĐENJU URINARNIH DERIVACIJA NAKON TOTALNE CISTEKTOMIJE: PETNAESTOGODIŠNJE ISKUSTVO

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Cilj našeg istraživanja bila je analiza trendova urinarnih derivacija (UD) kod bolesnika koji su bili podvrgnuti radikalnoj cistektomiji (RC) od 2008. do 2023. godine, s posebnim osvrtom na vreme pandemije kovida 19. Ukupno 283 bolesnika (243 muškarca i 40 žena, prosečne starosti 65,07 godina $\pm$ 7,73 godine) klasifikovana su prema tipu UD-a u sledeće grupe: *Mainz pouch* II (MP II) – 85 bolesnika; ileumski provodnik (engl. *ileal conduit* – IC) – 89 bolesnika; ureterokutaneostomija (engl. *ureterocutaneostomy* – UCS) – 105 bolesnika; perkutana nefrostomija (engl. *percutaneous nephrostomy* – PCN) – 4 bolesnika. Relativni udeo inkontinentnih i kontinentnih UD-a iznosio je 70% i 30%, redom. MP II je bio najzastupljeniji UD od 2008. do 2013. godine, IC od 2014. do 2019. godine, a UCS od 2019. do 2023. godine. Najzastupljeniji UD bio je UCS (37,1%), a onda slede IC (31,4%), MP2 (30,0%) i PCN (1,4%). Trendovi UD-a menjali su se kroz vreme, s tim što su MP II i IC imali relativno stabilan udeo na godišnjem nivou, a UCS najmanji udeo do izbijanja pandemije kovida 19; tada je značajno povećan udeo UCS-a, a vrhunac je dostigao 2022. godine. Analiza trenda pokazuje značajno povećanje UCS-a između 2008. i 2023. godine ($p < 0,001$), dok značajnije promene trendova u preostalim grupama nisu zabeležene ($p > 0,05$). Trendovi kontinentnih i inkontinentnih UD-a nakon RC-a predstavljeni u ovoj studiji odgovaraju podacima iz savremene literature.

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Ključne reči: urinarne derivacije, trendovi, *Mainz pouch* II, ileumski konduit, ureterokutaneostomija

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ECHOCARDIOGRAPHIC ESTIMATES OF LEFT VENTRICULAR REMODELING IN MITRAL REGURGITATION ACCORDING TO THE TYPE OF SURGICAL CORRECTION: MITRAL VALVE REPAIR OR REPLACEMENT

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The pathophysiology of chronic primary mitral regurgitation (MR) is primarily based on a degenerative process. Inadequate adaptation of the left ventricle (LV) due to volume loading leads to gradual dilatation and weakness. The only complete therapeutic option in this case is surgical intervention. This study was aimed at evaluating early echocardiographic parameters of LV remodeling in chronic primary MR between two types of operative correction, a mechanical mitral valve replacement (MVR) and mitral valve prosthetic ring annuloplasty (MVA). Using 2D and M-mode echocardiography, a number of variables were measured or calculated. In addition, the variables are assessed according to early postoperative LV dysfunction (LVD). Ejection fraction (EF) was $< 50\%$. Thirty-six asymptomatic patients with primary severe MR (grade 3–4) undergoing surgical correction were included. The LV end-diastolic diameter improved significantly (6.11 ± 0.9 vs. 5.50 ± 0.7 cm) in both groups ($p < 0.006$) after interventions, while there were no significant differences in LV volumes between the groups. Immediate postoperative LV systolic dysfunction showed similar incidence in the groups (43%). A significant distinction between the groups was revealed in patients without LVD, that is, a higher preoperative forward LVEF in MVA compared to MVR patients. Interestingly, the opposite direction of forward LVEF change was seen in LVD between the groups. We can conclude that there are subtle differences in early postoperative echocardiographic parameters between the MVA and MVR procedures, reflecting subtle specificities of early LV remodeling in patients with chronic primary MR.

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Key words: annuloplasty, end-diastolic diameter, ejection fraction, left ventricular dysfunction, end-systolic volume

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Introduction

Mitral regurgitation (MR) is the second most common valvular lesion, with its prevalence rising with age. Considering an increase in population ageing, MR prevalence is likely to increase further (1–4). Mitral regurgitation develops due to a failure of the mitral leaflets during left ventricular

(LV) contraction. It can be classified as primary, related to valve abnormalities, or secondary, due to distortion of LV shape. According to the disease course, MR can present as an acute or chronic disorder. The etiology of chronic secondary MR is either ischemic or non-ischaemic, resulting in annular dilatation and the papillary muscles' displacement, causing mitral valve failure (3–5).

Primary MR is considered a degenerative disease in about two-thirds of all cases, without defined causative factors. Nevertheless, long-term exposure to elevated blood pressure is observed to predispose to a substantial share of these patients. Specifically, each 20 mmHg increment in systolic blood pressure was associated with a 26% higher risk of MR. Similar associations are reported for each 10 mmHg increase in diastolic blood pressure. The effects were mediated directly and secondarily to high BP complications (4).

During the insidious development of chronic primary MR, there is progressive LV dysfunction and a reactive remodelling in an attempt to provide an adequate forward stroke volume (SV).

Initial hemodynamic changes, such as an increase in left atrial (LA) volume, a reduction in forward SV, and a rise in LV preload, further respond by eccentric hypertrophy. The compensation attempt implies the increase in wall thickness and increased LV filling, which normalizes forward SV. However, this is found insufficient to fully compensate for the wall stress (3).

The inadequate hypertrophy is supposed to occur partly because volume overload in MR does not trigger substantial increases in the rate of protein synthesis. Instead, the early wall thickening is due to a decrease in the rate of protein degradation (6). Other mechanisms include altered cytoskeletal changes, an increase in reactive oxygen species, a change in matrix metalloproteinase expression and activation, initiation of inflammatory and apoptotic pathways, and others. Ensuing disruption of the myocardial extracellular matrix architecture results in sliding displacement of cardiomyocytes and cell death. Adversely remodeled and dilated LV progresses to the decompensated MR with a thin myocardial wall and an increase in wall stress (3, 7, 8).

Therefore, surgical repair or mitral valve replacement (MVR) is currently the recommended therapy for severe primary MR, in patients who are surgical candidates. Without proper treatment, severe mitral valve regurgitation can cause abnormal cardiac rhythm and inevitably lead to heart failure (3, 4).

Aim

This study was aimed at evaluating the first week's echocardiographic parameters of LV remodeling between two types of operative correction in patients with chronic primary MR: a mechanical mitral valve replacement (MVR) and mitral valve prosthetic ring annuloplasty (MVA).

Materials and Methods

We conducted a prospective study that included 36 consecutive patients diagnosed as having grade 3–4 MR according to the 2020 American College of Cardiology/American Heart Association (ACC/AHA) guideline for the management of patients with valvular heart disease and using appropriate validated techniques (1, 9, 10). The patients were recruited at the Clinic for Cardiac Surgery, University Clinical Center of Serbia (University of Belgrade, Serbia), between 2019–2021.

Inclusion criteria comprised asymptomatic patients with primary severe MR, of non-ischemic and non-rheumatic causes, with a routine transthoracic echocardiography performed with quantification of MR. Excluded patients were those with ischemic cardiomyopathy, acute cardiac events, those who underwent bypass surgery, a percutaneous angioplasty, or had an aortic valve disease, mitral stenosis or other heart diseases.

Criteria for quantitative gradation of severe primary MR (grade 4), based on 2D echocardiography, included: effective regurgitant orifice area (EROA) (2D proximal isovelocity surface area (PISA), mm^2) of $\geq 40 \text{ mm}^2$, regurgitant volume (mL/beat) of $\geq 60 \text{ mL}$, and regurgitant fraction (%) $\geq 50\%$ (1, 9).

Patient-centred and integrative approach to evaluation of a specific lesion causative of MR was applied in accordance with current guidelines. The approach provides prognostic implications and is of vital importance for assessing possibilities of surgical or transcatheter valve repair (1, 10).

Patients were divided into two age-matched groups of those who underwent a MVR with a mechanical prosthesis and those with restrictive prosthetic ring annuloplasty (downsizing of 2.7 ± 0.8 ring sizes). As per guidelines, MVR is commonly performed in patients with severe MV disease unsuitable for surgical repair (11).

All patients were routinely assessed by transthoracic echocardiography before and three to four days after the surgical procedure (early follow-up). The obtained echocardiographic parameters were compared between these two periods and between the groups (MVA vs. MVR).

Using 2D and M-mode echocardiography, a number of variables were measured or calculated (through the Teicholz method), including dimensions (end-diastolic (EDD), end-systolic (ESD)) and volumes of the LV (EDV, ESV, SV), thickness of the myocardial wall and septum in diastole. Ejection fraction (EF%) and shortening fraction (FS%) were also evaluated, as well as derived measures of forward LVEF ($100 \times \text{forward stroke volume/LV EDV}$), right ventricle systolic pressure (RVSP) and left ventricle wall stress (WS).

We further assessed differences in echocardiographic parameters in relation to an early postoperative LV dysfunction, which is defined as LV EF $< 50\%$ after mitral valve surgery.

Statistical Analysis

Results are expressed as mean $\pm$ standard deviation (SD) or median $\pm$ interquartile range (IQR), or percentages, as required. The Wilcoxon signed-rank test, Mann–Whitney U test or independent/paired t-tests were used, based on the normality distribution of data (assessed by the Shapiro–Wilk test). The Pearson correlation coefficient test was also used. A significance threshold was set at $p < 0.05$. Statistical analysis was carried out using SPSS 25.0 software (SPSS Inc., Chicago, IL, USA).

Results

Out of 36 patients included in the study, 21 (58.3%) underwent MVA, while 15 (41.7%) had MVR intervention. There were 28 (77.8%) males and 8 (22.2%) females, median years of age 66.5 ± 12 . Demographic data and risk factors were

comparable between the groups. Baseline characteristics of the patients are shown in Table 1. There was no significant difference in the type of procedure according to gender or body mass index (BMI). All patients recovered in the postoperative period. Mitral regurgitation grades at discharge were 1.18 ± 0.34 (mean $\pm$ SD).

Early Echocardiographic Parameters of LV Remodeling

There was a significant difference in EF% before and after the MR surgery (57.53 ± 12.32 vs. 49.78 ± 10.46 , $p = 0.000$; $r = 0.537$), as well as in FS% (36.27 ± 7.23 vs. 30.73 ± 4.71 , $p = 0.045$; $r = 0.668$), but with no significant difference between the type of surgical correction.

Forward LVEF values were significantly higher after the procedure (32.67 ± 10.97 vs. 43.49 ± 16.77 , $p = 0.005$), but again, similar according to the type of procedure. The postoperative forward LVEF positively correlated with RVSP ($r = 0.541$, $p = 0.025$).

End-diastolic diameter of the LV improved significantly (6.11 ± 0.9 vs. 5.50 ± 0.7) in both groups (MVA $p = 0.000$, MVR $p = 0.006$) ($r = 0.654$, $p = 0.000$) after interventions, while ESD measurements remained mostly unaffected (3.85 ± 1.0 vs. 3.95 ± 0.9 , $p > 0.05$) ($r = 0.754$, $p = 0.000$). There were no differences in the LV dimensions between the groups in this early period. Similarly, left atrial (LA) diameters decreased postoperatively in both groups (5.30 ± 1.33 vs. 4.60 ± 0.88 , $p = 0.02$).

We determined a significant difference in the median between EDV, before and after the MR correction (190.46 ± 59.12 vs. 147.4 ± 40.14 ml, $p = 0.000$) ($r = 0.701$, $p = 0.000$), but not for end-systolic volumes (67.96 ± 45.58 vs. 67.95 ± 34.21 ml, $p > 0.05$) ($r = 0.805$, $p = 0.000$).

Similar findings were obtained for the mean level of SV before and after the surgery (115.95 ± 34.92 vs. 77.62 ± 24.53 ml, $p = 0.000$) in both groups ($p < 0.003$), but without correlation. There were no distinct differences in EDV, ESV or SV according to the type of MR surgery.

Right ventricular systolic pressure was elevated (≥ 30 mmHg) before (48.9 ± 15.0 vs. 49.6 ± 13.5 mmHg) and after (37.7 ± 8.8 vs. 42.3 ± 9.6 mmHg) the interventions in MVA and MVR groups, respectively, but with no significant difference between the two periods or between the groups. Also, the LV WS remained almost the same in the two periods (116.54 ± 0.65 vs. 116.16 ± 14.92 kdynes/cm², $p > 0.05$).

After the treatment, the mitral velocity propagation (Vp) recordings were different between the groups, with higher velocity in MVR (1.400 ± 0.345 vs. 1.681 ± 0.259 m/s, $p = 0.015$), which was related to characteristics of mechanical valves.

A Comparison According to Postoperative LV Systolic Dysfunction (LVD)

Out of 36 patients, 8 (22.2%) had EF% lower than 50% prior to the mitral correction and therefore were excluded from analysis of postoperative LVD. Twelve out of 28 patients (42.9%) with adequate preoperative EF developed early LVD, half of them had underwent MVA (6/15) and another half had MVR procedure (6/7) ($p > 0.05$). There was no significant difference in the number of subjects that developed LVD depending on the type of MR correction.

In total, patients with higher preoperative EDD showed a tendency to develop LVD (6.39 ± 0.77 vs. 5.95 ± 0.40 , $p = 0.06$), but with no group difference. Postoperative EDD was also higher in those with LDV (5.45 ± 0.56 vs. 5.38 ± 0.39 , $p > 0.05$).

A significantly higher preoperative EDD was determined in MVA patients who progressed to LVD (6.50 ± 0.27 vs. 6.01 ± 0.46 , $p = 0.038$), as well as in the measurements of postoperative ESD (4.26 ± 0.43 vs. 3.73 ± 0.38 , $p = 0.035$). In the MVR group, there was a tendency for higher preoperative ESD values and higher postoperative EDV in those who developed LVD (Table 2).

In both groups, a significantly lower postoperative FS% were determined in patients with LVD compared to those with stable LV function, that is, in MVA 23.69 ± 6.76 vs. $31.79 \pm 4.13\%$, $p = 0.016$, and in MVR 25.22 ± 6.23 vs. $33.11 \pm 3.24\%$, $p = 0.044$, respectively.

In total, preoperative forward LVEF was lower in LVD patients (31.45 ± 13.06 vs. 37.41 ± 8.34 , $p > 0.05$). There was a significant difference in preoperative forward LVEF measures according to the group in those who did not develop LVD. Specifically, MVA patients with normal postoperative LV function had higher preoperative forward LVEF compared to MVR patients (38.63 ± 8.73 vs. 29.50 ± 4.14 , $p = 0.045$). Interestingly, we documented the opposite direction of forward LVEF change related to LVD between the groups. Mean values of forward LVEF were lower in MVA patients who developed LVD compared to those who did not (28.5% vs. 38.63%), while the values were higher in MVR patients with LVD versus those with stable function (35.00% vs. 29.5%).

Table 1. Baseline characteristics of the patients (total)

Gender (male), n (%)	28 (77.8%)
BMI (kg/m ²)	26.72 ± 3.18
MR duration (months)	12 ± 11
TA systolic (mmHg)	131.28 ± 15.895
TA diastolic (mmHg)	80.0 ± 18.0
Hypertension arterial, n	26 (72.2%)
Atrial fibrillation, n	21 (58.3%)
Type 2 diabetes mellitus, n	7 (19.4%)
Cigarette smoking, n	11 (30.6%)
BMI – body mass index, MR – mitral regurgitation, TA – tension arterialis	

Table 2. Echocardiographic parameters according to the early postoperative left ventricular dysfunction (LVD) and the type of operative procedure

		LVD	Non-LVD	P value
EDD preop (cm)	1	6.50 ± 0.27	6.01 ± 0.46	p = 0.038
	2	6.28 ± 1.10	5.87 ± 0.32	ns
EDD post (cm)	1	5.63 ± 0.26	5.47 ± 0.35	ns
	2	5.22 ± 0.78	5.27 ± 0.44	ns
ESD preop (cm)	1	4.23 ± 0.39	3.87 ± 0.47	ns
	2	4.13 ± 0.68	3.51 ± 0.48	p = 0.080
ESD post (cm)	1	4.26 ± 0.43	3.73 ± 0.38	p = 0.035
	2	3.90 ± 0.62	3.53 ± 0.37	ns
EDV preop (ml)	1	216.28 ± 77.85	185.11 ± 31.40	ns
	2	207.40 ± 34.59	176.36 ± 31.39	ns
EDV post (ml)	1	174.11 ± 90.12	148.74 ± 20.62	ns
	2	164.03 ± 23.59	134.72 ± 24.9	p = 0.053
ESV preop (ml)	1	100.54 ± 78.28	64.72 ± 21.84	ns
	2	87.15 ± 31.61	66.38 ± 31.61	ns
ESV post (ml)	1	79.29 ± 59.56	72.84 ± 37.15	ns
	2	84.66 ± 29.13	61.68 ± 18.72	ns
FLVEF preop (ml)	1	28.50 ± 16.01	38.63 ± 8.73	ns

	2	35.00 ± 8.80	29.50 ± 4.14*	ns
FLVEF post (ml)	1	39.95 ± 20.61	41.94 ± 17.63	ns
	2	43.17 ± 15.47	48.04 ± 15.57	ns
FS% preop (%)	1	34.86 ± 5.51	35.49 ± 7.62	ns
	2	33.65 ± 7.72	40.25 ± 6.30	ns
FS% post (%)	1	23.69 ± 6.76	31.79 ± 4.13	ns
	2	25.22 ± 6.23	33.11 ± 6.23	ns
RVSP preop (mmHg)	1	56.00 ± 12.99	44.67 ± 16.01	ns
	2	55.17 ± 12.92	50.00 ± 11.73	ns
RVSP post (mmHg)	1	36.80 ± 9.45	34.44 ± 8.92	ns
	2	45.50 ± 10.89	40.64 ± 9.73	ns
1 - mitral valve annuloplasty, 2 - mechanical mitral valve, EDD – end-diastolic dimension, ESD – end-systolic dimension, EDV – end-diastolic volume, ESV – end-systolic volume, FLVEF – forward LV ejection fraction, FS – fractional shortening, RVSP – right ventricular systolic pressure * p = 0.045 compared to MVA				

Discussion

Severe MR (4+) is associated with progressive ventricular remodeling that results from chronic volume overload. Several studies demonstrated better survival of these patients when mitral valve operative intervention was performed before the onset of symptoms. However, due to established risks of surgery, only patients with a severe degree of MR are selected for the intervention (12–14). The estimation approach using M-mode–derived dimensions is simple, noninvasive, and successful, which allows it to be recommended as a criterion for intervention in mitral valvulopathy. Moreover, LV diameters, volumes, EF% and wall stress are commonly used to assess LV remodeling process (9).

An individualized consideration needs to be performed when deciding on the type of surgical correction for MR, including both patient-related status and specificities of procedure with its potential adverse effects. Studies report that mitral valve replacement is associated with lower occurrence of valve-related complications but greater rates of thromboembolic events and structural impairment of the valve that reflects on ventricular tethered loop and LV contraction (8, 15, 16).

Despite no significant difference in mortality between MVA and MVR for ischemic mitral valve incompetence by the end of 7-year follow-up in the study by Micovic et al. (17), the 30-day

mortality rates were significantly higher in the MVR group (9.6% vs. 5.8%). Similarly, in a meta-analysis, clinical outcomes of repair and replacement for severe ischemic MR showed lower perioperative mortality in MVA patients and no differences in long-term survival. However, MVA was associated with a higher recurrence of MR compared to the MVR procedure (15).

We detected subtle changes in the early postoperative period between the two types of procedures for degenerative severe MR, specifically when comparing mitral valve correction with implantation of annuloplasty ring with MVR using a mechanical valve. Following both types of intervention, EDD and EDV decreased significantly compared to initial values, as well as LA diameter, but these were not distinctly different between the groups. Patients with MVA who progressed to LVD (defined as EF < 50%) had significantly higher preoperative EDD (p = 0.038), while other patients showed the same tendency. Postoperative LVD was associated with higher postoperative ESD in MVA patients, while there was a tendency of higher preoperative ESD in the mechanical valve replacement group.

Left ventricular contractile dysfunction is an early and frequent complication of MR surgery. It interferes with and prolongs LV recovery and influences patients' survival. Nevertheless, the issue of early LVD following mitral valve surgery is still poorly evaluated (14, 18, 19). Despite preoperative EF ≥ 60%, it is suggested that there is an ongoing myofibrillar degeneration and diffuse interstitial fibrosis in the myocardium with MR

leading to contractile dysfunction that remains asymptomatic and unrecognised until surgical correction. The mitral valve surgery removes the confounding effects of regurgitant volume, reduces preload but increases afterload pressures, thereby unmasking the actual state of LV function (12, 14, 20).

In a study that evaluated short- and long-term changes in postoperative LVEF after mitral valve repair, preoperative LVEF and ESD were found important in defining these changes. There was a greater decrease in LVEF after repair, the higher the preoperative LVEF was, along with preoperative ESD greater than 40 mm compared to < 40 mm (21).

Forward LVEF was shown to be superior to the total EF in predicting outcomes in MR, with higher risk for adverse events in those patients having forward LVEF less than 50%. A preoperative forward EF less than 40% was found to be associated with the risk of developing LV systolic dysfunction after MR corrective procedure (12). Nevertheless, LVD occurs in patients with previously normal LV function as well (14, 18). This parameter could predict early LVD, 3–4 days after an MR repair, with an optimal cut-off of 31.8% reported in patients with preoperative LVEF $\geq 60\%$. It also correlated with mitral regurgitant volume and fraction (14).

We determined a significant difference in preoperative forward LVEF values according to the type of intervention in patients with preserved postoperative LV function. Those who underwent annuloplasty presented with higher preoperative forward LVEF than those who underwent valve replacement. Although we did not find statistical significance, our MVA patients with postoperative LVD had lower forward LVEF compared to non-LVD. This change had the opposite direction in MVR patients.

Preoperative forward LVEF of less than 40% accurately predicts postoperative LV dysfunction three months after surgery for chronic organic MR (22). Both our groups with LVD had forward LVEF below this limit. Additionally, the results of MVA patients are comparable to the study of Kim et al., who determined an even lower cut-off of 31% for forward LVEF in those progressing to LVD (14).

Other factors reported to be associated with LVD after MR surgery include enlargement in systolic dimension, midwall and stress-corrected midwall FS%, decrease in RVSP after a repair, preoperative RVSP, LA diameters, global longitudinal strain and global circumferential strain (14, 18, 19, 23).

Similarly to others (14, 21), we detected a significantly reduced postoperative FS% compared to the preoperative in our patients (36.3 vs. 30.7%). A fall in FS% indicates LV systolic dysfunction. Also, LVD was associated with

significantly lower postoperative FS% in both our groups (23.7% in MVA and 25.2% in MVR). It is suggested that midwall FS may represent preoperative intrinsic LV contractility that is not obvious before surgical MR correction due to a compensation mechanism for LV volume overload (14, 21).

Left atrial dimensions decreased after MR correction in both groups. There were slightly lower dimensions of LA in LDV patients in both groups, but still higher than normal (> 4 cm). Left atrial size is a simple parameter affected by MR severity and thus may identify patients who are suitable for early elective valve surgery. Moreover, it is found to be the strongest independent echocardiographic predictor of outcome in severe but asymptomatic MR (23).

The information presented here provides valuable insight into the subtle specificities of early LV remodeling according to the type of operative MR correction. Besides MR correction and prevention of recurrence, types of operative intervention represent a new basis that later on defines long-term outcomes.

Conclusion

We can conclude that there are subtle differences in preoperative and early postoperative echocardiographic parameters between the MVA and MVR procedures of surgical MR correction in patients with chronic primary MR. Forward LVEF values were significantly higher after the procedure in general. Left ventricle EDD improved significantly after the interventions, while no significant differences in LV volumes were determined between the groups.

There was a similar incidence of immediate postoperative LV systolic dysfunction in the groups. Each group had distinct alterations to LVD: a significantly higher preoperative EDD and postoperative ESD were present in MVA patients, while the MVR group showed only a tendency towards higher preoperative ESD and postoperative EDV.

A significant distinction between the groups was revealed in patients without LVD, that is, a higher preoperative forward LVEF in MVA compared to MVR patients. Interestingly, the opposite direction of forward LVEF change was seen in LVD between the groups.

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EHOKARDIOGRAFSKA PROCENA REMODELOVANJA LEVE KOMORE U MITRALNOJ REGURGITACIJI PREMA TIPU HIRURŠKE KOREKCIJE: REPARACIJE ILI ZAMENE MITRALNE VALVULE

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Patofiziologija hronične primarne mitralne regurgitacije (engl. *mitral regurgitation* – MR) uglavnom se zasniva na degenerativnom procesu. Neadekvatna adaptacija leve komore (engl. left ventricle – LV) usled zapreminskog opterećenja dovodi do postepene dilatacije i slabosti. Jedinu adekvatnu terapijsku opciju u ovom slučaju predstavlja hirurška intervencija. Cilj ovog istraživanja bio je da proceni rane ehokardiografske parametre remodelovanja LV-a u hroničnom primarnom MR-u u dvema vrstama operativne korekcije: mehaničkoj zameni mitralne valvule (engl. *mitral valve replacement* – MVR) i anuloplastici mitralnog zaliska protetskim prstenom (eng. *mitral valve annuloplasty* – MVA). Ehokardiografske varijable su izmerene ili izračunate korišćenjem 2D i M tehnike. Pored toga, varijable su procenjene u odnosu na ranu postoperativnu disfunkciju LV-a (engl. *left ventricular dysfunction* – LVD). Ejekciona frakcija (engl. *ejection fraction* – EF) bila je < 50%. Istraživanje je obuhvatilo 36 asimptomatskih pacijenata sa primarnim teškim MR-om (stepen 3–4) koji su bili podvrgnuti hirurškoj korekciji zalistaka. Krajnji dijastolni prečnik LV-a značajno se poboljšao ($6,11 \text{ cm} \pm 0,9 \text{ cm}$ prema $5,50 \text{ cm} \pm 0,7 \text{ cm}$) u obema grupama ($p < 0,006$) posle intervencija. Kada je reč o zapreminama LV-a, nisu zabeležene značajne razlike između grupa. Neposredna postoperativna sistolna disfunkcija LV-a pokazala je sličnu incidenciju u grupama (43%). Značajna razlika između grupa uočena je kod pacijenata bez LVD-a, odnosno zabeležen je veći preoperativni prednji LVEF kod pacijenata sa MVA nego kod pacijenata sa MVR-om. Nasuprot tome, kod pacijenata sa LVD-om uočen je suprotan smer promene prednjeg LVEF-a. Može se zaključiti da postoje suptilne razlike u ranim postoperativnim ehokardiografskim parametrima između MVA i MVR procedura koje odražavaju suptilne specifičnosti ranog remodelovanja LV-a kod pacijenata sa hroničnim primarnim MR-om.

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Ključne reči: anuloplastika, krajnji dijastolni dijametar, ejekciona frakcija, disfunkcija leve komore, end-sistolni volumen

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ANTIOXIDANT, ANTIMICROBIAL, AND SPASMOLYTIC EFFECTS OF THE CLARY SAGE (*SALVIA SCLAREA* L.) HYDROETHANOLIC EXTRACTS PREPARED BY DIGESTION

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In traditional medicine, clary sage (*Salvia sclarea* L.) is known as an aromatic and medicinal plant used in the treatment of respiratory, digestive, and menstrual issues, as well as various inflammatory processes such as gingivitis, stomatitis, canker sores, and arthritis. This study examined the antioxidant, antimicrobial, and spasmolytic effects of the hydroethanolic extracts from the aerial parts of *S. sclarea* collected in Malča, near Niš, Serbia. Two extracts were prepared using the digestion method with 80% and 60% ethanol (HE80D and HE60D, respectively). The extracts were chemically characterized, revealing significant amounts of phenolic compounds, with rosmarinic acid being the most dominant. The tested extracts showed considerable antioxidant potentials in the 2,2-diphenyl-1-picrylhydrazyl and β -carotene/linoleic acid systems. The results indicated that the extracts were particularly effective as antilipoperoxidant agents. The most pronounced antimicrobial effects of the extracts were observed against *Staphylococcus aureus*, with moderate effects against *Bacillus cereus* and *Listeria monocytogenes*. The extracts demonstrated significant inhibition of rat ileum spontaneous smooth muscle contractions. At the maximum concentration of 1.5 mg/ml, the HE80D and HE60D extracts reduced ileum contractions by $40.45 \pm 2.06\%$ and $40.60 \pm 1.22\%$, respectively. Further research on *S. sclarea* extracts should be directed towards more detailed *in vivo* and clinical studies to assess their potential use in rational phytotherapy.

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Key words: *Salvia sclarea* L., hydroethanolic extracts, antioxidant effects, antimicrobial effects, spasmolytic effects

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Introduction

Traditional medicine has historically been vital in both the prevention and treatment of diseases (1). It is based on the action of various pharmacologically active compounds with therapeutic effects confirmed through the experience and knowledge gained during the long history of disease treatment, and can also be

approved by clinical trials (2). Numerous plant species from the Lamiaceae family are commonly utilized in traditional medicine, and their pharmacological effects have been validated through various studies (3). The most prevalent species in this family are from the sage genus, *Salvia* L., which is represented in many of the world's pharmacopoeias. The range of traditional uses for *Salvia* species in folk medicine is remarkably extensive. They are most often used as antiseptics, spasmolytics, carminatives, and agents for rinsing the mucous membranes of the oral cavity and throat in inflammatory processes, as well as for healing skin wounds and skin care. Alongside *Salvia officinalis* L., the most frequently used and researched species, there has been growing interest in other species of this genus, including *Salvia sclarea* L. known as clary sage (4). It is employed as a treatment for digestive and respiratory conditions, such as dyspepsia, painful spasms, flatulence, colds, and coughs, in addition to gingivitis, polyarthritis, rheumatism, and menstrual issues (5–14).

Recently, there has been considerable interest in the use of natural antioxidant agents, prompted by studies suggesting potential adverse effects associated with synthetic antioxidants. A variety of plant materials are recognized as natural sources of antioxidants, and the growing interest in these natural components stems from their biological value and economic impact (15). Furthermore, natural antioxidants are crucial for antioxidant defense and redox regulation in living cells, serving as potential protectors against diseases such as atherosclerosis, diabetes, neurodegenerative disorders, cancer, infections, chronic inflammatory diseases, and autoimmune conditions (16). *Salvia* species are sources of various compounds with powerful antioxidant activity finding a promising place in industry and healthcare products (17).

The increasing prevalence of drug-resistant pathogens underscores the need to identify and isolate new substances from medicinal plants, offering innovative and effective strategies to combat pathogenic bacteria. Many medicinal plants have been historically recognized and extensively used as antiviral, antifungal, antibacterial, or antiparasitic agents (18). *Salvia* species, similar to many other plants, tend to inhibit the growth of Gram-positive bacteria more effectively than Gram-negative bacteria, a difference attributed to the synergistic action of phytochemicals, primarily flavonoids, phenolic acids, tannins, and volatiles (1, 19–21).

Most aromatic drugs are excellent spasmolytic and carminative agents used for treating many gastrointestinal complaints such as colics, flatulence, irritable bowel syndrome, congestive dyspepsia, slow digestion, chronic inflammations, and infections. These medicinal plants and their herbal products have been used for generations due to their multiple nutritional and therapeutic benefits, which are attributed to their active chemicals (22). *S. officinalis* is traditionally used in herbal medicinal products to alleviate mild dyspeptic complaints, including heartburn and bloating (23). Nowadays, other *Salvia* species are currently being studied extensively for their effects on gastrointestinal complaints, particularly on digestive motor function.

With all this in mind, this study intended to demonstrate the pharmacological properties of less-studied species within the *Salvia* genus, *S. sclarea*, growing in the area of Niš, Serbia. We sought to show the antioxidant, antimicrobial, and spasmolytic activities of its extracts prepared with hydroethanolic solvents by the digestion technique, estimating their possible application in functional foods and dietary supplements.

Materials and Methods

Plant material and extraction

The aerial parts of *S. sclarea* (including the stem, leaves, and flowers) were collected in Malča, near Niš, in July during full bloom. Dr. Bojan Zlatković (the Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš) identified the plant material. The herbarium specimen was submitted to the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac" and assigned collection number 17077.

The material was dried in a dark, well-ventilated space and stored in paper bags until it was analysed. The dried plant material was powdered on a mechanical mill and extracted using the digestion (D) method and two hydroethanolic (HE) solvents (ethanol: water (v/v) ratios of 80:20 and 60:40), in a drug: solvent ratio of 1:10. Thus, two extracts were obtained, HE80D and HE60D. The digestion method involved extracting the powdered plant material for three hours in a water bath at 40 °C. After filtration, the resulting liquid extracts were completely evaporated using a vacuum evaporator. All dry extracts were stored in sealed glass vials at 4 °C until analysis.

Chemical characterization of the extracts

Determination of the content of total phenols

The content of total polyphenols was determined by the colorimetric method according to Hagerman et al. (2000) (24). The method involves the use of the commercially available Folin-Ciocalteu reagent, which is diluted with the same volume of distilled water immediately before the analysis. Distilled water (480 µl), Folin-Ciocalteu reagent (250 µl), and Na₂CO₃ × 10H₂O solution (20%, 1250 µl) were added to the diluted extract samples (20 µl) in a test tube. The contents of the test tubes were thoroughly mixed and then allowed to stand at room temperature for 40 minutes. The absorbance was measured at 725 nm, using a UV-Vis spectrophotometer (Evolution 60 Thermo Scientific) with distilled water serving as a blank instead of the diluted extract solution. Prior to analysing the extracts, a calibration curve was established using various dilutions of gallic acid following the same procedure. The results are reported as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

Determination of the content of total tannins

Total tannin contents in the extracts were assessed as a continuation of the previous experiment using the Folin-Ciocalteu solution (24). Polyvinyl polypyrrolidone (PVPP, 100 mg), which binds the tannins from the extract, was added to a test tube containing 1 ml of distilled water and 1 ml of the diluted sample. The

mixture was shaken thoroughly and cooled for 15 minutes at 4 °C. It was then centrifuged for 10 minutes at 3000 revolutions per minute. The supernatant, containing all phenolic compounds except tannins, was used to determine non-tannic polyphenols using the previously described method. The tannin content in the extracts was calculated as the difference between the total phenols and the non-tannic polyphenols.

Determination of the content of total flavonoids

The total flavonoid contents in the extracts were determined using the colorimetric AlCl_3 method (25, 26). The extract solution (0.5 ml, 5 mg/ml) was mixed with 1.5 ml of concentrated ethanol, 0.1 ml of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution (10%), 0.1 ml of a 1M sodium acetate solution, and 2.8 ml of water. After 30 minutes, the solutions were transferred to microtiter plates (200 μl), and their absorbance was measured at 405 nm using an ELISA reader (Multiskan Ascent No354, Thermo Labsystems, Finland). The blank consisted of a mixture without the AlCl_3 solution, replaced by an equivalent volume of water. The results are presented as micrograms of luteolin equivalents per milligram of extract (mg LE/g). Before analysing the extracts, a calibration curve was established using various dilutions of luteolin following the same procedure.

Determination of the content of total hydroxycinnamic acid derivatives

The content of total hydroxycinnamic acid derivatives (THAD) was determined according to the prescription from the 11th European Pharmacopoeia (Ph. Eur. 11.0, 2023), which is described in the monograph of the drug *Rosmarini folium* (27).

HPLC analysis of the extracts

The analysis of the chemical composition of the *S. sclarea* extracts using HPLC was performed according to the procedure previously described by Kostić et al. (2017) (28).

Determination of the antioxidant activity of the extracts

Antiradical activity in the DPPH (2,2-diphenyl-1-picrylhydrazyl) system

The ability of the extract to neutralize and "capture" free radicals was estimated in the DPPH system, which was carried out according to the method reported by Kostić et al. (2017) (28). Increasing concentrations of dissolved extracts (40 μl) were added to the wells of a 96-microtiter plate that already contained 120 μl of methanol. Next, 40 μl of a methanol solution of DPPH (0.2 mg/ml) were added to each well. The microtiter

plates were briefly mixed on a shaker and then kept in the dark for 30 minutes. The absorbances of the mixtures in the wells were measured at 550 nm using an ELISA reader (Multiskan Ascent No354, Thermo Labsystems, Finland). The percentage of free radical inhibition was calculated using the following formula:

$$\% \text{ inhibition of DPPH} = ((\text{AK}-\text{AA})/(\text{AK}-\text{AS})) \times 100$$

In the equation, AK represents the absorbance of the solvent (methanol) + DPPH solution, AA is the absorbance of the analysed extract solution + methanol + DPPH solution, and AS is the absorbance of methanol. The concentration of the extract that neutralizes 50% (IC_{50}) of free radicals was determined based on a curve constructed with the inhibition percentages and the extract concentrations. Vitamin E (α -tocopherol) and vitamin C (ascorbic acid) were used as the standards.

Antilipoperoxidant activity in the β -carotene-linoleic acid system

The capacity of the extracts to inhibit the process of lipid peroxidation was investigated according to the method of Kostić et al. (2017) (28). This method assesses the extract's effectiveness in protecting linoleic acid molecules from lipid peroxidation, thereby reducing the reliance on β -carotene, which would otherwise function as an antioxidant in the absence of the extract. β -Carotene (2 mg) was dissolved in 10 ml of chloroform, and one milliliter of this solution was transferred to a round-bottom flask along with linoleic acid (25 μl) and Tween 20 (180 mg). After the complete evaporation of chloroform, 50 ml of oxygenated water was added to the flask with shaking to form an emulsion. The emulsion without β -carotene was used as a blank. This aqueous emulsion (200 μl) was pipetted into each well of a microtiter plate, and mixed with 25 μl of the tested extracts with increased dilutions. The plates were then incubated for 2 h at 55 °C. The absorbances of the obtained mixtures in the wells of the plates were measured with an ELISA reader (Multiskan Ascent No354, Thermo Labsystems, Finland) at 450 nm before incubation (A0) and after 2 h (120 minutes) (A120). The contents of the microtiter plates were mixed on a shaker before measuring the absorbances. The percentage of inhibition was determined according to the formula:

$$\% \text{ inhibition} = (\text{A120}/\text{A0}) \times 100\%$$

The concentration of the extract that neutralizes 50% of free radicals (IC_{50}) was calculated based on a curve created from the inhibition percentages and extract concentrations. Ascorbic acid and α -tocopherol served as the standards.

Spasmolytic effects of the extracts on rat ileum spontaneous contractions

Experimental animals

The experimental procedures adhered to the guidelines outlined in the European Directive 2010/63/EU for animal experiments and received approval from the Veterinary Directorate of the Ministry of Agriculture and Environmental Protection (decision number 323-07-00073/2017-05/04). A male Wistar albino rat, weighing about 200 g and aged 10 weeks, was obtained from the vivarium of the University of Niš Faculty of Medicine. One week before the experiments, the rat was housed individually in a stainless steel cage with a room temperature of 20 to 24 °C and a 12-hour light-dark cycle. Throughout the study, the rat had *ad libitum* access to food and water, except for a 24-hour fasting period before the experiments.

Isolation and preparation of rat ileum

Following anesthesia, thoracic and aortic dissection was performed to isolate the rat's ileum. The ileal sections were carefully separated from the mesentery and connective tissue. These segments were then placed in an organ bath containing 20 mL of Tyrode's solution, maintained at 37 °C with a continuous supply of a gas mixture composed of 95% O₂ and 5% CO₂. A stabilization period of thirty minutes was allowed for the ileum in the organ bath (29). Contractile changes were measured using a transducer (Transducer-TSZ-04-E, Experimetria Ltd., Budapest, Hungary), and the data were analysed with the SPEL Advanced ISOSYS Data Acquisition System software.

Experimental design with rat ileum

This experiment aimed to evaluate the impact of the hydroethanolic *S. sclarea* extracts on the spontaneous contractions of isolated rat ileum. Following an adaptation period, the extracts were added in incremental doses (ranging from 0.005 to 1.5 mg/mL), and the dose-dependent response was determined from the resulting curve. The spasmolytic effect of each extract concentration was expressed as a percentage relative to the initial spontaneous activity of the isolated ileum without the presence of the tested extract (29). Papaverine, an opium alkaloid known for its spasmolytic activity, served as the positive control (0.01–3 µg/mL).

Antimicrobial effects of the extracts

The antimicrobial efficacy of the tested *S. sclarea* extracts was assessed using laboratory control strains sourced from the American Type Culture Collection (ATCC). The assay encompassed Gram-positive and Gram-negative bacteria and a fungus. *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 9433, *Streptococcus pneumoniae* ATCC 6301,

Streptococcus pyogenes ATCC19615, *Bacillus cereus* ATCC 11778, and *Lysteria monocytogenes* ATCC 15313 were used as the representatives of Gram-positive bacteria. *Pseudomonas aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 12453, *Salmonella enteritidis* ATCC 13076, *Escherichia coli* ATCC 8739, *Enterobacter aerogenes* ATCC 13048, and *Klebsiella pneumoniae* ATCC 10031 were used for the testing of antimicrobial properties against Gram-negative bacteria. Antifungal effects of the extracts were assessed against a yeast, *Candida albicans* ATCC 24433.

The antibacterial and antifungal activities were evaluated using the microdilution method following the Clinical and Laboratory Standards Institute (30). Overnight cultures of the tested microorganisms were prepared to achieve a suspension with a standard turbidity of 0.5 McFarland, equivalent to 1.5×10^8 colony-forming units (CFU)/mL for bacteria and 1.5×10^7 CFU/mL for *C. albicans*. The extracts were dissolved in a 10% aqueous solution of dimethyl sulfoxide, and serial double dilutions (ranging from 0.1 to 100 mg/mL) were prepared in 96-well microtiter plates containing inoculated nutrient broth. After incubation at 37 °C for 24 hours for bacteria or 25 °C for 48 hours for *C. albicans*, microbial growth was detected by adding 20 µL of a 0.5% aqueous solution of 2,3,5-triphenyl tetrazolium chloride to each well (31). The minimum inhibitory concentration (MIC) was defined as the lowest concentration at which no visible growth of microorganisms was observed. The minimum bactericidal concentration (MBC or MFC) was determined as the lowest concentration of the extract capable of killing 99.9% of the investigated bacteria or fungus. To ascertain the MBC/MFC, the broth from each well with no visible growth was inoculated into Mueller-Hinton agar at 37 °C for 24 hours for bacteria or Sabouraud-dextrose agar at 25 °C for 48 hours for *C. albicans*. Positive controls containing chloramphenicol, streptomycin, or nystatin (0.008–16 µg/mL) were employed.

Statistical analysis

The collected data were analysed statistically, and the results were presented as mean values from three measurements for chemical composition or six measurements for spasmolytic activity, along with standard deviations (SD), except for antimicrobial activity and extraction yields. An effective concentration EC₅₀ and an inhibitory concentration IC₅₀, representing the concentrations causing 50% of maximal response or inhibition, were calculated via regression analysis. Statistical significance between the means was determined using Student's t-test/one-way ANOVA, followed by Duncan's post hoc test ($p < 0.05$ or $p < 0.01$). The statistical analyses were conducted using the SPSS 20.0 statistical package (SPSS, Inc., Chicago, IL, USA).

Results

Plant extraction and chemical characterization of the extracts

The yields of extractions in the present study were high and very similar, exceeding 20% (Table 1). Further, the *S. sclarea* extracts were chemically characterized by the determination of total phenolics, tannins, flavonoids, and hydroxycinnamic acid derivatives (Table 1), and by HPLC analysis, which was used for the precise definition of present phytocompounds (Table 2). Significant amounts of total phenols and tannins were quantified in the extracts, whereby HE60D contained their higher values, 125.07 ± 3.88 mg GAE/g and 31.85 ± 0.88 mg GAE/g, respectively. Hydroxycinnamic acid derivatives were more dominant in HE80D, with a value of $7.47 \pm 0.30\%$ of rosmarinic acid, while flavonoids were extracted with both hydroethanolic solvents in a similar quantity.

All tested extracts were chemically characterized using a precise HPLC technique. The recorded chromatograms showed the dominance of rosmarinic acid in relation to all other compounds. Caffeic acid, flavonoid aglycones (apigenin, luteolin and salvigenin), and flavonoid heterosides (apigenin-7-O-glucoside and luteolin-7-O-glucoside) were identified and then quantified (Table 2). Retention times (RT) were as follows: caffeic acid 20.5 min, luteolin-7-O-glucoside 29 min, apigenin-7-O-glucoside 32.5 min, rosmarinic acid 33.5 min, luteolin 39.3 min, apigenin 42.7 min, and salvigenin 53 min. Figures 1 and 2 present HPLC chromatograms of each of the extracts. The highest amounts of rosmarinic and caffeic acid were recorded in HE80D with the values of 167.81 ± 1.10 µg/mg and 1.32 ± 0.06 µg/mg, respectively. All three flavonoid aglycons (apigenin, luteolin and salvigenin) were better isolated using 80% ethanol. Flavonoid heterosides, luteolin- and apigenin-7-O-glucoside, were more present in HE60D.

Antioxidant activity of the extracts

The antioxidant activity of the extracts was investigated in two complementary test systems, DPPH and β -carotene/linoleic acid (BCL). Table 3 displays the IC_{50} (µg/ml) values of the extracts and positive controls. The extracts produced very similar effects in the antiradical activity assay, while the HE60D extract was the stronger antioxidant agent in the BCL system, with the IC_{50} value of 13.36 ± 0.00 µg/ml. This antilipoperoxidant effect was even better than that of ascorbic acid, which was, on the other hand, unassailable as a free radical scavenger.

Antimicrobial effects of the extracts

Gram-positive bacteria exhibited greater sensitivity to the tested extracts compared to Gram-negative bacteria. While inhibitory concentrations were notable, bactericidal concentrations of all extracts were not significant, reaching 100 mg/ml or higher (Table 4). The most remarkable antibacterial effects were observed against *S. aureus*, with minimum inhibitory concentrations of 6.25 mg/ml. Moderate activity was observed against *B. cereus* and *L. monocytogenes*, while weaker effects were noted against *E. faecalis*, *S. pneumoniae*, and *S. pyogenes*. These clary sage extracts showed poor effects against Gram-negative bacteria. A more significant antimicrobial effect can be attributed to the HE80D extract, which was active against *P. aeruginosa* and *E. aerogenes* with Minimum inhibitory concentration (MIC) values of 50 mg/ml. Against *C. albicans*, the extracts showed insignificant effects due to high MIC and Minimum fungicidal concentration (MFC) values.

Spasmolytic effects of the extracts on spontaneous rat ileal contractions

Extracts HE80D and HE60D demonstrated notable dose-dependent relaxation of ileal smooth muscle contractions (Figure 3). At the concentrations of 1.5 mg/ml, they decreased the ileal contractions by $40.45 \pm 2.06\%$ and $40.60 \pm 1.22\%$, respectively (Table 5). Although the spasmolytic effects of the extracts were significant, they were less potent in comparison to the non-specific muscle relaxant, papaverine. Papaverine, at the concentration of 0.003 mg/ml, inhibited $97.00 \pm 0.02\%$ of contractions, with the EC_{50} value of $1.2 \times 10^{-4} \pm 0.1 \times 10^{-4}$ mg/ml.

Table 1. Yields of extractions (%), the contents of total phenols and tannins (mg GAE/g), hydroxycinnamic acid derivatives (% of rosmarinic acid), and flavonoids (mg LE/g) in *Salvia sclarea* L. hydroethanolic extracts

EXTRACT	Y	TP	TT	THAD	TF
	%	mg GAE/g	mg GAE/g	% RA	mg LE/g
HE80D	21.76	107.22 ± 1.72^a	27.30 ± 1.72^a	7.47 ± 0.30^a	27.13 ± 0.99^a
HE60D	22.01	125.07 ± 3.88^b	31.85 ± 0.88^b	5.82 ± 0.08^b	26.49 ± 1.17^a

Results show the mean of three measurements $\pm$ standard deviation. Different lowercase letters in the columns indicate a statistically significant difference in the content of total phenols, tannins, hydroxycinnamic acid derivatives and flavonoids between the extracts (Student's t-test, $p < 0.05$).

Table 2. The contents of phenolic acids and flavonoids ($\mu\text{g}/\text{mg}$) in *Salvia sclarea* L. hydroethanolic extracts

EXTRACT	RA	CA	L	A	S	LG	AG
	$\mu\text{g}/\text{mg}$						
HE80D	167.81 ± 1.0^a	1.32 ± 0.06^a	1.21 ± 0.03^a	0.97 ± 0.01^a	1.86 ± 0.02^a	6.30 ± 0.26^b	6.68 ± 0.20^c
HE60D	136.16 ± 0.11^b	0.96 ± 0.03^b	0.78 ± 0.04^b	0.69 ± 0.01^b	1.16 ± 0.05^b	6.82 ± 0.43^b	6.70 ± 0.22^c

RA – rosmarinic acid; CA – caffeic acid; L – luteolin; A – apigenin; S – salvigenin; LG – luteolin-7-O-glucoside; AG – apigenin-7-O-glucoside. Results show the mean of three measurements $\pm$ standard deviation. Different lowercase letters in columns indicate a statistically significant difference in compound content between the extracts (Student's t-test, $p < 0.05$).

Table 3. Antioxidant activity of *Salvia sclarea* L. hydroethanolic extracts in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β -carotene/linoleic acid (BCL) assay

EXTRACT	DPPH	BCL
	IC_{50} ($\mu\text{g}/\text{ml}$)	
HE80D	35.13 ± 1.01^a	24.78 ± 1.88^a
HE60D	36.07 ± 1.17^a	13.36 ± 0.00^b
AA	4.74 ± 0.34^b	22.95 ± 1.52^c
α -TOC	10.40 ± 1.73^c	0.15 ± 0.00^d

AA-ascorbic acid; α -TOC- α -tocopherol. The results show the mean values of three measurements $\pm$ standard deviation. Different lowercase letters in columns indicate a statistically significant difference in IC_{50} values among extracts and standards (Duncan's t-test, $p < 0.05$).

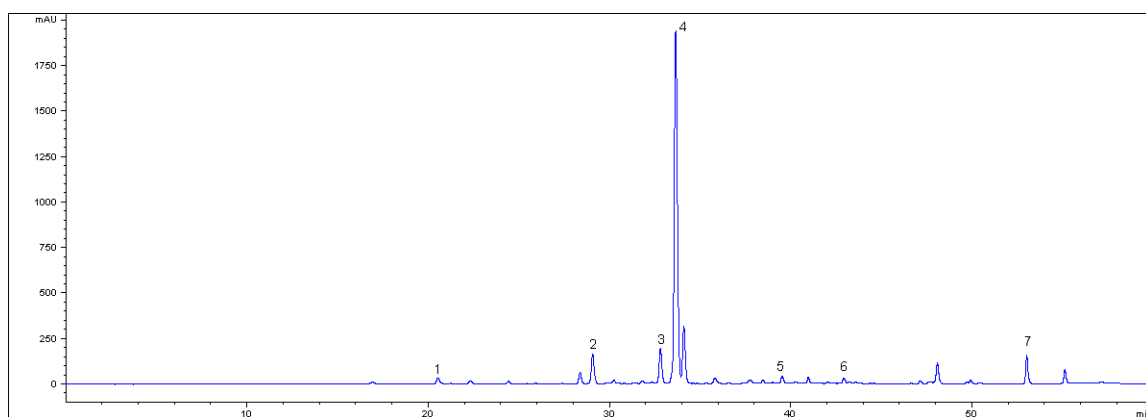
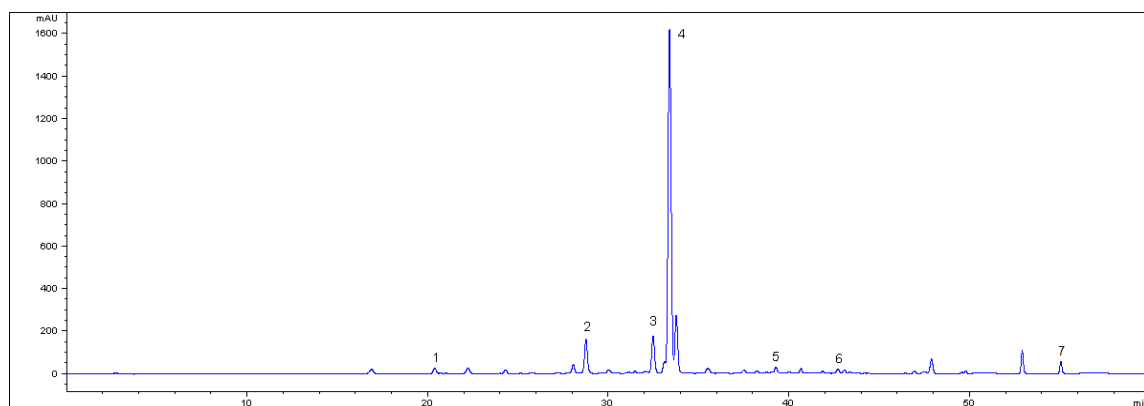
Table 4. Minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentrations of the *Salvia sclarea* L. extracts bacterial Gram-positive and Gram-negative strains and yeast

EXTRACTS	HE80D	HE60D	S
Bacterial strains	MIC / MBC (mg / ml)		MIC / MBC ($\mu\text{g}/\text{ml}$)
Gram (+)			Chloramphenicol
<i>Staphylococcus aureus</i>	6.25/>100	6.25/100	7.81/15.61
<i>Enterococcus faecalis</i>	100/>100	50/100	3.91/7.81
<i>Streptococcus pneumoniae</i>	>100/>100	>100/>100	0.06/0.12
<i>Streptococcus pyogenes</i>	100/>100	100/>100	0.25/0.49
<i>Bacillus cereus</i>	12.5/>100	12.5/>100	7.81/15.61
<i>Listeria monocytogenes</i>	12.5/100	25/100	0.25/0.49
Gram (-)			Streptomycin
<i>Pseudomonas aeruginosa</i>	50/>100	100/>100	0.60/0.60
<i>Proteus mirabilis</i>	>100/>100	>100/>100	0.30/0.30
<i>Salmonella enteritidis</i>	>100/>100	100/>100	0.30/0.30
<i>Escherichia coli</i>	>100/>100	>100/>100	0.16/0.16
<i>Enterobacter aerogenes</i>	50/>100	100/>100	0.60/0.60
<i>Klebsiella pneumoniae</i>	100/>100	100/>100	0.30/0.30
Fungal strain	MIC / MFC (mg / ml)		MIC / MBC ($\mu\text{g}/\text{ml}$)
Yeast			Nystatin
<i>Candida albicans</i>	>100/>100	100/>100	3.91/7.81

Table 5. Spasmolytic effects of the *Salvia sclarea* L. extracts and positive control (papaverine) on the spontaneous contractions

EXTRACT	EC ₅₀	max response
	mg / ml	%
HE80D	1.89 ± 0.12 ^b	40.45 ± 2.06 ^a
HE60D	1.97 ± 0.11 ^b	40.60 ± 1.22 ^a
papaverine	1.2·10 ⁻⁴ ± 0.1·10 ^{-4c}	97.00 ± 0.02 ^b

The results represent the mean of the six measurement ± the standard deviation. Different lower case letters in the columns indicate a statistically significant difference among EC₅₀ values of the extracts and the control (Duncan's test, $p < 0.05$)

**Figure 1.** HPLC chromatogram of the *Salvia sclarea* L. extract HE80D (330 nm). 1. caffeic acid, 2. luteolin-7-*O*-glucoside, 3. apigenin-7-*O*-glucoside, 4. rosmarinic acid, 5. luteolin, 6. apigenin, 7. salvigenin**Figure 2.** HPLC chromatogram of the *Salvia sclarea* L. extract HE60D (330 nm). 1. caffeic acid, 2. luteolin-7-*O*-glucoside, 3. apigenin-7-*O*-glucoside, 4. rosmarinic acid, 5. luteolin, 6. apigenin, 7. salvigenin

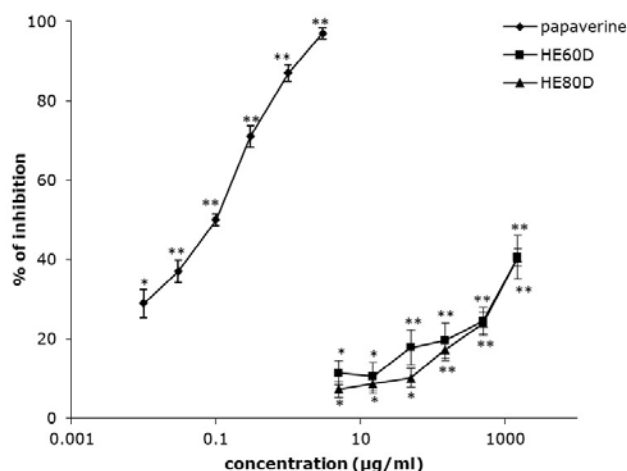


Figure 3. Relaxant effects of the *Salvia sclarea* L. extracts (HE80D and HE60D) and papaverine on spontaneous contractions of isolated rat ileum. Each point represents the average percentage of relaxation relative to spontaneous contractions in Tyrode solution (control) $\pm$ standard deviation (Student's t-test, * $p < 0.05$, ** $p < 0.01$ vs. control)

Discussion

Many factors play important roles in extraction efficiency, such as extraction technique, type of solvent, temperature, time, pH, and particle size (32). Water, as a solvent, has a great extraction capacity, but it also has certain disadvantages. It can cause hydrolytic and fermentative decomposition, lead to the adsorption of the active substance on the swollen drug, dissolve ballast substances, and enable the development of microorganisms. Due to these shortcomings of water, ethanol-water mixtures are used more often. Ethanol is a much more selective solvent because it perfectly isolates active and less ballast substances (33). Increasing the water proportion in the solvent system causes swelling of the plant material and results in an increase in the contact between the plant matrix and the solvent, as well as an increase in the yield of extraction (34). Considering this fact, the ethanol-water mixture of 60% was a more effective solvent in this study. In addition, the selection of the appropriate extraction technique is very important for the isolation of the desired active ingredients. Changing the temperature can improve the extraction process and reduce the required amount of solvent for extraction (17, 35). Therefore, we chose to use the digestion

method, presenting one-time extraction that uses heat at up to 50 °C for the extraction of phytocompounds (36).

The investigated *S. sclarea* extracts contained high amounts of total phenols and tannins, with a preference for extraction using a more polar solvent, 60% ethanol. Phenols are widely found in the plant kingdom and constitute the most abundant group of secondary metabolites, featuring over 8,000 phenolic structures. These range from simple molecules, like phenolic acids, to complex, highly polymerized compounds, such as tannins (37). Polyphenols, as compounds with more than one phenolic hydroxyl group, are gaining special importance because of their possible roles in the prevention of various diseases such as cancer, cardiovascular or neurodegenerative diseases (38). Hydroxycinnamic acid derivatives form a sizable category of simple phenolic acids, acting also as precursor molecules for various compounds including stilbenes, chalcones, flavonoids, lignans, and anthocyanins (39). Hydroxycinnamic acid derivatives were found to be more abundant in the extract prepared with 80% ethanol, whereas flavonoids were extracted in similar quantities using both hydroethanolic solvents.

HPLC analysis of the *S. sclarea* extracts identified two phenolic acids, rosmarinic and

caffeic, along with three flavonoid aglycones of the flavone type: apigenin, luteolin, and salvigenin, and two heterosides: apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside. Among all these compounds, rosmarinic acid is identified as the most abundant. The extraction of rosmarinic and caffeic acid was significantly more efficient with 80% ethanol compared to 60%. The importance of the high content of rosmarinic acid is reflected in potential pharmacological activities such as antioxidant, antibacterial, antiviral, antimutagenic, anti-inflammatory, anti-thrombotic, anti-aggregation, anxiolytic, spasmolytic, renoprotective, etc. (40, 41–43). Our previous study of the chemical profiles of *S. sclarea* methanol extracts prepared from the same plant material revealed significant quantities of rosmarinic acid. In four methanolic extracts, its values were even larger and ranged from 171.99 ± 1.88 to 197.48 ± 2.00 $\mu\text{g}/\text{mg}$ (44). In our another investigation, concentrate ethanol (96%, v/v) was shown to be a weak solvent for the extraction of rosmarinic acid, which was present in the concentration of 85.65 ± 0.38 $\mu\text{g}/\text{mg}$ (42). According to the literature data, other phenolic acids were also identified in *S. sclarea* extracts, such as gallic, chlorogenic, ferulic, ellagic, *p*-coumaric, *o*-coumaric, fumaric, vanillic, syringic, quinic, tannic, salvianolic B and K, salvianolic A, lithospermic B, caffeoylmalic, cinnamic, benzoic, 3-hydroxybenzoic, 3,4-dihydroxybenzoic, and *p*-hydroxybenzoic acid (45–55).

Less polar forms of flavonoids, aglycones, were better extracted from *S. sclarea* with a solvent with a lower water fraction (80% ethanol). In addition, flavonoid glucosides were more present in the HE60D extract prepared with a more polar solvent and a higher water fraction (60% ethanol). Considering these and the results from our previous studies (42, 44), it can be concluded that concentrated ethanol (96% ethanol) is the optimal solvent for isolating apigenin, luteolin and salvigenin from this plant material, while hydroalcoholic mixtures are suitable for isolating glucosides. According to previous literature data, flavones are mostly present in *S. sclarea*. Flavones such as 4'-methylapigenin, 6-hydroxyluteolin-6,7,3',4'-tetramethyl ether, 6-hydroxyapigenin-7,4'-dimethyl ether, genkwanin, chrysin, acacetin, jaceosidin, viscosine, cirsimaritin, eupatorin, hispidulin and chrysoeriol were identified in different *S. sclarea* extracts, along with apigenin, luteolin and salvigenin. In addition, other types of flavonoids can be found in *S. sclarea* such as kaempferol, myricetin, quercetin, isoquercitrin, rutin, pinocembrin, naringenin, hesperidin, genistein and catechin (40, 45, 48, 50–52, 54–57).

It is widely recognized that sages contain polyphenolic and terpenic compounds that contribute to their strong antioxidant effects (1). While the antioxidant activity of polyphenols is

linked to various mechanisms, their heightened reactivity towards free radicals is regarded as the most significant one (58). Rosmarinic acid, caffeic acid, apigenin, luteolin, and salvigenin have been proven to be excellent antioxidants (59–62) contributing to the same activity of plant extracts. This study assessed the antioxidant activity of *S. sclarea* extracts using two *in vitro* test systems. The DPPH test was used to measure the free radical scavenging capacity of the extracts. During the experiment, the phenolics contained in the extracts reacted with the stable purple DPPH• radical, which after receiving a proton (hydrogen) was converted into the DPPH reduced form, followed by a color change from purple to yellow (63). The investigated *S. sclarea* extracts showed a strong activity in this test system with similar IC_{50} values, but they were slightly weaker antioxidant agents in comparison to the extract prepared from the same plant material, with 80% ethanol and ultrasound method, from our previous study ($\text{IC}_{50} = 27.82 \pm 2.05$ $\mu\text{g}/\text{ml}$). The second method of testing the antioxidant efficiency of clary sage extracts was related to examining the antilipoperoxidant activity in the BCL system. This type of antioxidant activity test relies on the competitive decolorization of β -carotene during the oxidation of linoleic acid in the presence of oxygenated water. As an effective antioxidant, this carotenoid prevents the formation of linoleic acid peroxide by discoloring itself. The addition of potential antioxidants, which are being evaluated for their effectiveness, halts the degradation of β -carotene and maintains the yellow color of the system (58). The HE60D extract, which contained higher amounts of luteolin- and apigenin-7-*O*-glucoside, demonstrated a stronger antilipoperoxidant activity. Its effect was also better than the effect of the extract from our previous study, made by the ultrasound method and 80% ethanol ($\text{IC}_{50} = 19.13 \pm 1.70$ $\mu\text{g}/\text{ml}$).

The antimicrobial activities of *Salvia* species are primarily ascribed to the synergistic actions of their volatile compounds, flavonoids, phenolic acids, and tannins (19, 64, 65). The examined *S. sclarea* extracts inhibited the growth of Gram-positive bacteria more effectively than that of Gram-negative bacteria, demonstrating greater bacteriostatic activity compared to bactericidal activity. Considering the high prevalence and growing resistance of staphylococci (66), good antibacterial properties against *S. aureus* (MIC = 6.25 mg/mL) are very important. The extracts were also effective against *B. cereus* and *L. monocytogenes*, which is noteworthy considering their role as significant causative agents of gastrointestinal infections (67). Although the effects against Gram-negative bacteria are weak, moderate bacteriostatic activity against *P. aeruginosa* and *E. aerogenes* can be distinguished for HE80D. These pathogens belong to the group of nosocomial and opportunistic bacteria in medical centers, and their treatments

are quite difficult due to their multi-resistant nature. In our study, *C. albicans* was found to be resistant to the *S. sclarea* extracts, indicating that they cannot be considered effective antifungal agents. Similar antimicrobial effects were reported for other ethanolic and methanolic *S. sclarea* extracts from our previous studies (42, 44). Polyphenols are known for their strong antimicrobial properties, and their presence in extracts plays a significant role in inhibiting the growth and destroying microorganisms (68). Numerous *in vitro* studies have shown that the polyphenols identified in the tested extracts exhibit these effects, particularly against Gram-positive bacteria (42, 69–71).

Salvia species have a long history of use in treating various gastrointestinal complaints, thanks to their exceptional spasmolytic properties (72). Todorov et al. (1984) (73) were the first to demonstrate the *in vitro* spasmolytic properties of *Salvia* species by showing their ability to inhibit ileum contractions induced by acetylcholine, histamine, serotonin, and barium chloride. The investigated *S. sclarea* extract significantly reduced spontaneous rat ileum contractions. The isolated intestinal preparation contraction is induced by an increase in the concentration of free calcium ions in the cytoplasm, which activates the contractile elements. This rise in intracellular calcium concentration occurs through voltage-dependent L-type calcium channels or through release from the sarcoplasmic reticulum. Periodic depolarization governs spontaneous movements, and at the peak of depolarization, an action potential is generated due to a rapid influx of calcium ions through these channels (74, 75). The first evidence of the spasmolytic activity of *S. sclarea* was presented by researchers Lis Balchin and Hart (1997) (76), who found that its essential oil significantly reduced the contractions of the small intestine of guinea pigs. Furthermore, the spasmolytic actions of the *S. sclarea* extracts were revealed in our two studies. Namely, the ethanolic and methanolic extracts prepared by maceration and ultrasound extraction expressed relaxant effects on

spontaneous, but also on KCl- and acetylcholine contractions. The extract made of concentrated ethanol showed the best spasmolytic effects on spontaneous contractions with EC_{50} of 1.33 ± 0.09 mg/ml (42). In addition, docking analysis indicated that apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside exhibited the highest binding affinity to voltage-gated calcium channels (44).

Conclusion

The investigated hydroethanolic *Salvia sclarea* extracts are significant sources of polyphenolic compounds, and are particularly notable for their high content of rosmarinic acid. In addition to it, caffeic acid and flavonoids of the flavone type were also determined. These extracts can be characterized as excellent antioxidant agents because they successfully suppressed free radical production in both model systems used. In addition, they exhibited moderate antibacterial effects, acting more as bacteriostatic than bactericidal agents and could, therefore, serve as supportive agents in managing some infectious disorders. It is of great importance that the extracts successfully reduced spontaneous rat ileal contractions in *in vitro* conditions, and could be beneficial in managing associated gastrointestinal complaints. Hence, *S. sclarea* and its extracts hold promise as potential herbal remedies in modern phytotherapy. Nevertheless, additional studies are necessary to validate their efficacy in human subjects.

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doi: 10.5633 / amm.2025.0203**ANTIOKSIDATIVNI, ANTIMIKROBNI I SPAZMOLITIČKI
EFEKTI HIDROETANOLNIH EKSTRAKATA MUSKATNE
ŽALFIJE (*SALVIA SCLAREA* L.) PRIPREMLJENI
METODOM DIGESTIJE**

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Muskatna žalfija (*Salvia sclarea* L.) poznata je u tradicionalnoj medicini kao aromatična i lekovita biljka koja se koristi u tretmanu respiratornih, digestivnih i menstrualnih tegoba i različitih inflamatornih procesa poput gingivitisa, stomatitisa, afti i artritisa. Cilj ovog rada bio je da ispita antioksidativne, antimikrobne i spazmolitičke efekte vodeno-etanolnih ekstrakata nadzemnih delova biljke *S. sclarea*, koji su sakupljeni u Malči, u okolini Niša u Srbiji. Pripremljena su dva ekstrakta tehnikom digestije sa etanolom od 80% i etanolom od 60% (HE80D i HE60D, redom). Ekstrakti su bili hemijski okarakterisani i tada je utvrđeno da sadrže značajne količine fenolnih jedinjenja, među kojima je ruzmarinska kiselina bila najdominantnija. Ispitivani ekstrakti su ispoljili značajne antioksidativne potencijale u sistemima 2,2-difenil-1-pikrilhidrazil i β -karoten/linolenska kiselina. Rezultati su pokazali da su ekstrakti naročito efikasni kao antilipoperoksidativni agensi. Ekstrakti su najizraženije antimikrobne efekte imali na bakteriju *Staphylococcus aureus*, dok su oni koje su ispoljili prema bakterijama *Bacillus cereus* i *Listeria monocytogenes* bili umereni. Ekstrakti su pokazali značajnu inhibiciju spontanijih kontrakcija glatkih mišića ileuma pacova. Pri maksimalnoj koncentraciji HE80D i HE60D ekstrakata od 1,5 mg/ml kontrakcije ileuma bile su smanjene za $40,45 \pm 2,06\%$ i $40,60 \pm 1,22\%$, redom. Dalja istraživanja ekstrakata biljke *S. sclarea* treba usmeriti na detaljnija *in vivo* ispitivanja i kliničke studije kako bi se sagledala mogućnost njihove upotrebe u racionalnoj fitotetapiji.

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Ključne reči: *Salvia sclarea* L., vodeno-etanolni ekstrakti, antioksidativni efekti, antimikrobni efekti, spazmolitički efekti

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EPIDEMIOLOGICAL CHARACTERISTICS OF HOSPITAL INFECTIONS CAUSED BY *CLOSTRIDIUM DIFFICILE* AT THE UNIVERSITY CLINICAL CENTRE OF NIŠ

Bojan Stanojević¹, Nikola Milenković¹, Sonja Novak¹, Ivica Grozdanović², Goran Maksić³, Zvonko Zlatanović⁴

Hospital (nosocomial, hospital-acquired) infections represent the greatest health challenge in more developed countries worldwide. Infections caused by the bacterium *Clostridium difficile* have been a serious medical problem for years. Despite the constant progress and improvement of diagnostic and therapeutic procedures, they still represent one of the most frequent and most severe hospital-acquired infections, and their incidence rate, mortality rate, as well as severity of their clinical picture are all on the rise. This paper aimed to analyse the epidemiological situation surrounding hospital-acquired infections caused by the bacterium *Clostridium difficile* at the University Clinical Centre of Niš between 2015 and 2019. In this study, *Clostridium difficile* was the most frequent cause of hospital-acquired gastrointestinal infections and was identified in as many as 48% of the studied samples. Preventive measures to control the spread of *Clostridium difficile* in a hospital environment are based on identifying transmission routes. In 1995, the first prevention and control guidelines were issued, while the Center for Disease Control and Prevention in Atlanta (CDC) modified the guidelines to control the spread of *Clostridium difficile* in health facilities, which were re-evaluated in 2005, while hygiene guidelines following contact with a patient infected with *Clostridium difficile* were added. The European Centre for Disease Prevention and Control (ECDC) published a new set of guidelines in 2008, while the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) published the most recent set of guidelines in 2010. A comprehensive examination into the interplay of different aspects of important factors dealing with *C. difficile* would provide a more detailed understanding of the various factors that could inform academics and practitioners and improve theoretical knowledge and actual practices in this really important area of public health. In that sense, the study of potential and significant factors which could have a significant impact on the health of the population, especially illnesses caused by the bacterium *C. difficile* in a hospital environment, should be an imperative in the scientific and professional sphere.

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Key words: hospital-acquired infections, *Clostridium difficile*, disease prevention and control

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Introduction

Hospital (nosocomial, hospital-acquired) infections (HIs) represent the greatest health challenge in the developed countries of the world.

They include infections contracted by both patients and staff in hospitals or other health facilities. They can manifest as a local or a systemic illness (conditions) which represents the body's response to the presence of an infective agent (one or more) or its toxins, not found in the patient, when the patient was not in an incubation period before admission to the hospital or some other health institution (1–3).

Despite the considerable progress in the development of medicine, as well as the progress made in further developing hospital epidemiology, according to the World Health Organization (WHO), hospital-acquired infections are to this day a global problem which considerably increases overall morbidity and mortality, lengthens treatment time, and increases the overall cost of healthcare. The spread of multidrug-resistant

pathogen bacteria is a particular problem, as is the transmission of infections onto healthcare workers and into the environment (4, 5).

Infections caused by the *Clostridium difficile* (*C. difficile*) bacteria have been a serious medical problem for years. Despite the constant progress and improvement of diagnostic and therapeutic procedures, *C. difficile* infections (CDIs) still represent one of the most frequent and severe hospital-acquired infections, and their incidence rate, mortality rate, and the severity of the clinical picture of a CDI are all on the rise (5, 6). *C. difficile* can normally be found among 3% of the adult population, while the carriage of this bacterium in the digestive tract of hospitalized patients is much higher and reaches up to 21% (8, 9).

This paper aimed to examine the epidemiological situation related to hospital-acquired infections caused by the bacterium *C. difficile* at the University Clinical Centre of Niš from 2015 to 2019. By determining the basic epidemiological characteristics, we obtained the necessary elements to form guidelines for the measures and procedures aimed at preventing and controlling hospital-acquired infections caused by these bacteria at the University Clinical Centre of Niš.

Materials and Methods

This paper includes data from the archives of the Institute of Public Health Niš, the Centre for Disease Control and Prevention, as well as reports of the Department of Sanitary and Epidemiological Supervision of the University Clinical Centre of Niš, including reports on trends in nosocomial infections caused by the bacterium *C. difficile* at the University Clinical Centre of Niš from 2015 to 2019, reports on trends in nosocomial infections caused by the bacterium *C. difficile*, and reports on patients hospitalized at the University Clinical Centre of Niš.

A descriptive method was used to analyse the data. All the data were statistically processed using Microsoft Office and Excel (version 2019).

Results

The University Clinical Centre of Niš consists of 36 organizational units (OUs) of which 14 OUs are clinics of particular surgical branches. In 2018, the General Surgery Clinic was reorganized, and the following clinics were formed: the Abdominal Surgery Clinic, Endocrine Surgery and Breast Surgery Clinic, Vascular Surgery Clinic, Digestive Surgery Clinic, and as a special unit, the Anaesthesiology and Intensive Therapy Clinic.

The data analysis is shown in Table 1, and based on it, we can conclude that in 2015, most HIs caused by the bacterium *C. difficile* were reported in the Physical Medicine and Rehabilitation Clinic (16 infections) and the Gynaecology and Obstetrics Clinic (15), with the highest incidence rate reported in the Physical Medicine and Rehabilitation Clinic (14.16%) and the Urology Clinic (10.68%). The Endocrinology, Haematology, Infectious Disease Clinics, as well as the Vascular, Cardiology, and Paediatric Surgery Clinics, had no instances of infections caused by the bacterium *C. difficile* (Table 1).

In 2016, most hospital-acquired infections caused by *C. difficile* were reported in the Cardiology Clinic (12), while the highest incidence rate was reported in the Physical Medicine and Rehabilitation Clinic (5.38%). The Endocrinology, Haematology, Nephrology, Oncology, Infectious Disease, Paediatric, Gynaecology, as well as the Vascular and Paediatric Surgery Clinics had no instances of hospital-acquired infections caused by the bacterium *C. difficile*.

In 2017, the greatest number of HIs caused by the bacterium *C. difficile* were reported in the Cardiovascular Surgery Clinic (10) and the Nephrology and Neurosurgery Clinics (8). The highest incidence rate for that year was reported in these clinics. The Oncology and Paediatric Clinics, as well as the Vascular and Paediatric Surgery Clinics, had no infections caused by the bacterium *C. difficile*.

Based on the analysis of data from 2018, most of the HIs caused by the bacterium *C. difficile* originated from the Neurosurgery Clinic (10), which had the highest noted incidence rate (6.39%). In the remaining clinics, a significantly lower number of incidences were noted.

Finally, in 2019, most of the HIs caused by the bacterium *C. difficile* were reported in the Nephrology Clinic (7), which also had the highest incidence rate (5.87%). In the Endocrinology, Haematology, Paediatric, Oncology, and Neurology Clinics, as well as the Vascular and Paediatric Surgery Clinics, no hospital-acquired infections caused by the bacterium *C. difficile* were reported.

Epidemics in the University Clinical Centre of Niš

From 2015 to 2019, there were several epidemics at the University Clinical Centre of Niš, two of which were epidemics in the operating theatres, in 2015 and 2016, at the General Surgery Clinic.

Table 1. The number of findings and the incidence rate of HI caused by the bacterium *Clostridium difficile* from 2015 to 2019

CLINIC	The number of findings/ incidence rate in 2015	The number of findings/ incidence rate in 2016	The number of findings/ incidence rate in 2017	The number of findings/ incidence rate in 2018	The number of findings/ incidence rate in 2019
CARDIOLOGY	6/1.13	12/2.31	6/1.13	1/0.18	2/0.35
GASTROENTEROLOGY AND HEPATOLOGY	6/2.52	8/3.32	2/0.93	2/1.07	2/1.08
ENDOCRINOLOGY	0.00	0.00	1/0.80	0.00	0.00
HAEMATOLOGY	0.00	0.00	1/0.88	0.00	0.00
NEPHROLOGY	10/9.16	0.00	8/7.92	1/1.00	7/5.87
ONCOLOGY	2/0.69	0.00	0.00	0.00	0.00
INFECTIOUS DISEASE	0.00	0.00	4/3.82	2/2.18	4/5.27
PEDIATRIC	3/0.37	0.00	0.00	0.00	0.00
PULMOLOGY	3/1.13	3/1.16	1/0.41	0.00	2/1.43
NEUROLOGY	10/5.28	0.00	6/3.39	0.00	0.00
REHABILITATION	16/14.16	6/5.38	3/2.91	1/0.94	5/4.94
GENERAL SURGERY	10/3.37	2/0.66	2/0.67	0.00	0.00
NEUROSURGERY	13/8.80	3/2.01	8/	10/6.39	4/2.33
ORTHOPEDIC	3/1.46	2/1.02	4/2.03	0.00	1/0.49
UROLOGY	10/10.68	8/4.20	3/1.74	0.00	3/1.61
VASCULAR SURGERY	0.00	0.00	0.00	0.00	1/2.13
PLASTIC SURGERY	2/2.27	3/3.75	1/1.03	0.00	0.00
CARDIOSURGERY	0.00	5/3.56	10/7.69	1/1.22	1/2.34
GYNAECOLOGY	15/2.28	0.00	6/0.85	0.00	1/0.15
PEDIATRIC SURGERY	0.00	0.00	0.00	0.00	0.00
EMERGENCY AND THORACIC SURGERY	2/1.75	4/2.63	1/0.72	1/1.77	1/1.36

An epidemic caused by the bacterium *Clostridium difficile* in the Neurosurgery

Clinic of the University Clinical Centre of Niš in 2015

A hospital epidemic caused by the bacterium *C. difficile* in the Neurosurgery Clinic of the University Clinical Centre of Niš was declared on April 28, 2015. An epidemiological study determined that the epidemic began on April 22, 2015, when three patients contracted the infection

(two females and one male). The number of exposed patients and health workers totalled 95. Following adequate and rapid implementation of measures, the spread of the bacteria in hospital conditions was prevented, and the epidemic was called off on May 6, 2015. There were no casualties.

The source of the contagion was not determined. The patients exhibited the following clinical symptoms: watery stools, weakness, and fatigue. The proposed measures to control the epidemic were: reporting the epidemic, reporting

the infected patients, epidemiological testing, and isolation of the infected; acquiring stool samples from all the exposed patients for laboratory analysis; spatial and personal isolation; heightened measures of general and personal hygiene of the patients and staff; more frequent cleaning of the wards with bleach-based chemical products; the use of gloves and single-use lab coats (PVC aprons); reduced visits; prohibiting outside food intake; as needed, consulting with an infectious disease specialist; health-related and educational work with everyone involved.

An epidemic caused by the bacterium *Clostridium difficile* at the Neurology Clinic of the University Clinical Centre of Niš in 2015

A hospital epidemic caused by the bacterium *C. difficile* in the Neurology Clinic of the University Clinical Centre of Niš was declared on July 14, 2015. Through an epidemiological analysis, it was determined that the epidemic broke out on June 24, 2015, when three patients became infected (one female and two males). The number of exposed patients and healthcare workers amounted to 57. After adequate and rapidly implemented measures, the spread of the bacteria in hospital conditions was stopped, and the epidemic was called off on July 28, 2015. There were no casualties.

The source of the contagion was not determined. The patients had the following clinical symptoms: watery stools, weakness, and fatigue. The proposed measures to control the epidemic covered the following steps: reporting the epidemic; reporting the infected patients; epidemiological testing and isolation of the infected; acquiring stool samples from all the exposed patients for laboratory analysis; spatial and personal isolation; heightened measures of

general and personal hygiene of the patients and the staff; more frequent cleaning of the wards with bleach-based chemical products; the use of gloves and single-use lab coats (PVC aprons); reduced visits; prohibiting outside food intake; as needed, consulting with an infectious disease specialist; health-related and educational work with everyone involved.

Discussion

The problem of hospital infections emerged with the introduction of hospital treatment. Little was known about the causes and means of transmission of HIs, and virtually nothing was known about disinfection, sterilization, and aseptic techniques (10–12). The work of Ignaz Philipp Semmelweis provided significant progress in that respect, and remains an example of the importance of epidemiology in the prevention and control of HIs, a full decade before the microbiological discoveries of Pasteur and Koch. Specifically, Semmelweis noted the difference in the maternal mortality rate in two different clinics of the maternity ward of the University Hospital in Vienna. A higher maternal mortality rate was registered in the clinic, which was part of the university hospital, where the doctors and students often, straight from performing autopsies, without previous preparations, took part in deliveries (Figure 1) (13, 14). In the clinic with a lower maternal mortality rate, the midwives were in charge of births. After implementing Semmelweis's guidelines on hand hygiene (hand disinfection using calcium hypochlorite), the maternal mortality rate was reduced significantly in both clinics, in the one that was part of the university hospital by 89%, and the one run by midwives by 52% (15, 16).

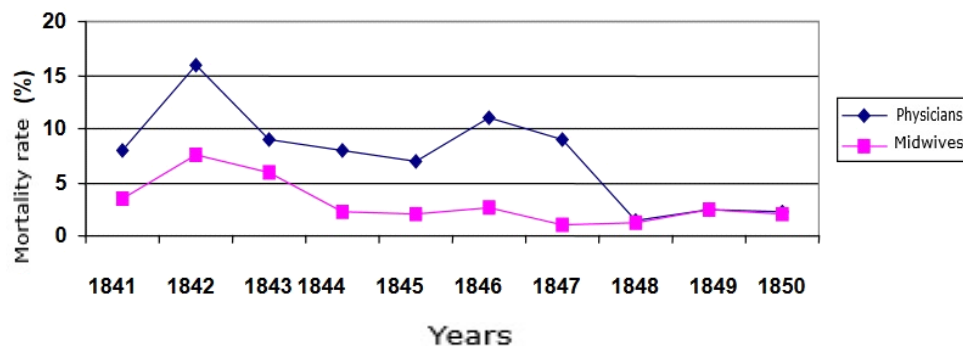


Figure 1. The maternal mortality rate due to infection in the University Hospital in Vienna, Austria, 1841–1850 (7)

All this is an indication that infections caused by the bacterium *C. difficile* have been a serious medical problem for years, especially when it comes to hospital-acquired infections. Considerable efforts have been invested into developing and improving national health strategies, considering that it is known that the quickest means of transmission is through direct contact with the source of the contagion or indirect contact involving the hands of the staff or contaminated medical equipment. It is estimated that at least 20% of HIs can be prevented by implementing specific means of supervision and control. In order to increase the quality of healthcare and the safety of the patients, it is necessary to gain as detailed an insight as possible into the nature and scope of the existing problem. Precisely because of that, epidemiological and microbiological studies of these infections are more than welcome. Their implementation is considerably advocated for and supported by European and American associations (the CDC, ECDC, and ECMID). Thus, this study too was carried out in accordance with their guidelines and with the desire to improve the quality of healthcare at the University Clinical Centre of Niš.

In this study, *C. difficile* was the most frequent cause of hospital-acquired infections of the gastrointestinal system, and it was identified in as many as 48% of the studied samples. Great differences were noted in the prevalence of HA-CDIs among various countries, which is a consequence of the varying frequency of testing, as well as the varying sensitivity and the specific nature of the diagnostic tests. This is probably a result of a lack of funding, but also diagnostic guidelines. Even though numerous European countries have developed regional and/or national networks for reporting CDIs, based on the data provided by the European Commission, monitoring is not standardized or legally binding in all European countries (17–22).

In order for *C. difficile* to cause diarrhoea, at least three conditions need to be met: a change in the normal ratio of bacteria inhabiting the digestive tract, the production of the *C. difficile* toxin, and particular characteristics on the part of the host. Normal intestinal flora among adults usually prevents the onset of colonies of *C. difficile*. *C. difficile* secretes two main toxins: enterotoxin (toxin A) and cytotoxin (toxin B). Previously, it was considered that the strain which only secretes toxin B (A-/B+) cannot cause damage to the mucus tissue of the colon. However, recent studies have shown that toxin B is the main determinant in the virulence and that avirulent strains only secrete toxin A (23, 24). Kuehne et al. (25, 26), based on a genetic analysis, determined that the production of one or both toxins indicates cytotoxin activity both *in vitro* and *in vivo*, so that the current hypothesis regarding the importance of both toxins for the onset of the illness has been adopted. Research done in the relevant laboratories for anaerobic

infections of the Institute of Public Health Niš in 2014 resulted in the first isolates of *C. difficile* from 175 samples taken from the environment (23, 24, 27). In soil samples from within the University Clinical Centre of Niš, the parks in the city of Niš, and mud and sand samples from unregulated sewer systems in the municipality of Niška Banja, small numbers of the *C. difficile* bacteria which produce toxins (A+B+) as well as non-toxigenic isolates (A-B-) were registered. The role of the third toxin, known as the binary toxin, has still not been sufficiently studied, even though it caused epidemics associated with a very serious clinical picture (24, 28, 32). The *C. difficile* ribotype 027, which is toxin A-positive and toxin B-positive, also has a gene for a binary toxin, as well as an 18 bp deletion and a mutation on the *tcdC* gene, leading to a disruption in the expression of toxins A and B. This strain leads to the considerable production of the toxin *in vitro* and is particularly important as it is resistant to fluoroquinolones. A study carried out in 38 hospitals from 14 European countries indicated that the prevalence of the epidemic-inducing 027 strain of *C. difficile* was 6.2%, that all the strains were positive for the binary toxin, resistant to erythromycin and moxifloxacin, but also sensitive to metronidazole and vancomycin. However, the infected patients had a much more severe form of the illness (29). The model of origin of the CDI is as follows: patients are admitted to the hospital, and although intermittently exposed to spores of *C. difficile*, they have a negligible risk of the occurrence of the condition until the application of antibiotics. After they have been included, especially when it comes to broad-spectrum penicillins, cephalosporins, and clindamycins, the normal intestinal flora is disrupted, so that when *C. difficile* enters the digestive tract of such patients, it can proliferate and colonize the mucus tissue without causing any kind of symptoms of the illness (asymptomatic colonization), in the case of a strain which does not secrete toxins or a strain which secretes toxins but where an immunological response has been activated through the secretion of IgG. However, if there is no immunological response, symptomatic conditions will occur (30–32).

A high rate of possible carriage persists over the first 8 months of life, until normal intestinal flora is achieved, and then it decreases to about 2–3% among adults. A study carried out in Sweden indicated a carriage rate of 1.9% in the case of 594 volunteers, while Philips and Rogers (33) determined a rate of 2% after analysing 100 randomly selected stool samples, which were negative for other pathogens of the digestive tract. A special aspect of transmitting *C. difficile* is the carriage of this bacterium in the digestive tract of hospitalized patients. Carriage among hospitalized individuals is considerably higher (up to 21%) (5). In prospective studies in which patients were systematically examined for the presence of the bacteria during admission and one

or possibly two weeks after admission to hospital in Western Europe, the rate of *C. difficile* carriage after a stay in hospital ranged from 5.9 to 11%. This is a much higher rate than the 3% which occurs among the healthy adult population and could be a consequence of using antibiotics or colonisation during previous hospitalizations. A study carried out in Niš also indicated a higher carriage rate of *C. difficile* among hospitalized patients (7%), as well as the presence of toxigenic strains in the intestines of 2% of hospitalized patients with formed stool (34).

The incidence rates of CDIs vary depending on the type of hospital, country, and time of analysis. In all of North America, the incidence rate has been on the rise over the past fifteen years, despite preventive measures, with the highest noted in university hospitals. Over the past decade, the number of the infected has doubled (35, 36). In a prevalence study carried out in 2008 in 648 hospitals in the USA, it was determined that 13 out of 1,000 hospitalized patients (1.3%) were suffering from CDIs or were colonised (37, 38). That is a higher rate than previously predicted. This considerable increase can be ascribed to the occurrence of a very virulent ribotype 027 (NAP1/HI/27), which caused major epidemics in the USA and Canada from 2001 to 2003 (39–42). Even though the increase in the incidence rate can be a reflection of improved diagnostics and the identification of this condition, it is still clear that the incidence rate has actually increased. In Europe, the incidence rates also vary by country and hospital. It was recently estimated that the average incident rate in Europe was 4.1 per 10,000 hospitalized patients. It is important to note that, after many epidemics which were caused by ribotype 027, other ribotypes of *C. difficile* are now also prevalent in Europe. In a large-scale study which included data from 106 laboratories from 34 European countries (the aim was to compile data from at least one hospital in each country with a population of fewer than two million citizens, three hospitals in the case of two to twenty million citizens, and five hospitals in the case of more than twenty million citizens), according to the latest published data for November 2008, the most frequent ribotypes were 014 and 020 (identified in 19 countries), then 001 (in 13 countries), and 078 (in 18 countries). The PCR-ribotype 027 is sixth in terms of frequency of occurrence (41, 42). Few data exist on the frequency of occurrence of CDIs in the countries of South-East Europe. In the cited study for Europe, there are data for Bulgaria, Croatia, Slovenia, and Greece. The average incidence rate of hospital-acquired infections related to *C. difficile* ranges from three per 10,000 hospitalized patients in Bulgaria, six in Croatia, 19 in Slovenia and 29 in Greece (42, 44). In a study carried out during 2008 in Niš, in 12.4% of clinical stool samples obtained from patients experiencing diarrhoea, a cause was isolated. Of the overall number of

positive isolates (351), *C. difficile* was isolated in 27.1% (45).

Even though most of the patients with CDIs are hospitalized, this condition should not be linked to medical interventions, since it can also occur in institutions for long-term care, housing for the elderly, and also in day hospitals. A reservoir could also be colonized (asymptomatic) or ill patients, and the hospital environment. Cases of CDAD noted in a health institution, where there are also numerous asymptomatic carriers in the same room, or on the same ward, should also be taken into consideration, as they represent a significant source of hospital diarrhoea. Still, routine screening is not recommended as a preventive measure. All surfaces and objects contaminated by spores of *C. difficile* (the floor, furniture, and medical equipment) are also a significant reservoir. The rate of contamination usually correlates with the epidemic nature of the illness.

According to the results of a study of European countries, serious comorbidities and antibiotic therapy were considered risk factors for older age patients (approximately two-thirds of those infected were over the age of 65). Almost all the patients who took antibiotics for three months before a *C. difficile* infection usually received cephalosporins, quinolones, and beta-lactamase inhibitors (28). Almost all the antibiotics administered either orally or parenterally can be linked to CDIs. The first antibiotic linked to this illness was clindamycin. More recent studies indicate that this illness is linked to therapy involving cephalosporins and fluoroquinolones (45, 46). The more recent occurrence of the resistance of *C. difficile* to fluoroquinolones is linked to their broad application (45, 46). Ceftriaxone is especially marked as an antibiotic that disrupts normal gastrointestinal flora, and has no effect on *C. difficile* itself, which promotes the occurrence of CDIs during its use in the recovery stage of intestinal flora. However, not all patients taking antibiotics will develop a CDI as well. What is also important is the ability of the immune system to produce the serum IgG anti-toxin A. This explains why CDIs are more frequent among the elderly. It should be mentioned that using more antibiotics at the same time is linked to a higher risk of CDI occurrence (42, 45).

The incidence rate of CDADs varies and ranges from 0.1 to 2% (47–51). This incidence rate includes patients who are hospitalized due to a *C. difficile* infection, as well as patients with a hospital-acquired *C. difficile* infection. Most researchers agree that the infections caused by *C. difficile* occur in a hospital environment. Insufficient attention is paid to the role and prevalence of *C. difficile* as the cause of diarrhoea in non-hospital environments.

Measures aimed at preventing the spread of *C. difficile* in hospital environments depend on the identification of transmission routes. Back in 1995, the first guidelines for the prevention and control

of CDIs were provided. The CDC in Atlanta published guidelines for the prevention and control of *C. difficile* in healthcare facilities, which were renewed in 2005, and recommendations for hand hygiene following contact with a patient with a CDI were added (52–54). The European Centre for Disease Prevention and Control (ECDC) published new guidelines in 2008, while SHEA and IDSA published the most recent set of guidelines in 2010 (55–57).

Conclusion

Illnesses caused by the bacteria *C. difficile* in a hospital environment represents a great challenge for modern-day medicine. An increase in the number of more severe cases and the irresponsible use of antibiotics are the main factors which contribute to their spread. Healthcare workers and health service providers

are facing the important task of adopting suitable and proven preventive measures and, where necessary, therapeutic measures, so that the spread of the illness caused by *C. difficile* could fully be brought under control. A comprehensive examination into the interplay of different aspects of important factors dealing with *C. difficile* would provide a more detailed understanding of the various factors that could inform academics and practitioners and improve theoretical knowledge and actual practices in this really important area of public health. In that sense, the study of potential and significant factors which could have a significant impact on the health of the population, especially illnesses caused by the bacterium *C. difficile* in a hospital environment, should be an imperative in the scientific and professional sphere.

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EPIDEMIOLOŠKE KARAKTERISTIKE BOLNIČKIH INFEKCIJA IZAZVANIH BAKTERIJOM *CLOSTRIDIUM DIFFICILE* U UNIVERZITETSKOM KLINIČKOM CENTRU NIŠ

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Bolničke (nozokomijalne, intrahospitalne) infekcije predstavljaju najveći izazov u zdravstvu u razvijenim zemljama. Infekcije izazvane bakterijom *Clostridium difficile* ozbiljan su medicinski problem već godinama. I pored konstantnog napredovanja i usavršavanja dijagnostičkih i terapijskih procedura, ova infekcija je i dalje jedna od najčešćih i najtežih infekcija stečenih u bolničkoj sredini. Došlo je i do porasta incidencije, obolevanja, smrtnosti, kao i težine kliničke slike. Cilj ovog rada bio je da se sagleda epidemiološka situacija u vezi sa bolničkim infekcijama izazvanim bakterijom *Clostridium difficile* u Univerzitetskom kliničkom centru u Nišu u periodu od 2015. do 2019. godine. Ovo istraživanje je pokazalo da je bakterija *Clostridium difficile* bila najčešći uzročnik bolničkih infekcija gastrointestinalnog sistema; dokazana je čak kod 48% ispitanih uzoraka. Mere sprečavanja širenja bakterije *Clostridium difficile* u bolničkoj sredini zasnivaju se na poznavanju puteva prenošenja. Godine 1995. date su prve preporuke za prevenciju i kontrolu, a Centri za prevenciju i kontrolu bolesti u Atlanti objavili su preporuke za prevenciju širenja bakterije *Clostridium difficile* u zdravstvenim ustanovama, koje su dopunjene 2005. godine. Dodate su i preporuke za higijenu ruku posle kontakta sa bolesnikom sa bakterijom *Clostridium difficile*. Evropski centar za prevenciju i kontrolu bolesti publikovao je 2008. godine nove preporuke. Godine 2010. SHEA (engl. *Society for Healthcare Epidemiology of America*) i IDSA (engl. *Infectious Diseases Society of America*) izdale su najnovije preporuke. Sveobuhvatna istraživanja i objašnjenja u vezi sa međusobnim odnosima različitih aspekata važnih za borbu sa efektima prouzrokovanim bakterijom *Clostridium difficile* omogućila bi da se bolje i jasnije razumeju razni faktori koji su od značaja za unapređenje znanja ne samo članova akademske zajednice nego i profesionalaca u praksi. Na taj način mogla bi se proširiti teorijska saznanja, ali i poboljšati načini reagovanja u praksi u veoma važnoj oblasti javnog zdravlja. Dakle, studije o potencijalnim i značajnim faktorima koji utiču na zdravlje ljudi, a posebno o oboljenjima prouzrokovanim bakterijom *Clostridium difficile* u bolničkim uslovima, moraju se postaviti kao svojevrsan imperativ, kako u naučnoj sferi, tako i u medicinskoj praksi.

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Ključne reči: intrahospitalne infekcije, *Clostridium difficile*, prevencija i kontrola bolesti

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RESULTS OF THE COMPARATIVE STUDY ON THE ONSET AND FREQUENCY OF OCCUPATIONAL ALLERGIC CONTACT DERMATITIS AMONG WORKERS IN THE RUBBER AND CONSTRUCTION INDUSTRIES

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Occupational skin diseases are changes in the skin and its adnexa which occur under the influence of various exogenous and endogenous factors. They can be caused by irritants or allergens (irritant or allergic contact dermatitis). To examine the impact of the work environment and occupational hazards concerning the occurrence of Occupational Allergic Contact Dermatitis (OACD), a study on workers in the rubber and construction industries diagnosed with contact dermatitis (CD) was conducted. The structure of the workers was presented according to sex, place of residence, age, qualifications, years of service, type of allergen and work position. The importance of medical, occupational, and social rehabilitation was emphasized, as well as prevention measures (professional orientation, education and health education of the workers). The following conclusion was reached—in the construction industry, men are more frequently affected and there is a higher risk of OACD, although a longer time of exposure is required. The causing allergens are varied. It is imperative to register those affected by the disease and to undertake the corresponding measures of treatment and prevention.

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Key words: occupational dermatitis, allergic contact dermatitis, allergens

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Introduction

Occupational diseases of the skin are changes in the skin and its adnexa, which occur while performing the profession under the influence of varied exogenous (physical, biological, or chemical) or endogenic (sex, age, race, genetic diseases, atopic) factors. They are among the top five registered occupational diseases in the EU, making up between 10% and 40% (1) or even 40–50% (2) of the total number of registered cases and represent a significant health issue in Europe, while for instance, the one in Austria includes 52, in Italy 58, in Germany 67, in England 70, in France 98 (3).

Occupational contact dermatitis (OCD), which occurs as a consequence of contact with chemicals, is the most frequent amongst occupational skin diseases (4), (as much as 90–95% of these diseases with irritant contact

dermatitis (ICD) in 80% and allergic contact dermatitis (ACD) in 20% of cases (5). It is estimated that more than 85,000 chemicals in human surroundings can act as irritants or allergens and cause irritant or allergic CD (6).

ACD occurs after repetitive contact of the sensitized person with the sensitizer after a IV type allergic reaction (7), which plays out in two stages—induction stage (primary response) and elicitation stage (secondary response) (8–10). Contact sensitization occurs in 20% of the population (11). The frequency of CD in the USA is 13.6% per 1,000 residents. Clinical signs vary depending on the stage of the disease and the containment of the changes. In the acute stage, the place of contact is affected by erythema, papulae, vesicles, pustules, possibly blisters with continuous oozing, creation of scabs and flaking of the skin. In the case of chronic disease, the skin is rough, thick, with dark reddish color, lichenified, with rare papulae, flakes and sometimes cracks (12).

Occupational allergic contact dermatitis occurs within the work environment and has the same characteristics (13), as well as many differences depending on the environment and the occupation. We performed examinations on workers from the rubber industry and construction, two very different work environments, and we received specific results. After the examination, extensive clinical history was written out, and a finding was made that the

changes in the skin are of the contact dermatitis typem (14). Epicutaneous (patch) testing was performed, using suitable allergens. For the workers in the rubber industry, it was a "rubber series" of 30 allergens, with the addition of standard and domestic series as needed (15). Workers from the construction industry were tested with a series according to the substances they come in contact with while performing their jobs. Further action was dependent on the results of the testing (16).

Aim

A group of workers in the rubber and construction industries who had skin changes of the CD type were tested to examine the effect of the working environment and occupational hazards with regard to the occurrence of OACD. It is only after the causes of the disease are determined that the appropriate treatment measures and appropriate therapy can be applied to the diseased person, as well as prevention and protection at the workplace, including leave of absence, possibly even a change of job or work conditions.

Materials and Methods

At the Rubber Products Factory in Niš, 1,032 workers were examined. Signs of CD were registered in 90 (8.72%) workers. Epicutaneous testing of rubber chemicals was conducted on 80 workers.

In the period of 10 years, the Dermatovenerology Clinic in Niš tested 1,237 subjects. Four hundred and ninety-four workers tested positive. Amongst the tested workers, there were 160 (12.93%) construction workers. It was proved that 51 (75%) people who have OACD, have hypersensitivity to one or more allergens that they come into contact with while working. This amounts to 86.44% of all workers with ACD and 4.84% of all examined workers.

Examinations and epicutaneous testing were accompanied by certain parameters (sex, place of residence, age, years of service, qualification structure, work position, etc.), and they were presented in a chart.

Results and Discussion

Out of the 80 workers tested for chemicals found in rubber in the Rubber Products Factory, 59 tested positive—73.75% workers with CD, which was 5.71% of the total number of examined workers. Eight out of these 59 workers had no contact with chemicals in rubber; therefore, it was proved that 51 (75%) had hypersensitivity to one or more allergens with which they came into contact at the workplace and had ACD. This summed up to 86.44% of all workers with ACD and 4.84% of all examined workers.

Ninety-one construction workers (56.87%) tested positive, i.e., they represented 19.42% of the total number of workers who tested positive. A number of construction workers reacted to allergens that were not related to the profession. OACD was found in 61 workers, 38.12% of all construction workers, while 67.03% of construction workers who tested positive were diagnosed with ACD.

Table 1 shows structure of workers with OACD in the rubber and construction industries according to sex and place of residence

OACD was registered in 52.94% of men and 47.05% of women in the rubber industry workers. If we take into consideration the fact that out of the total number of tested workers, 74% were men and 26% were women, and 57% men had ACD, whereas 43% of women had ACD, it is clear that women are more sensitive to chemicals in the workplace and beyond.

There were no women with ACD or OACD among the construction workers.

Workers in the rubber industry were more frequently residents of the town (60.78%) rather than a village (39.20%), while the number of workers in the construction industry with OACD who reside in a village was slightly higher (56.87%).

Table 2 shows age structure and Years of Service of workers with OACD in the rubber and construction industries

In the rubber industry, OACD was the most frequent among workers in their fifth decade of life, followed by those in their fourth and then in their third decade of life. In the construction industry, it was the most frequent among workers in their fifties, then in their sixties and forties.

In the rubber industry, OACD was most frequently reported by workers with 21 to 30 years of service, followed by those with 11 to 20 years, then those with 6 to 10 years, and finally those with up to 5 years of service. It was the least common among those with over 30 years of service. OACD was recorded among workers with the first 10 years of service, at approximately 33.32%.

The situation was similar in the construction industry, with the most common occurrence in those with over 30 years of service, less frequent in those with between 6 and 10 years of service and the least common in those with up to 5 years of service. OACD was registered in 14.75% of workers in the first 10 years of service. This means that in the construction industry, OACD occurs after prolonged exposure.

Table 3 shows number and percentage of positive tests and order of allergens of workers with OACD in the rubber and construction industries

Table 1. Structure of Workers with OACD in the Rubber and Construction Industries according to Sex and Place of Residence

Sex	Male (Number %)	Female (Number %)	Total (Number %)
Rubber industry	27 (52.94)	24 (47.05)	51 (99.99)
Construction	61 (100.00)	-	61 (100.00)
Place of residence	Village (No %)	Town (No %)	Total (No %)
Rubber industry	20 (39.20)	31 (60.78)	51 (99.98)
Construction	35 (56.87)	26 (43.13)	61 (100.00)

Table 2. Age Structure and Years of Service of Workers with OACD in the Rubber and Construction Industries

Age	Rubber	Construction	Years of service	Rubber	Construction
	Number %	Number %		Number %	Number %
Up to 20	-	-	Up to 5	6 (11.76)	4 (6.55)
21–30	11 (21.58)	3 (4.92)	6–10	11 (21.56)	5 (8.20)
31–40	16 (31.37)	10 (16.39)	11–20	13 (25.49)	17 (27.86)
41–50	20 (39.21)	25 (40.98)	21–30	16 (31.36)	26 (42.62)
51–60	4 (7.84)	23 (37.70)	Over 30	5 (9.80)	61 (99.98)
Total	51 (100.00)	61 (99.99)	Total	51 (99.97)	61 (99.98)

Table 3. Number and Percentage of Positive Tests and Order of Allergens of Workers with OACD in the Rubber and Construction Industries

Rubber industry Type of Allergen	Number %	Construction Type of Allergen	Number %
Antioxidants	50 (40.98)	Potassium dichromate	60 (66.67)
Organic dissolvents	22 (18.03)	TMT (accelerator)	8 (8.89)
Vulcanising accelerants	17 (13.93)	Cobalt chloride	4 (4.44)
Glues	13 (10.66)	Phenergan	3 (3.34)
Synthetic rubber	6 (4.92)	Nickel sulfate	3 (3.34)
Natural rubber	5 (4.07)	Aniline	2 (2.22)
Mastication agent	4 (3.28)	Formaldehyde	2 (2.22)
Smoke	4 (3.28)	Other	x 1 (11.11) 8 (8.88)
Colophonium	1 (0.28)	(IPPD (antioxidant), Ursol, synopen, lanoline, glue, cement, white cement, grout mortar)	
Total	122 (100.00)	Total	90 (100.00)

In each group of chemicals in the rubber series with which the workers were tested, several allergens were present: 5 antioxidants with 50 positive tests, 4 organic dissolvents with 22 positive tests, 7 vulcanizing accelerators with 17 positive tests, 3 glues with 13 positive tests, 4 synthetic rubbers with 6 positive tests, 2 natural rubbers with 5 positive tests, Masticit VII and smoke with 4 positive tests each and colophonium with 1 positive test. In 51 workers with OACD, 122 positive reactions to 27 allergens were registered, which is an average of 2.19 reactions per worker.

In the construction industry, 90 positive reactions were registered in 61 workers, an average of 1.47 positive tests per worker.

Therefore, the conclusion is that polysensitization is somewhat more common among rubber workers than it is among construction workers.

The leading allergen among construction workers was potassium dichromate, which made up to 66.67% of all positive tests. A positive test was registered in 60 of the 61 workers with OACD (98.36%). Potassium dichromate is a hexavalent chromium salt which easily penetrates the skin. This is in fact a ubiquitous allergen found in cement and construction material, in products for protection against corrosion, in explosives, wood ash, in dyes and pigments, in synthetic perfumes, photographic and lab chemicals, glue, leather and textile (16). It is the cement that acts as the irritant at first, allowing penetration and sensitization. Metals: Cr, Co, and Ni have been labeled as a high-risk factor for the occurrence of ACD (17–19). As for cobalt, the metal itself and its salts can be factors of sensitization. It is used in the chemical and pharmaceutical industries and is found in dyes, cement, resin, glass, ceramics, etc. A simultaneous hypersensitivity to Cr, Co and Ni can occur due to the bonding of hapten to the same place of protein carrier (20).

Table 4 shows number and percentage of positive tests and order of allergens of the rubber series among workers with OACD

As for the order of allergens according to the number of positive tests, the Antioxidant 4010 Na or IPPD (N-isopropyl-N-phenyl-p-phenylene diamine) holds the first place with 13 + 17 (m + f) positive tests. It is a derivative of para-phenylenediamine, a sensitizer from the group of aromatic amines (21). It frequently causes crossed-sensibilization (aniline and azo dyes, sulphonamides, local anesthetics and other aromatic amines found in hair dye, food, cosmetic products (22).

From the list of antioxidants, we can list PAN (phenyl alpha naphthylamine) with 6 + 4 (m + f) positive tests, PBN (phenyl-beta-naphthylamine) with 4 + 1 (m + f) and DOD (4,4-dihydroxydiphenyl) with 2 + 2 (m + f) positive tests. The sensitization to antioxidants usually occurs in workers with longer years of service (23).

There were 17 reactions to vulcanizing accelerators: Vulkacid D 1 + 6 (m + f), to MBT 2

+ 2 (m + f), to Vulkacid DM 2 + 0 (m + f), to TMT 0 + 1 (m + f), to Vulkacid CZ 1 + 0 (m + f), to Vulkacid ZDBC 0 + 1 (m + f) and to Vulkacid H30 0 + 1 (m + f).

The reactions to organic dissolvents were as follows: to acetone 6 + 3 (m + f), xilol 1 + 4 (m + f), gasoline 3 + 2 (m + f), Toluene 1 + 2 (m + f).

Reactions to glues: Hemosil 20 2 + 6 (m + f), Hemosil 21 2 + 3 (m + f), white glue 0 + 1 (m + f).

Reactions to natural rubber: SMR 10 3 + 1 (m + f), SMR 20 1 + 0 (m + f).

Reactions to synthetic rubber: Perbunan 4 + 2 (m + f)

Reactions to Masticit VII 4 + 3 (m + f), to smoke 3 + 1 (m + f), to colophonium 0 + 1 (m + f).

Table 5 shows work positions of workers with OACD in the rubber and construction industries

Work positions of workers with OACD in the rubber industry, showed a great variety: 6 workers come from the laboratory and manufacture of rubber footwear, 3 people worked on lining sewing, 2 of each worked on pressing boots, packing boots, pressing rubber, separating foil from rubber, coating metal parts, phosphatization, packing finished products and production of conveyor belts (a total of 16 workers) and 20 more workers in various other work positions.

In the construction industry, OACD is mostly registered among bricklayers (27 workers), followed by tile installers (19 workers), concrete mixers (6), façade makers (4), carpenters (3) and terrazzo masons (2).

In respect to the qualification structure, workers with OACD in the rubber industry are most frequently qualified (40 workers), semi-qualified (4 workers), unqualified (1 worker) and people with a high-school or university diploma (3 of each category).

In the construction industry, these are mostly qualified and semi-qualified workers.

After making the diagnosis of contact, and in particular, allergic and occupational allergic dermatitis, symptomatic treatment needs to be administered depending on the stage of the disease, the intensity of the clinical signs and containment of the changes (24).

This would include medical rehabilitation by applying protective measures and/or taking sick leave, making attempts to influence the behavior of the affected person and work conditions (25).

The next step would be occupational rehabilitation—referral of the patient to the Disability Board, where the assessment of his/her work abilities is to be conducted (lowered work ability – III category disability or loss of work ability), and the Board is to give an opinion in regards to vocational retraining (26).

Social rehabilitation depends on the characteristics of the affected person, as well as the financial situation and his or her social standing in the family and society in general.

Prevention measures are imperative (27, 28). The process of manufacture with specific technology is very important (machinery, automation, closed base system of transport), different working conditions and exposure to various aggressive chemicals, with assessment of the possibility of replacing them with less aggressive substances.

However, we must not forget the individual with his or her characteristics.

Professional orientation is very important, education of the workers as well as health education, use of personal protection means, and health control in terms of conducting general medical examinations or periodical examinations of the workers in risky work positions.

Table 4. Number and Percentage of Positive Tests and Order of Allergens of the Rubber Series among Workers with OACD

Allergen	Men	Women	Allergen	Men	Women
Order	Number %	Number %	Order	Number %	Number %
1. 4010 Na (antioxidant)	13 (48)	17 (71)	13. MBT (accelerator)	2 (7)	2 (8)
2. PAN (antioxidant)	6 (27)	4 (17)	14. Masticit VII (mastic)	4 (15)	2 (8)
3. Acetone (org.dissolvent)	6 (22)	3 (12)	15. Smoke	3 (11)	1 (4)
4. Hemosil 20 (glue)	2 (7)	6 (25)	16. Toluene (org. dissolvent)	1 (4)	2 (8)
5. Vulkacid D (accelerator)	1 (4)	6 (25)	17. Vulkacid DM (accelerator)	2 (7)	0 (0)
6. Perbunan (synthetic rubber)	4 (15)	2 (8)	18. SMR 20 (nat. rubber)	1 (4)	0 (0)
7. PNB (antioxidant)	4 (15)	1 (4)	19. MBI (antioxidant)	0 (0)	1 (4)
8. Xylol (org. dissolvent)	1 (4)	4 (17)	20. White glue	0 (0)	1 (4)
9. Gasoline (org.dissolvent)	3 (11)	2 (8)	21. TMT (accelerator)	0 (0)	1 (4)
10. SMR 10 (nat. rubber)	3 (11)	1 (4)	22. Vulkacid CZ (accelerator)	1 (4)	0 (0)
11.DOD (antioxidant)	2 (7)	2 (8)	23. Vulkacid ZDBC (accelerator)	0 (0)	1 (4)
12. Hemosil 21 1 (glue)	2 (7)	2 (8)	24. Vulkacid H30 (accelerator)	0 (0)	1 (4)
			25.Colophonium	0 (0)	1 (4)
			All	M	F
				61 (50)	61 (50)
			Total	122 (100.00)	

Table 5. Work Positions of Workers with OACD in the Rubber and Construction Industries

Rubber industry		Construction	
Work position	Number %	Work position	Number %
Laboratory	6 (11.76)	Bricklayer	27 (44.26)
Production of rubber footwear	6 (11.76)	Tile installer	19 (26.23)
Lining sewing	3 (5.88)	Concrete mixer	6 (9.84)
Pressing boots	2 (3.92)	Façade maker	4 (6.56)
Packing boots	2 (3.92)	Carpenter	3 (4.92)
Pressing	2 (3.92)	Terrazzo mason	2 (3.28)
Separating foil from rubber	2 (3.92)	Total	61 (99.99)
Coating of metal parts	2 (3.92)	/	/
Phosphatisation	2 (3.92)	/	/
Final product packing	2 (3.92)	/	/
Production of conveyor belts	2 (3.92)	/	/
All	20 (39.33)		
Total	51 (99.98)	Total	61 (99.99)

Conclusion

In the construction industry, there is a high risk of the occurrence of OACD, although longer exposure is required, while the rubber industry is an environment of medium risk.

In the construction industry, it is more frequent for men to get the disease, while in the rubber industry, it is more frequent among women.

The causing allergens are varied and depend on the production technology.

The rubber and construction industries are two very different industry branches. In the rubber industry, many employees are placed in the same

position; production is quite complicated; a large number of various chemicals, many of which have allergenic potential, are used; both men and women are employed, and they come from towns and villages.

In the construction industry, jobs are mostly performed by men. It is a matter of a number of specific jobs that are not very different among each other; fewer types of materials are used and contact dermatitis occurs more frequently, but after prolonged exposure.

Be as it may, it is of great importance to register the affected individuals and administer appropriate treatment and prevention measures.

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doi: 10.5633 / amm.2025.0205**REZULTATI UPOREDNOG ISPITIVANJA NASTANKA I
UČESTALOSTI PROFESIONALNOG KONTAKTNOG
ALERGIJSKOG DERMATITISA KOD GUMARSKIH I
GRAĐEVINSKIH RADNIKA***Mirjana Paravina¹, Marija Nedeva²*¹Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija²Univerzitet u Nišu, Medicinski fakultet, student doktorskih studija, Niš, SrbijaKontakt: Mirjana Paravina
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Profesionalne bolesti kože predstavljaju promene na koži i njenim adneksima, koje nastaju prilikom obavljanja određenog posla, i to pod uticajem različitih egzogenih i endogenih faktora. Mogu ih izazvati iritansi ili alergeni (iritativni ili alergijski kontaktni dermatitis). Kako bi se sagledao uticaj radne sredine i profesionalnih noksi na kožu u pogledu nastanka profesionalnog alergijskog kontaktnog dermatitisa (PAKD), ispitani su gumarski i građevinski radnici na čijoj su koži uočene promene koje izaziva kontaktni dermatitis (KD). Radnici su klasifikovani prema polu, mestu stanovanja, starosti, kvalifikacijama, dužini radnog staža, vrsti alergena i radnom mestu. Istaknut je značaj medicinske, profesionalne i socijalne rehabilitacije, kao i mera prevencije (profesionalna orijentacija, edukacija i zdravstvena osvešćenost radnika). Utvrđeno je da u građevinarstvu obolevaju muškarci i da postoji veći rizik za nastanak PAKD-a, s tim što do toga dolazi tek nakon duže izloženosti alergenima. Uzročni alergeni su raznoliki. Važno je registrovati obolele i preduzeti odgovarajuće mere lečenja i prevencije.

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INFLUENCE OF VITAMIN C AND RIBWORT PLANTAIN EXTRACT ADDITION TO THE PROPOLIS EXTRACT ON THE VIABILITY OF FIBROBLASTS IN CELL CULTURE *IN VITRO*

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Propolis is a honey bee product rich in biologically active substances that have been proved to have beneficial effects on human health. It is widely used in traditional medicine for the treatment of various not only respiratory but also skin disorders. Propolis can be used alone as a pure extract or with the addition of multiple plant extracts and antioxidants to achieve synergistic effects. The aim of this study was to examine the effects of different propolis extracts, commercially available on the market, on the viability of fibroblasts in cell culture *in vitro*. We examined the effect of three different propolis extracts: pure propolis extract (25%), propolis extract (10%) with added vitamin C and propolis extract (10%) with Ribwort Plantain extract and added vitamin C, on the viability of L929 fibroblasts, using the MTT test and microscopically. Concentration-dependent effect of all examined propolis extracts on the viability of fibroblasts was observed. Also, differences in the effect of examined extracts on cell viability were noticed related to the additions to the propolis extract, and the pattern was different in lower compared to the higher examined concentrations. The addition of vitamin C and Ribwort Plantain extract influences the effects of pure propolis. Using propolis in combination with plant extracts and bioactive substances may have beneficial effects, but it should be considered based on the indications for which these products are intended and the effects to be achieved.

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Key words: *propolis, ribwort plantain, vitamin C, fibroblasts, in vitro*

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Introduction

Propolis is a natural, resinous substance produced by bees from substances they collect from different parts of plants and exudates. The chemical composition of propolis, biological and pharmacological activity vary greatly depending on the plant species from which it originates and the geographical origin (1). Propolis is mainly composed of resins and vegetable balsam (50%), wax (30%), essential oils (10%), pollen (5%), sugars, amino acids, vitamins, and minerals (5%)

(2–9). Except for resins and waxes, the main groups of chemical compounds found in propolis are phenols (e.g., flavonoids, polyphenols, phenolic acids and other phenolic compounds) and their esters, terpenes and terpenoids, steroids, aromatic acids, aromatic esters, aldehydes, alcohols, sugars and their alcohols and acids, amino acids, vitamins, fatty acids, hydrocarbons, mineral elements and alcohols (7, 9–12). Flavonoids are a major group of phenolic compounds in propolis which greatly contribute to the biological and pharmacological activities of propolis. Propolis has been known in the traditional medicine of many countries for its therapeutic properties for a long time, so in some countries, it is used to treat a wide range of diseases and is also used for cosmetic purposes (13). It is known that propolis has an antibacterial, antiviral and antifungal activity (8, 9, 13), so it is used to treat infections of the upper respiratory tract as well as superficial wounds in the form of topical formulations (14). Propolis acts as an antioxidant and anti-inflammatory agent, so it is also used in the treatment of inflammatory processes and disorders (8, 9, 13). Since propolis has been shown to have a beneficial effect on wound

healing (13, 15), it is used in the form of topical formulations for healing wounds and burns on the skin and in dental preparations for the treatment of gingivitis (16). Nowadays, propolis is used in the form of drops and sprays for oral and *per os* use, in the form of creams, gels, lotions, capsules, lozenges, toothpastes and others.

Vitamin C is a well-known antioxidant that is added to various products in order to achieve a synergistic effect and to potentiate their antioxidant capacity. In addition to its antioxidant effect, vitamin C stimulates fibroblast proliferation and collagen synthesis (17), so it is often added to preparations for skin care and wound healing. In cosmetology and dermatology, vitamin C is commonly used as a component of various creams and serums because it has a protective effect on skin damage caused by various harmful effects and slows down the ageing process (18). The addition of vitamin C to various formulations can contribute to and stimulate their beneficial properties.

Ribwort Plantain (*Plantago lanceolata* L.) has been known for its beneficial effects on human health since ancient times. *Plantago lanceolata* L. is a species from the genus *Plantago*, family Plantaginaceae, that is the most commonly used for medicinal purposes. Ribwort Plantain is registered as a natural medicine (herbal medicinal product) by the European Medicines Agency (EMA). EMA defines the oral, oromucosal and cutaneous use of the leaf of Ribwort Plantain (lat. *Plantaginis lanceolatae folium*) for the following indications: as a demulcent for the symptomatic treatment of oral or pharyngeal irritations and associated dry cough, for the relief of cough associated with cold and for the treatment of minor inflammation of the skin (19). In many countries, it is used in combination with other plant species to treat various disorders. It is used in the form of extracts, syrups, teas, lozenges and tablets by oral and topical route for the treatment of inflammation of the upper and lower parts of the respiratory tract, inflammation of the skin and for the healing of skin and mucous membranes wounds (20). Ribwort Plantain has been shown to possess anti-inflammatory (21), antioxidant (22), antibacterial (23), and antiviral activity (24), to stimulate epithelization and to act as a spasmolytic agent (25). It is one of the main plants used in cough remedies and has anti-inflammatory effects, protects the liver, and is used in the treatment of cancer (26). The main biologically active compounds of *Plantago lanceolata* L. are: iridoid glycosides (aucubin and catalpol), phenylethanoid glycosides, polysaccharides, flavonoids (apigenin and luteolin), polyphenols, alkaloids, terpenoids, fatty acids, phytosterols, phenylethanoids (acteoside, plantamajoside) and tannins, organic acids, mucilaginous substances, mineral salts, and pectins (26–29). Ribwort Plantain extract can

successfully be used as an effective ingredient in cosmetic products (30).

In biomedical research, where biological activities of various substances and pharmaceutical products are examined, studies on cell cultures *in vitro* are of fundamental importance and represent the first step in examining the biological activities. In order to investigate the potential effects, such as wound healing, on the skin and mucous membranes, the most commonly used model for this type of investigation is fibroblast cultures. The cell line that is most suitable for this purpose is the L929 cell line, a permanent cell line of fibroblasts obtained from the mouse skin.

The aim of this study was to examine the influence of the addition of vitamin C and Ribwort Plantain extract to the propolis extract on the viability of fibroblasts in cell culture *in vitro*.

Materials and Methods

Propolis extracts

Three different propolis ethanol extracts available on the market were tested: highly purified propolis extract (25%) in ethanol solution (Propolis drops—extra 25%), highly purified propolis extract (10%) in ethanol solution with added vitamin C (Propolis drops with vitamin C), highly purified propolis extract (10%) and *Plantaginis lanceolatae folium* extract in an ethanolic solution with added vitamin C (Propolis drops with Ribwort Plantain and vitamin C), all from producer Sinefarm d.o.o. Serbia. Extracts were diluted in complete DMEM, and final dilutions 1/100, 1/200, 1/500, 1/1,000 and 1/2,000 (v/v) were examined.

Cell culture and viability assay

The effect on cell viability was examined on the L929 cell line (obtained from the American Type Culture Collection—ATCC), which was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, antibiotic-antimycotic solution and stable glutamine (which makes complete DMEM medium), all purchased from Capricorn Scientific GmbH, Germany. Cells were seeded in 96-well culture plates (Greiner Bio-One, Germany) in complete DMEM so that 20,000 cells were seeded per well per 100 µl of medium, and left for 24 hours in an incubator with 5% CO₂, in humidified atmosphere, at 37 °C, to adhere and to adapt to the environment. After that, 100 µl of prepared dilutions of examined propolis extracts (twice as high as those finally tested) were added to the cells. Cells were incubated with different concentrations of the examined propolis extracts for the next 24 hours, and after that MTT test was performed to assess the effect of the extracts on cell viability. MTT test is commonly used for viability testing and is based on the

ability of viable cells to reduce the yellow tetrazolium salt MTT by mitochondrial dehydrogenases to violet formazan crystals, which are further dissolved in 2-propanol. The intensity of the colored solutions obtained from the dissolved formazan crystals was in direct correlation with the number of viable cells. Absorbance was measured on a multichannel spectrophotometer, Multiscan Ascent (ThermoLab Systems, Finland), at the wavelength of 540 nm with the correction at 650 nm. Each concentration was tested in tetraplicates and the experiment was repeated twice. As a control, we used cells that were incubated under the same conditions in complete medium but without the examined extracts (untreated cells).

Microscopical analysis

Cells were analyzed microscopically using an inverted light microscope (Observer Z1, Carl Zeiss, Germany), under phase contrast. The images of cells were acquired before incubation with extracts and 24 hours after incubation, before the MTT test, using the camera AxioCam HR in the software ZEN 2 blue edition (Carl Zeiss, Germany).

Statistical analysis of the results

The results of the MTT test are shown as a percentage of cell viability in relation to the control (untreated cells), for which the viability was considered to be 100%, with relative standard deviations. The percentage of cell viability was obtained from the mean values of the measured absorbances for each examined concentration and control. Statistically significant differences were analyzed by one-way ANOVA (analysis of variance) test, and values for which $p < 0.05$ were considered significant.

Results

The effects of examined propolis extracts on the viability of L929 fibroblasts were assessed by MTT test and microscopically. The results of the MTT test are shown in Figure 1.

Statistically significant difference in the effect of examined propolis extracts on L929 cell

viability was observed at all examined dilutions. Significant decrease in cell viability, i.e., cytotoxicity, was noticed for all examined dilutions of P-25 and PC extracts, with more pronounced cytotoxic effect of dilutions from 1/100 to 1/1,000. PCP extract was shown to be the least cytotoxic among the examined extracts. Dilutions 1/1,000 and 1/2,000 of PCP extract were not cytotoxic for L929 cells. The trend of action of examined extracts in dilutions 1/100 and 1/200 was the same; the PCP extract was the most cytotoxic, while the P-25 extract was the least toxic at these concentrations. With a decrease in the concentration of all three extracts, i.e., increase in dilution, the trend of action changed, so the most cytotoxic effect was noticed for the P-25 extract, while the least cytotoxic was the PCP extract.

The effect of the examined extracts on cell morphology and number was examined microscopically, and the results are shown in Figure 2. In higher examined concentrations (dilutions from 1/500 to 1/100), cells were round in shape, which indicates toxic effects, with some cells becoming elongated in the case of 1/500 dilution of PCP extract. At dilution 1/1,000 differences among examined extracts were noticed, with the most pronounced cytotoxic effect reflected on the cell morphology in the case of P-25 extract, followed by PC extract where there were some cells elongated in shape and PCP extract with most of the cells elongated in shape, typical of fibroblasts. These observations were in accordance with the results of the MTT test. At dilution 1/2,000, differences in cell density besides differences in cell morphology could be noticed, which were in accordance with the results of MTT test in terms that PCP extract did not exert toxic effects, which was visible as the highest cell density on microscopical images and morphology of cells typical for fibroblasts.

Solvent (ethanol at dilution 1/100) neither influenced the cell morphology and number of cells, nor acted cytotoxic (% of cell viability measured by the MTT test was 112% which was slightly, but significantly stimulated parameter of cell viability compared to the control). This means that solvent did not cause cytotoxic effects on cells.

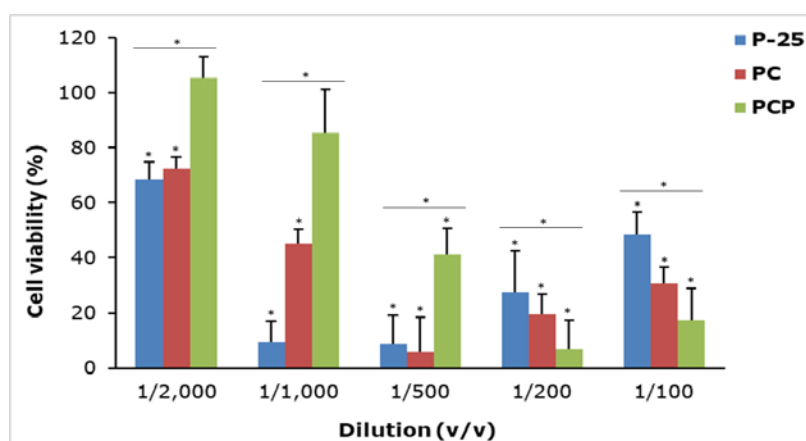


Figure 1. The effect of different propolis extracts: pure propolis extract 25% (P-25), propolis extract with added vitamin C (PC) and propolis extracts with Ribwort Plantain and added vitamin C (PCP), on the viability of L929 cells expressed as a percentage of cell viability compared to the control (untreated cells); (*) $p < 0.001$

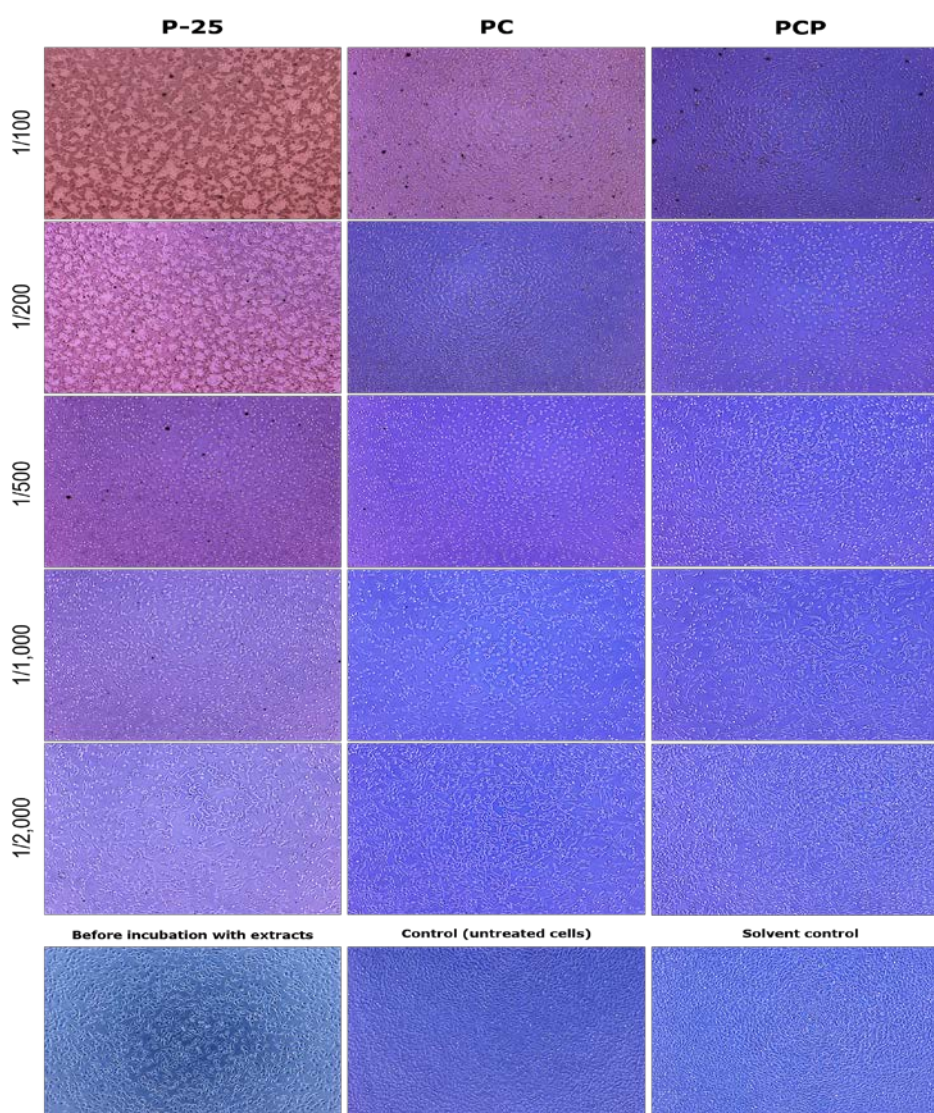


Figure 2. Morphological appearance of L929 fibroblasts before incubation with extracts, 24 hours after incubation with different dilutions of pure propolis extract 25% (P-25), propolis extract with added vitamin C (PC), propolis extract with Ribwort Plantain and added vitamin C (PCP), control (untreated cells) and solvent control.

Discussion

The obtained results show that all examined propolis extracts exhibited a concentration-dependent effect. A different effect on cell viability was obtained in the treatment of cells with different propolis extracts. At higher dilutions of the extracts, e.g., lower concentrations of the extracts, the protective effect of vitamin C and Ribwort Plantain extract on cell viability was pronounced, compared to the effect of pure propolis extract. At lower dilutions, i.e., higher concentrations of extracts, the cytotoxic effect was present regardless of the type of extract used. In order to rule out the potential effect of the solvent used for extract preparation, the cells were incubated with the same dilutions of ethanol, which was used as a solvent in all examined extracts, where even the highest concentration of ethanol, a dilution of 1/100, was not cytotoxic for L929 fibroblasts, moreover, it slightly increased the reduction of MTT by fibroblasts. The concentration of ethanol in all examined dilutions of propolis extracts was less than 1%.

The cytotoxic and antiproliferative effect of propolis and extracts of *Plantago* spp. was demonstrated on cancer cell lines, which gives these extracts a potential application in chemotherapy (31, 32). This effect was mainly demonstrated on epithelial cancer cells.

The ethanolic extracts of Malaysian and Brazilian red propolis show potential to assist in wound healing, depending on their concentration, in an *in vitro* cell model using normal human fibroblast cell line CRL-7522 (33). In the study where Portuguese 30% propolis ethanolic extract was examined on human dermal fibroblasts and keratinocytes, it was found that concentrations of propolis extract below 1 mg/mL were well-tolerated by fibroblasts and moderately tolerated by keratinocytes, which, together with good antimicrobial and antibiofilm effect, suggests that propolis extract could have a good applicability in the form of topical formulations for antibacterial treatment of infected skin disorders (14).

Vitamin C is important for collagen synthesis by fibroblasts and is a very popular addition to creams and other topical formulations for preventing skin damage and ageing, as well as different products for preventing gum retraction. In a study where human gingival fibroblasts were rinsed with 0, 10, 20 and 50 µg/mL of L-ascorbic acid for 7 min, three times per day in the experiment that lasted for two days, it was shown that rinsing the fibroblasts with 50 µg/mL of L-ascorbic acid significantly reduced the cell viability, evaluated by MTT test, while concentrations below 50 µg/mL did not influence the cell viability (34). In our study, propolis extract with added vitamin C (PC) led to decreased cell viability at dilutions from 1/100 to 1/500, compared to the pure propolis extract (P-25) without vitamin C. The concentration of

vitamin C in extracts PC and PCP examined in our study, was 3 g/100 mL according to the data available on the website of the manufacturer, which means that 1/500 dilution of PC extract contains 60 µg/mL of vitamin C which is close to the concentration of vitamin C used in the mentioned study. In lower concentrations (dilutions 1/1,000 and 1/2,000) propolis extract with added vitamin C was shown to be less cytotoxic compared to the pure propolis extract. The important difference was also that in the mentioned study, fibroblasts were only rinsed with vitamin C, while in our study, fibroblasts were incubated for 24 hours with propolis extracts containing vitamin C. Also, we used a permanent cell line, while in the mentioned study, a primary culture of gingival fibroblasts was used.

Extracts from *Plantago ovata* have been shown to have a beneficial effect and to stimulate the proliferation of fibroblasts *in vitro* (35). Extracts of *Plantago lanceolata* L. were shown to be a valuable source of bioactive substances that have beneficial effects on fibroblasts due to high antioxidant properties, UV protecting activity and stimulation of skin regeneration, which makes those extracts a good additive to the pharmaceutical formulations that are used as natural cosmetics (26). Wound healing activity of aqueous extract of Ribwort Plantain was demonstrated in an open wound rat model (36). It was also shown that different solvents influence different effects of the Ribwort Plantain extracts on fibroblasts. For example, extracts using glycol and glycerin allowed the isolation of some bioactive compounds that were not present in ethanolic and aqueous extracts, while the ethanolic extract of Ribwort Plantain at concentrations of 50 mg/mL reduced the viability of fibroblasts by 45% (26). In our study, propolis ethanolic extract with Ribwort Plantain and added vitamin C was examined and was shown to be the least cytotoxic among examined propolis extracts, with absence of cytotoxic activity at lower examined concentrations (1/1,000 and 1/2,000), while pure propolis extract and propolis extract with added vitamin C were cytotoxic at those dilutions.

There are numerous propolis extracts on the market. Differences in the action of propolis extracts that can be found in the literature can be explained by the different composition of propolis from different countries, which is inevitable, because the composition of propolis depends primarily on the type of plants and their parts from which bees collect it. Combining propolis extract with plant extracts may have beneficial effects on human health if the combination is properly designed. It is also very important to consider the potential application of propolis extracts prior to mixing with other biologically active compounds. Further studies with propolis extracts that we examined are necessary, in order to analyze the effects of those extracts on

wound healing both *in vitro* and *in vivo*, before making any conclusions regarding the potential use of those extracts in the treatment of skin disorders.

Conclusion

Based on the obtained results, it can be concluded that there is a concentration-dependent effect of all examined propolis extracts and that there is a difference in the effect of the extracts concerning the composition. The addition of vitamin C to the propolis extract, as well as the combination of propolis extract with Ribwort Plantain, changes the effect of propolis on the viability of fibroblasts in cell culture. When choosing the propolis extracts with additions of other extracts or vitamin C, the potential indication and application of that extract should be considered. Further *in vitro* and *in vivo* studies are needed before recommendations on the use of these combinations in the treatment of skin disorders.

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**UTICAJ DODATKA VITAMINA C I EKSTRAKTA BOKVICE
EKSTRAKTU PROPOLISA NA VIJABILNOST
FIBROBLASTA U ČELIJSKOJ KULTURI *IN VITRO***

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Propolis je pčelinji proizvod izuzetno bogat biološki aktivnim supstancama za koje je dokazano da blagotvorno utiču na zdravlje ljudi. Propolis ima široku primenu u tradicionalnoj medicini u lečenju raznih respiratornih, kao i kožnih oboljenja. Propolis se može koristiti samostalno kao prečišćeni ekstrakt ili sa dodatkom raznih biljnih ekstrakata i antioksidanasa kako bi se ostvarili sinergistički efekti. Cilj ovog istraživanja bio je da se ispituju efekti koje različiti ekstrakti propolisa dostupni na tržištu imaju na održivost fibroblasta u ćelijskoj kulturi *in vitro*. Pomoću MTT testa ispitan je efekat triju različitih ekstrakata propolisa – čistog ekstrakta propolisa (25%), ekstrakta propolisa (10%) sa dodatkom vitamina C i ekstrakta propolisa (10%) sa bokvicom i dodatkom vitamina C – na održivost L929 fibroblasta. Uočeno je da je efekat svih ispitivanih ekstrakata propolisa na održivost fibroblasta zavisio od koncentracije. Takođe, uočene su razlike u uticaju ispitivanih ekstrakata na održivost ćelija koje su bile povezane sa dodatkom ekstraktu propolisa, a obrazac u nižim koncentracijama razlikovao se od onog u višim koncentracijama. Dodatak vitamina C i ekstrakta bokvice ekstraktu propolisa utiče na delovanje čistog propolisa. Premda upotreba propolisa u kombinaciji sa biljnim ekstraktima i bioaktivnim supstancama može imati blagotvorne efekte, te kombinacije najpre treba razmotriti na osnovu indikacija za koje su ovi proizvodi namenjeni i željenih efekata koje je potrebno postići.

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Ključne reči: propolis, bokvica, vitamin C, fibroblasti, *in vitro*

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IN VITRO WOUND HEALING ACTIVITY OF ALPHA-LIPOIC ACID

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Chronic wounds represent a worldwide healthcare and socio-economic problem which significantly affects the patients' quality of life. Healing of chronic wounds is a very complex process and requires constant wound management and treatment with agents that should stimulate wound healing. Alpha-lipoic acid (ALA) is a naturally occurring organosulfur compound with two thiol groups in its structure. It is a very potent antioxidant with other beneficial activities such as anti-inflammatory, anti-ageing and neuroprotective. This study aimed to investigate *in vitro* wound healing activity of ALA and its effect on the proliferation of L929 fibroblasts. Wound healing activity was examined using an *in vitro* 'scratch' assay, while the impact on cell proliferation was assessed using the MTT test. A concentration-dependent effect of ALA on fibroblasts' proliferation was observed. ALA stimulated the wound closure and migration of fibroblasts in used *in vitro* wound healing model, which suggests that ALA can be used as a potent agent in various pharmaceutical formulations for wound management and wound healing.

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Key words: wound healing, chronic wounds, alpha-lipoic acid, cell proliferation, fibroblasts

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Introduction

Wound healing (WH) represents a highly dynamic biological process in the human body (1). Interruptions, aberrancies, or prolongation in the programmed phases of WH, such as hemostasis, inflammation, proliferation, and tissue remodeling, can lead to delayed WH or a non-healing chronic wound (2). The complex and long-term process of chronic wound healing starts with wound

formation, can take months or years (3) and can result in a significant negative impact on healthcare systems with serious impacts on the life quality of patients (4). Recent estimations show that non-healing chronic wounds represent a huge socio-economic problem, and represent a silent epidemic that affects 1–2% of the world population (5).

Despite numerous research studies in the field of WH and chronic wounds, critical gaps in the impaired healing process remain (6). Therefore, there is an immediate need for further analysis of factors associated with delayed healing, as well as identification of new candidates that can stimulate the proliferation of fibroblasts, which are the cells that play a crucial role in all phases of WH (7). Chronic inflammation and oxidative stress are distinguished as important factors of healing dysfunctions (8). Therefore, regulation of redox balance through the modulation of reactive oxygen species (ROS) and antioxidant levels may improve the WH process. The interest in using antioxidants for wound treatment is growing, and numerous studies have been performed with the aim of developing and examining different compounds with antioxidant properties which can enhance the healing process (3). Among a large number of natural antioxidant compounds, alpha-lipoic acid (ALA) stands out due to its noteworthy effects, and is considered today as "the universal antioxidant" (9). The molecular nature of ALA puts this unique dithiol compound in

special focus, because ALA acts as an antioxidant and can exert immunomodulatory properties (10, 11). Therefore, our aim was to examine the effect of ALA on the proliferation of fibroblasts and WH activity *in vitro*.

Materials and Methods

Cell line and culturing

In vitro WH activity testing of alpha lipoic acid ((±)-α-Lipoic acid, purity ≥ 98.0%, Cat. No. 62320, Sigma Aldrich, Germany) was performed on L929 fibroblast cell line (mouse skin fibroblasts). L929 fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 2 mM stable glutamine, and antibiotic-antimycotic solution (which makes complete DMEM), at 37 °C in an incubator with humidified atmosphere and 5% CO₂. Cell culture media and reagents used in the study were purchased from Capricorn Scientific GmbH, Germany.

Cell Proliferation Assay

L929 cells were seeded in standard 96-well plates (purchased from Greiner Bio-One, Germany) at a density of 5,000 cells per well of the 96-well plate. Twenty-four hours after cultivation of cells, ALA was added in various concentrations (ranging from 0 to 1,000 μM). Concentrations of ALA for testing were prepared by diluting the stock solution of ALA with complete DMEM. A stock solution of ALA was prepared according to the manufacturer's instructions by dissolving ALA in methanol (purity p.a.) in a concentration of 100 mg/mL. The cells incubated only with complete DMEM, without ALA, were used as a control (untreated cells). Each tested concentration of ALA was examined in four to six replicates, and complete DMEM was used as a control as well. The cells were incubated with different concentrations of ALA or control medium for the next 72 hours. After the incubation period ended, the MTT test was performed. The experiment was performed twice under the same conditions. The MTT test is widely used for assessment of cell proliferation and is based on the reduction of tetrazolium salt MTT (purchased from Carl Roth, Germany) by mitochondrial dehydrogenases of living cells, resulting in formazan crystals that correspond to the number of viable cells. Prior to the addition of 100 μL of MTT solution at a concentration of 1 mg/mL, media with ALA were discarded, and cells were washed with phosphate buffer saline (DPBS, Capricorn, Germany). MTT substance was purchased from Carl Roth, Germany. The cells were incubated with MTT solution for the next three hours, followed by dissolution of the formed formazan crystals with 2-propanol (purchased from Thermo Fisher Scientific, USA). The absorbance of dissolved formazan was measured

on a Multiskan Ascent Photometric plate reader (Thermo Labsystems, Finland) at a wavelength of 540 nm with a reference wavelength of 650 nm for correction. The mean absorbance values were calculated for each tested sample, as well as for the control. The cell proliferation rate was calculated as: (absorbance value of cells treated with fibers/absorbance value of untreated cells) × 100.

In Vitro Wound Healing Assay

A "scratch" assay was performed to examine the *in vitro* WH effect of ALA, according to our previously published protocol (12–14). Briefly, L929 fibroblasts were seeded in 48-well plates and incubated under the standard cell culture conditions previously described in the section "Cell line and culturing". After reaching the complete confluence, a wound ('scratch') was created by a pipette tip in a cell monolayer, in the middle of each well. The cells were then washed with DPBS, and 100 μM of ALA, as well as complete DMEM (control), were added. ALA and complete DMEM were tested in three replicates, and the experiment was performed twice under the same conditions. Created "wounds" were incubated with ALA and complete DMEM for the next three days. A microscopic analysis of wounds' closure was then performed using an inverted light microscope, Observer Z1 (Carl Zeiss, Germany), and morphometric measurements were made in ZEN 2 (blue edition) software (Carl Zeiss, Germany) after imaging the "wounds". To assess the wound closure, we measured the width of the remaining wounded area after three days of incubation with the ALA as well as with complete medium (control), and compared it to the width of the area of initial wounds, before incubation with ALA. The WH activity was expressed as a percentage of wound closure. Additionally, the cell migration zone was measured starting from the initial edge of the wound.

Statistical Analysis

The results of the MTT test and morphometric measurements were analyzed using one-way analysis of variance (ANOVA). Results are expressed as a percentage of cell proliferation regarding the control culture, which represented cells incubated in complete DMEM (untreated cells) under the same conditions and for which we considered the cell proliferation rate to be 100%. Wound closure is expressed as a percentage with relative standard deviation. As statistically significant, we considered those values for which $p < 0.05$.

Results

The effect of ALA on the proliferation of fibroblasts

The effect of various concentrations of ALA is shown in Figure 1. Concentration-dependent effect of ALA on L929 fibroblasts proliferation was observed and was mostly pronounced in concentrations from 50 to 1,000 μM . A slight anti-proliferative effect at concentrations 250 μM and above was observed, with the most pronounced effect of the highest examined concentration, while lower concentrations did not exhibit an anti-proliferative effect instead, they even exerted a mild stimulatory effect.

In vitro wound healing activity of ALA

The wound healing effect of ALA, determined by *in vitro* 'scratch' assay, is shown in Figure 2. The effect of ALA was compared with the control, which represented complete DMEM without ALA. Three parameters were measured

after three days of incubation: cell migration zone (the extent of cell growth and migration starting from the initial edge of the wound), wound area after the treatment (width of remained wound area) and the percentage of wound closure (calculated by the formula: $100\% - (\text{width of remained wound area} - \text{width of the initial wound area}) \times 100\%$). For WH activity testing, we chose a concentration which did not alter fibroblasts' proliferation.

The stimulating WH effect of ALA was noticed, determined by an *in vitro* 'scratch' assay. Statistically significant greater migration zone, smaller width of the remained wound area and higher percentage of wound closure in the presence of ALA were observed when we compare ALA treatment with the control.

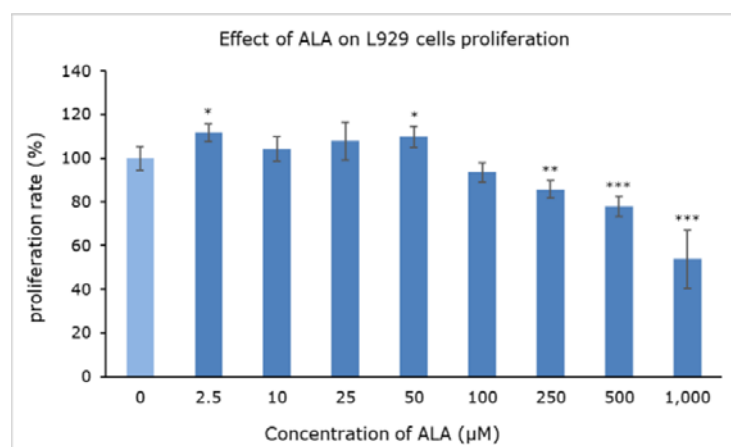


Figure 1. The effect of various ALA concentrations on L929 cells' proliferation, measured by MTT test. Results presented as mean % of cell proliferation compared to the control cell culture with relative standard deviation; (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

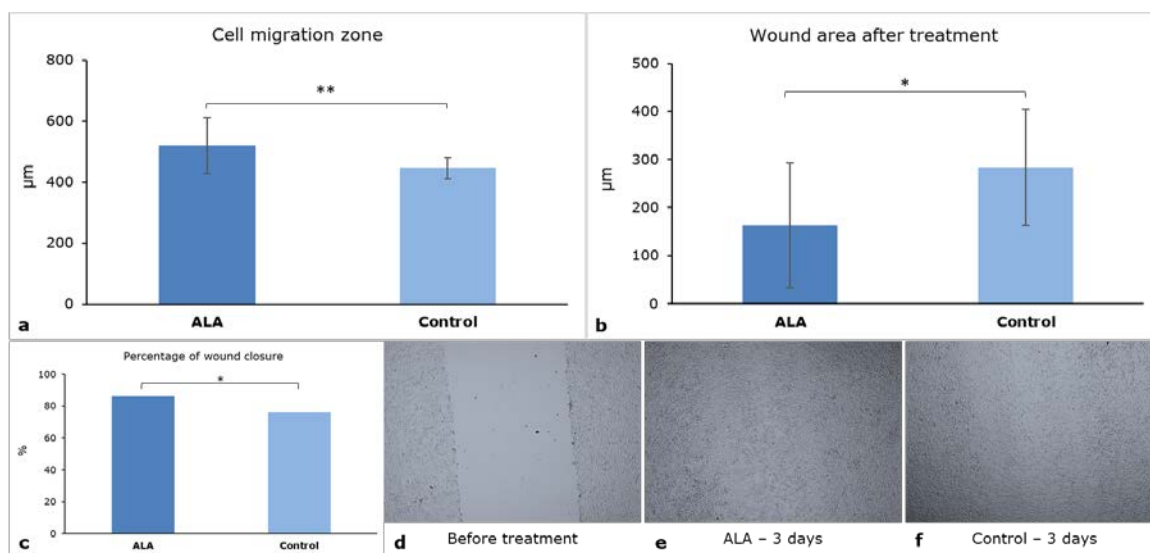


Figure 2. The wound healing effect of ALA, determined by the 'scratch' assay. Results presented as mean width (μm) $\pm$ SD of cell migration zone (a) and remained wound area after treatment (b) and % of wound closure after treatment (c); microscopical image of the wound before treatment (d), after three days of incubation with ALA (e) and control medium (f); (*) $p < 0.05$, (**) $p < 0.01$.

Discussion

A meticulous and very complex process of WH is achieved through several phases and requires the interaction and communication of different cell types present in the wound (15). Fibroblasts are distinguished as key effector cells in all phases of the WH process, with the note that accumulation and activation of the fibroblasts is closely related to and is responsible for the formation and deposition of granulation tissue, and finally leads to the wound contraction (16). Therefore, quick WH might be due to stimulation of proliferation and migration of fibroblasts, but it is also necessary to take into account a level of reactive oxygen species (ROS) playing a crucial role in the WH process (17). Growing evidence supports the fact that oxidative stress plays a significant role in WH phases and processes such as regulation of inflammation, angiogenesis, cell proliferation, formation of granulation tissue and the formation of extracellular matrix (ECM) (18). Because of the complexity of the WH process and the participation of oxidative stress in that process, various antioxidant compounds have become the focus of research (17).

Alpha-lipoic acid is a naturally derived organosulfur compound with two thiol groups in its structure, which may participate in redox reactions (19). It exists in oxidized and reduced forms and is characterized by unique antioxidant potential, since both endogenously and exogenously synthesized form is actively involved in ROS neutralization, restores the intrinsic antioxidant systems and supports their production (10, 19). ALA occurs usually in mitochondria, acting as a coenzyme for some enzyme complexes, and plays a major role in protein, carbohydrate and fatty acid metabolism, and manages gene activation (11). Its amphiphilic characteristic sets ALA apart from other antioxidants, and can elicit antioxidant and anti-inflammatory actions in both the cytosol and plasma membrane (20). Currently, it is attracting attention because of its distinctive antioxidant properties and influence on various cellular functions, leading to the beneficial effects on human health (21).

In this study, we analyzed the WH activity of ALA using a well-established *in vitro* cell 'scratch' assay. Before performing the WH assay, we investigated the effect of ALA on the proliferation of fibroblasts. We observed a concentration-dependent effect of ALA on the proliferation of L929 fibroblasts, with a slight anti-proliferative effect in concentrations above 250 μ M. Lower concentrations of ALA did not exhibit an anti-proliferative effect and they even had a mild stimulatory effect on fibroblasts' proliferation, which makes ALA a good candidate for topical applications (Figure 1). The cell scratch assay using skin cells, such as fibroblasts, is a widely used method as an *in vitro* WH model that provides information about the activity of different compounds and natural products (22). In the present study, we used this assay with a focus on

three parameters: cell migration zone, resting wound area and percentage of wound closure after three days of incubation with ALA. Representative images in Figure 2, statistically significant larger migration zone, smaller width of the remained wound area and higher percentage of wound closure comparing to the control, clearly show that ALA significantly stimulated WH in an *in vitro* cell model and may be used as a powerful WH agent in numerous topical pharmaceutical formulations intended for wound treatment and healing. The obtained results might be due to the strong and already confirmed antioxidative activity of ALA (22, 23), however, we assume that ALA exerts other activities that are involved in the stimulation of fibroblasts' proliferation and migration.

ALA has been in the research focus for several decades, and literature data reveals the effectiveness of ALA in the prevention of many oxidative stress-mediated pathologic conditions such as obesity, cardiovascular disease, diabetes and related complications, osteoporosis, cognitive dysfunction, malignant diseases, glaucoma, and many others (24). However, its potential application in WH has not yet been thoroughly investigated. There is a limited number of studies with different experimental designs making it difficult to compare the results (*in vitro*, animal models or human clinical studies) (23, 25, 26). Türkez et al. showed that ALA conjugated boron nanoparticles (concentrations of 50 μ g/mL and lower) enhanced WH and antimicrobial processes in human dermal fibroblasts cell culture (25). The group of authors from China showed that ALA enhanced injury repair in a dose-dependent manner in human colon epithelial cells NCM460 (27). Reported results of one study conducted by Külkamp-Guerreiro et al. confirmed that topical application of non-encapsulated ALA induces increased skin WH, which is shown using an *in vivo* model of experimentally induced skin wounds (23). Human clinical study conducted in Italy showed that supplementation with ALA, in combination with hyperbaric oxygen therapy, promotes progression of the healing process by down-regulating the growth factors and inflammatory cytokines production (26). The results of our study presented here, together with the literature data, indicate that ALA shows good potential to be used as a WH agent in wound treatment, and provide a basis for further investigation to clarify the therapeutic potential of ALA in WH. Further *in vivo* research on experimental animals is necessary to explain the precise role of ALA in the complex and chronic WH process.

Conclusion

We can conclude that alpha-lipoic acid, besides many beneficial effects, shows a great wound healing activity determined *in vitro* using the "scratch" assay on fibroblasts. This indicates that ALA can be used as a potent agent in various

pharmaceutical formulations for wound management and wound healing.

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doi: 10.5633/amm.2025.0207**IN VITRO EFEKAT ALFA LIPOINSKE KISELINE NA
ZARASTANJE RANA***Ivana Damnjanović¹, Milica Tomić², Nikola Jović³, Vesna Savić¹, Zoran
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Hronične rane predstavljaju svetski zdravstveni i socioekonomski problem koji umnogome utiče na kvalitet života bolesnika. Zarastanje hroničnih rana veoma je složen proces i zahteva stalnu obradu i tretiranje rana sredstvima koja treba da stimulišu njihovo zarastanje. Alfa lipoinjska kiselina je prirodno organsko sumporno jedinjenje koje u svojoj strukturi ima dve tiolne grupe. Veoma je moćan antioksidans, a zabeležena su i druga značajna svojstva koja ova kiselina ima: antiinflamatorna, antiejdžing i neuroprotektivna. Cilj ove studije bio je da se ispita efekat alfa lipoinjske kiseline na zarastanje rana *in vitro*, kao i efekat ove kiseline na proliferaciju L929 fibroblasta. Efekat na zarastanje rana ispitivao se korišćenjem *in vitro* testa koji podrazumeva pravljenje „ogrebotine“ u monosloju ćelija u kulturi, dok je za ispitivanje efekta na proliferaciju ćelija upotrebljen MTT test. Zabeleženo je da je efekat alfa lipoinjske kiseline na proliferaciju fibroblasta zavisio od koncentracije. Činjenica da je alfa lipoinjska kiselina stimulisala zarastanje, tj. zatvaranje načinjene rane (ogrebotine) i migraciju fibroblasta u korišćenom *in vitro* modelu zarastanja rana ukazuje na to da se alfa lipoinjska kiselina može koristiti kao snažan agens u različitim farmaceutskim formulacijama za lečenje i zarastanje rana.

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Ključne reči: zarastanje rana, hronične rane, alfa lipoinjska kiselina, proliferacija ćelija, fibroblasti

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COMPRESSIVE THERAPY SIDE EFFECTS DURING THE TREATMENT OF VENOUS LEG ULCERS

Maša Golubović^{1,2}, Iva Binić^{2,3}

Venous ulceration arises as an end stage of chronic venous insufficiency. The application of an adequate level of compression therapy is the gold standard of treatment. The study to assess the frequency of complications from compression therapy used to treat venous ulcerations of the lower limbs, in relation to age and the level of compression applied. The study included 102 outpatients with venous ulceration treated at the Niš University Clinical Center's Clinic of Dermatovenereology. Ulcers were monitored within 24 weeks from the start of treatment. There were two groups of patients, ≥ 65 years old and < 65 years old, divided into two subgroups in relation to the degree of compression therapy. The following were monitored: sex, age, body mass index, area, localization and number of ulcerations, degree of granulation tissue and fibrin in the ulceration, presence of dermatitis, lipodermatosclerosis, infection, and duration of the disease. It was found that there was a statistically significant difference in the number of previous ulceration episodes, the size of the ulcerations and the duration of the disease between the examined groups. Additionally, a difference was found in the level of pain in relation to the degree of compression, as well as the risk of paresthesia in relation to age. A higher degree of compression therapy in both age groups led to an increase in the rate of patients with paresthesia and a higher level of pain sensitivity. The study showed no statistically significant risk of superficial necrosis and skin discoloration related to the age of patients.

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Key words: venous ulcer, compressive therapy, paresthesia, pain

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Aim

This study aimed to assess the frequency of problems associated with compression therapy in the treatment of lower extremity venous ulcers in relation to age and compression level.

Material and Methods

The study involved 102 outpatients with venous ulceration treated at the Dermatovenereology Clinic of University Clinical Center Niš. Ulcers were monitored over 24 weeks from the start of treatment.

Before inclusion in the study, all patients underwent a color Doppler ultrasound (CDU) evaluation of the venous and arterial systems of the legs. Each patient's brachial-malleolar-ankle pressure index (ABPI) was also measured. CDU confirmed the venous origin of the ulcers. Patients with venous ulcers (VU) larger than 3 cm² and a disease duration exceeding three months were included in the trial. Patients with ABPI less than 0.8, with present heart failure (ejection fraction below 35%), pregnant women, patients with malignant diseases or diabetes, and those on immunosuppressive or corticosteroid therapy were excluded from the study. Patients with ulcerations associated with cutaneous vasculitis, pyoderma

Introduction

Venous ulceration arises as an end stage of chronic venous insufficiency (1). Applying an adequate level of compression therapy in treating venous ulcerations is equally essential for both patients and doctors. Compression therapy is associated with several potential risks and problems; however, there are no clear guidelines about contraindications, risk factors, or complications. Complications such as allergic skin reactions, eczema, paresthesia, and skin necrosis are rare (2). However, they represent a big problem because they prevent the application of compression therapy and thus compromise this type of therapy as one of the most important methods of treating venous ulcerations of the lower extremities.

gangrenosum, or other neutrophilic dermatoses were also excluded. The following patient data were monitored: sex, age, body mass index (BMI), ulcer size, localization and number of ulcers, degree of granulation tissue and fibrin in the ulcer, presence of dermatitis, lipodermatosclerosis, and ulcer infection, as well as disease duration. Groups were formed using simple randomization, by computer-generated random sorting of the patient list. Based on age, patients were divided into two groups: those 65 years and older formed the study group, while those under 65 years of age were the control group. Each group was further subdivided according to the degree of compression therapy applied in VU treatment. The first subgroup included patients treated with class III compression therapy, exerting a pressure of 35–40 mmHg. The second subgroup consisted of patients who received an additional elastic bandage over the class III compression device, resulting in a pressure exceeding 45 mmHg. Local treatment of ulcers involved the use of antiseptics and antibiotics. The standard regimen included wound debridement, with dressings changed from daily to weekly depending on exudate levels. After debridement and dressing application, the ulcer was bandaged. The first and second layers consisted of gauze without pressure. For the third layer, in the first subgroup, a tubular compression system was used to exert 30–40 mmHg on the ankle, with reduced pressure on the calf. In the second subgroup, an elastic bandage was applied via a tubular compression system, similarly exerting 30–40 mmHg. The bandage was applied spirally with 50% overlap in the supine position and with the foot in dorsiflexion. An elastic bandage measuring 15 cm in width and 5 m in length was utilized. Patients were advised to walk for 30 minutes after bandage application. Following wound healing, patients were instructed to continue using the class III tubular compression device to prevent recurrence.

Statistical analysis

Data analysis was performed using the following: Jandel SigmaStat version 2.0, for Fisher's exact test, Mann–Whitney rank sum test, and t-test. Comprehensive Meta-Analysis version 3.0, for calculating OR (odds ratio) with confidence interval (CI - Confidence interval 95%): Cox regression model for cross analysis of independent predictors (predictor variables) in all examined groups in relation to treatment, 2.2 Cox regression model for risk analysis of cases (< 65 years and ≥ 65 years) versus controls (< 65 years and ≥ 65 years).

Results

Out of the examined number of patients, there were 25 patients < 65 years in the subgroup with class III compression, while the < 65 years subgroup with class III+ consisted of 26 patients. The subgroup of elderly patients with class III compression consisted of 25 patients, and the subgroup of elderly patients with class III+ compression consisted of 26 patients. In all subgroups, the female gender was somewhat more represented. There was a statistically significant difference in previous episodes of ulcerations ($p < 0.05$) in groups with moderate pressure aged < 65 vs. ≥ 65 years. There was also a statistically significant difference in the size of the ulcer for the following groups: moderate pressure age < 65 vs. ≥ 65; ($p < 0.05$) moderate pressure age ≥ 65 vs. high pressure ($p < 0.05$); duration of the ulcer for the following groups: moderate pressure age < 65 vs. ≥ 65 ($p < 0.01$) (Table 1).

Pain sensitivity (Table 2) to compression therapy was monitored in < 65 years and ≥ 65 years' patients with class III and III+ compression therapy. A pain scale ranging from 0 to 10 was used. The highest pain level was in ≥ 65 years of patients with class III+ standard value $\pm$ SD (5.61 ± 1.39). The Mann–Whitney rank sum test determined a statistically significant difference ($p \leq 0.001$) between A, < 65 years III vs. < 65 years III+; B, ≥ 65 years III vs. ≥ 65 years class III+.

Localized complications associated with the use of compression therapy were monitored in patients aged under 65 and 65 years and older, who were receiving class III and III+ compression therapy. Using Fisher's exact probability test, it was determined that there was no statistically significant difference in the percentage of patients with paresthesia aged < 65 years and those ≥ 65 years treated with class III and III+ compression therapy (Figure 1).

Table 1. Baseline characteristics of patients by groups

Variable	< 65 years, class III n = (25)	< 65 years class III+ n = (26)	≥ 65 years, class III n = (25)	≥ 65 years, class III n = (26)	p
Gender					
Male	12 (48%)	12 (46%)	11 (44%)	12 (46%)	ns
Female	13 (52%)	14 (54%)	14 (56%)	14 (54%)	ns
BMI (mean ± SD)	30.40 ± 1.86	30.43 ± 1.58	29.94 ± 1.83	30.15 ± 1.65	ns
Previous episodes of ulceration (n%)	15 (60%)	16 (61.54%)	23 (92%)	22 (84.61%)	< 0.05 (class III < 65 vs. ≥ 65)
History of deep vein thrombosis (n%)	11 (44%)	10 (38%)	10(40%)	12 (46%)	ns
Number of ulcers (n%)	22 (88%) ^a ; 3 (12%) ^b	22 (85%) ^a ; 4 (15%) ^b	19 (76%) ^a ; 6 (24%) ^b	21 (81%) ^a ; 5 (19%) ^b	ns
Duration of ulcer disease (yrs ± SD)	6.59 ± 3.80	7.52 ± 4.79	11.56 ± 6.30	9.81 ± 6.78	< 0.01 (class III < 65 vs. ≥ 65) ^c
Size of the ulcer (cm ²) (mean ± SD)	17.84 ± 12.26	16.96 ± 10.39	11.8 ± 6.06	16.96 ± 10.80	< 0.05 (class III < 65 vs. ≥ 65) ^c ; < 0.05 (class III ≥ 65 vs. class III+) ^c
Dermatitis n (%)	13 (52%)	10 (38%)	13 (52%)	16 (61%)	ns
Lipodermatosclerosis n (%)	11 (44%)	19 (73%)	7 (28%)	9 (35%)	< 0.05 (class III+ < 65 vs. ≥ 65) ^d
Infection n (%)	17 (68%)	13 (50%)	18 (72%)	19 (73%)	ns

BMI, body mass index; ns, not significant; aone ulcer; btwo ulcers; cMann–Whitney test; dChi–square test

Table 2. Pain sensitivity in all groups

Pain sensitivity	Score	Mean ± SD	Mode
< 65 class III	0–10	2.84 ± 1.11	2
≥ 65 class III	0–10	3.24 ± 1.05	3
< 65 class III+	0–10	5.46 ± 0.95	2
≥ 65 class III+	0–10	5.61 ± 1.39	3
Comparisons	A***, B***		

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 (Mann–Whitney test)

(A, < 65 class III vs. < 65 class III+; B, ≥ 65 class III vs. ≥ 65 class III+; C, < 65 class III vs. ≥ 65 class III; D, < 65 class III+ vs. ≥ 65 class III+)

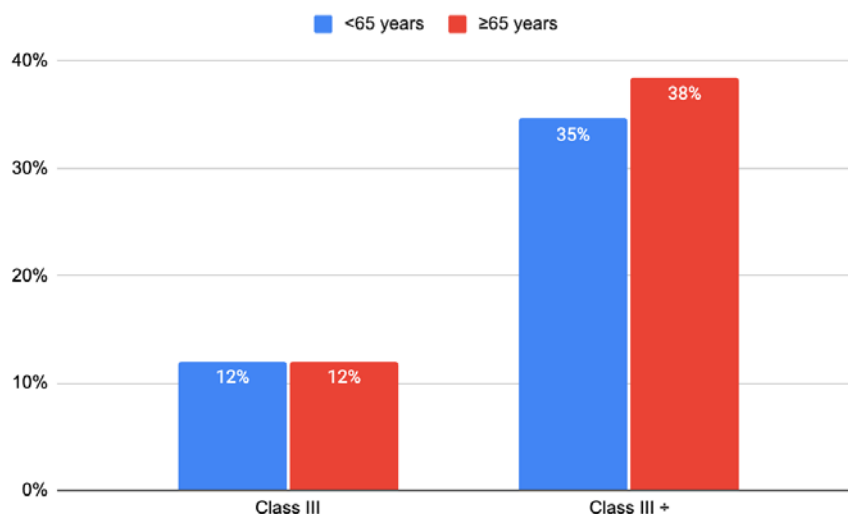


Figure 1. Percentage share (%) of patients with paresthesia < 65 years and ≥ 65 years, classes III and III+

The Cox regression model (OR, 95% CI) revealed a significant statistical risk of paresthesia in elderly patients treated with compression therapy class III + compared to class III, with an odds ratio (OR) of 4.58 (1.08–19.38; 95% CI; $p = 0.039$). This risk was not observed in patients under 65 years old treated with class III+ vs. class III compression therapy (Table 3).

The Fisher's exact probability test showed that there was no statistically significant difference between the groups of patients aged < 65 and ≥ 65 treated with class III and III+ compression treatment for superficial skin necrosis (Figure 2).

The Cox regression model (OR, 95% CI) was used to confirm that there was no statistically significant risk of superficial skin necrosis in patients aged ≥ 65 years treated with class III+

compared to class III compression therapy, as well as in patients aged < 65 years treated with class III+ compared to class III compression therapy (Table 4).

A statistically significant difference ($p \leq 0.05$) in the percentage of skin discoloration was seen between patients aged < 65 and ≥ 65 who received class III+ compression therapy (Graph 3).

The Cox regression model (OR, 95% CI) was used to evaluate whether there was a statistically significant risk of skin discoloration in patients aged ≥ 65 years treated with class III+ vs. III compression therapy compared to patients aged < 65 years treated with the same therapy (Table 5).

Table 3. Predictor variables (paresthesia) in the ≥ 65 years old/< 65 years old III+ compared to the ≥ 65 years old/< 65 years old III included in the Cox regression model (OR, 95% CI)

Variables	(OR, 95% CI)				
	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
≥65 years III+/III	4.583	1.084	19.383	2.069	0.039
<65 years III+/III	3.882	0.909	16.582	1.831	0.067
	0.01	0.1	1	1	100

OR, Odds Ratio; CI, Confidence Interval; Z-value, test statistic from regression model; p-value, probability value

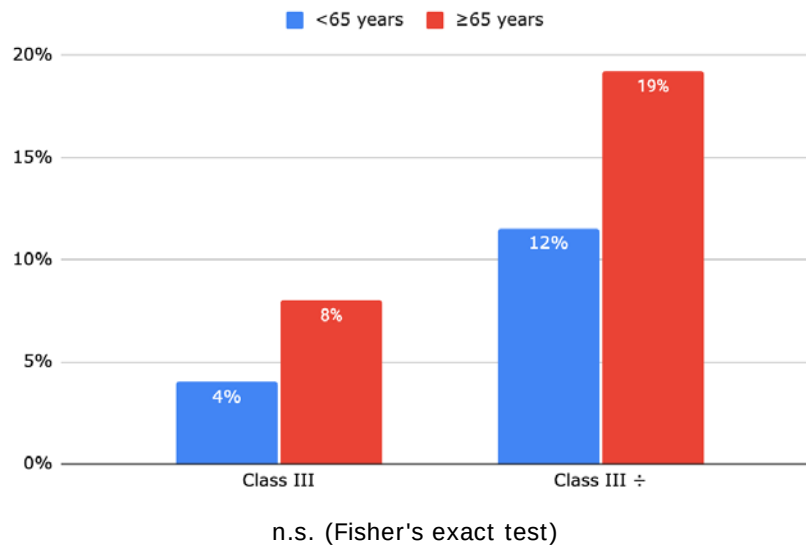


Figure 2. Percentage (%) of patients with superficial skin necrosis < 65 years and ≥ 65 years, classes III and III +

Table 4. Predictor variables (superficial skin necrosis) in the ≥ 65/<65 class III+ compared to the ≥ 65/< 65 class III, included in the Cox regression model (OR, 95% CI)

Variables	(OR, 95% CI)				
	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
≥65 y, Class III/III+	2.738	0.479	15.651	1.132	0.257
<65 y, Class III/III+	3.130	0.303	32.314	0.958	0.338

0.01	0.1	1	10	100
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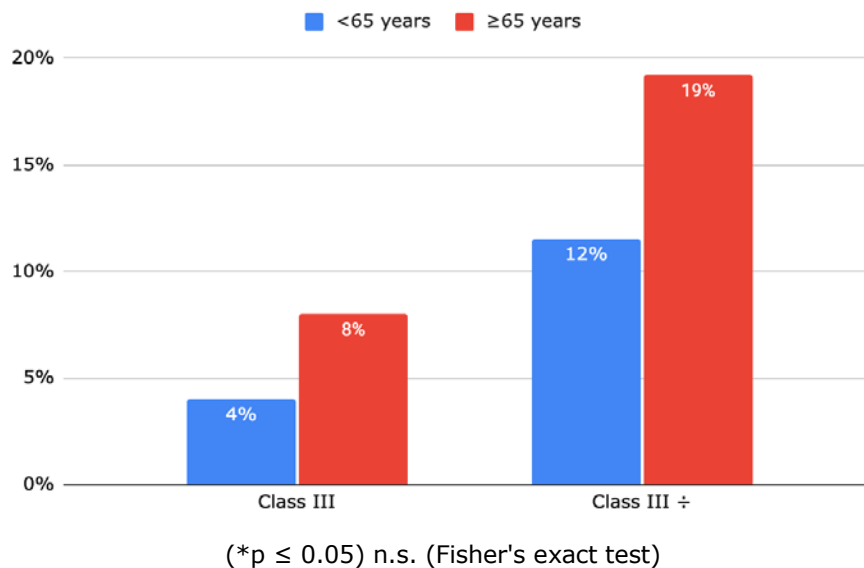
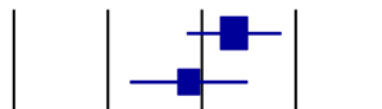


Figure 3. Percentage (%) of patients with skin discoloration aged < 65 years and ≥ 65 years, classes III and III +

Table 5. Predictor variables (discoloration of the skin) in the ≥ 65 / < 65 class III+ compared to the ≥ 65 / < 65 class III, included in the Cox regression model (OR, 95% CI).

Variables	(OR, 95% CI)				
	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
≥ 65 y, Class III/III+	2.204	0.688	7.065	1.330	0.184
< 65 y, Class III/III+	0.727	0.171	3.093	-0.431	0.666

0.0	1	0.1	1	10	100
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Discussion

Venous ulcerations affect approximately 1% of the global population, with a higher incidence in individuals aged 65 and above. The gender representation ratio indicates a much greater representation of women than men, with a ratio of 3:1 (women to men) (1). Chronic venous ulcerations occur in 0.6–3% of the population over the age of 60, and in patients over the age of 80, the percentage increases by another 5%. Chronic venous ulcerations are a prevalent cause of morbidity, and the prevalence of these ulcerations in communities ranges from 1.9% to 13.1%. (3–6). Ageing and the prevalence of atherosclerosis risk factors like smoking, obesity, and diabetes are believed to heighten the occurrence of ulceration. With a 2.5% mortality rate, over 10% of the population may experience a chronic wound at some point in their lives (5).

Venous ulcerations occur more frequently in patients ≥ 65 years than < 65 years, although the incidence and prevalence of chronic venous ulcerations in patients ≥ 65 years are not well established. Adequate compression therapy remains the gold standard in the treatment of venous ulcers. What cure rate can be expected when applying this type of treatment? The study by Karanikolic V et al. (7) on 102 patients treated with compression therapy showed that a higher degree of applied compression therapy leads to a statistically higher rate of venous healing and significantly faster healing.

Pain sensitivity to compression therapy was monitored in < 65 - and ≥ 65 -year-old patients with class III and III+ compression therapy. A pain scale ranging from 0 to 10 was used. The highest pain level was in elderly patients with class III+ standard value $\pm$ SD (5.61 ± 1.39). The Mann–Whitney rank sum test determined a statistically significant difference ($p \leq 0.001$) between A, participants aged < 65 years with stage III disease vs. participants aged < 65 years with stage III+ disease; and B, participants aged ≥ 65 years with stage III disease vs. participants aged ≥ 65 years with stage III+ disease. The

obtained results are consistent with other tests. The study by Hellström et al. (8) on 1,824 subjects with lower extremity ulcerations measured pain intensity during compression therapy. Pain intensity was measured by the Numeric Rating Scale (NRS). Pain level > 5 was present in 34.8% of subjects. Greater pain intensity correlated with the number of ulcerations, so patients with more ulcerations also experienced more intense pain.

A complication that may arise from utilizing a compressive bandage is paresthesia. Fisher's exact probability test was employed to ascertain that the proportion of patients aged < 65 and ≥ 65 years who experienced paresthesia while undergoing compression therapy of classes III and III+ does not differ statistically significantly. However, the Cox regression model showed a statistically significant risk of paresthesia among elderly patients treated with compression therapy class III+ vs. III, OR = 4.58 (1.08–19.38; 95% CI; $p = 0.039$), in contrast to those < 65 years treated with compression therapy class III+ vs. III. One of the relatively common complications of compressive therapy is superficial skin necrosis. Applying Fisher's exact probability test as a Cox regression model, it was concluded that there is no statistically significant difference in superficial skin necrosis between patients under 65 years old and patients aged 65 and above who received class III and III+ compression therapy.

Skin necrosis after applying compression therapy has been described in several studies dealing with this problem (9–12), including one Scottish study (13). This Scottish study documented the five-year experience of Scottish surgeons regarding skin necrosis in compression therapy-treated patients with ulcers of the lower extremities. Of 154 surgeons who participated in this study, one-third reported at least one case of skin damage, while 20% of the surgeons who participated in this study had more than one case. Our results are not in conflict with the results of other studies. Our study also showed the presence of superficial skin necrosis, but without statistical significance concerning old age. The ankle-brachial pressure index (ABPI) is used to measure the

condition of arterial circulation in order to administer compression therapy safely. Diagnosis of peripheral artery disease typically requires an ABPI of ≤ 0.90 at rest, with ABPI ≤ 0.50 suggesting significant limb ischemia. Most compression therapy guidelines for people with deep vein thrombosis advocate using compression stockings with a pressure of 23 mmHg (14). Few studies have addressed the adverse effects of compression therapy in patients treated for chronic venous ulceration. The above-mentioned side effects are reflected in the appearance of pain due to applying compression to the extremity, superficial skin necrosis, discoloration and other trophic changes caused by compression therapy. It is essential to point out that the quality of life is crucial for applying a therapeutic procedure. Also, the occurrence of side effects of therapy should be minimized so that this therapeutic procedure can be safely and successfully applied to many patients (15–17). When using a multi-layer compression bandage, and especially when applying a higher level of compression, it is essential to strictly adhere to the instructions for

applying this type of therapy. Before using compression therapy, one should strictly consider the state of the arterial system, i.e., the presence of chronic occlusive diseases. All this requires a modern concept and approach to treating patients with chronic venous ulcers. The peculiarities of each patient should be taken into account, and therapeutic procedures should be adjusted according to age, the presence of accompanying chronic diseases, and the state of the venous system of the lower extremities (18–23).

Conclusion

Increasing the intensity of compression therapy in both age groups results in a higher prevalence of patients experiencing paresthesia and increased pain sensitivity. The study found no statistically significant correlation between the age of patients receiving compression therapy and the likelihood of superficial necrosis and skin discoloration.

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Originalni rad

UDC: 616.14-002.44:615.81.06
doi: 10.5633 / amm.2025.0208**NEŽELJENI EFEKTI KOMPRESIVNE TERAPIJE U TOKU
LEČENJA VENSKIH ULCERACIJA DONJIH
EKSTREMITETA***Maša Golubović^{1,2}, Iva Binić^{2,3}*¹Univerzitetski klinički centar Niš, Klinika za dermatovenerologiju, Niš, Srbija²Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija³Univerzitetski klinički centar Niš, Klinika za psihijatriju, Niš, SrbijaKontakt: Maša Golubović
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Venske ulceracije čine jedno od najčešćih vaskularnih oboljenja među ljudima. Nastaju kao komplikacija hronične venske insuficijencije. Primena adekvatnog stepena kompresivne terapije u lečenju venskih ulceracija predstavlja zlatni standard u terapiji. Cilj ovog istraživanja bio je da se utvrdi učestalost komplikacija kompresivne terapije u lečenju venskih ulceracija donjih ekstremiteta u zavisnosti od starosti i primenjenog stepena kompresije. Ispitivanje je obuhvatilo 102 bolesnika starija od 18 godina sa venskim ulceracijama, koja su se lečila na Klinici za dermatovenerologiju Univerzitetskog kliničkog centra u Nišu. Ulceracije su praćene 24 nedelje od početka tretmana. Bolesnici su na osnovu starosti podeljeni u dve grupe: u prvoj grupi bili su oni sa ≥ 65 godina, a u drugoj oni sa < 65 godina. Svaka grupa bolesnika bila je podeljena na dve podgrupe na osnovu stepena kompresivne terapije. Kod bolesnika su se pratili pol, starost, *body mass index* (BMI), površina, lokalizacija i broj ulceracija, stepen prisutnog granulacionog tkiva i fibrina u ulceraciji, prisustvo dermatitisa, lipodermatoskleroze i infekcije i dužina trajanja bolesti. Utvrđeno je da postoji statistički značajna razlika u broju prethodnih epizoda ulceracija, veličini ulceracija i trajanju bolesti između ispitivanih grupa. Takođe, uočena je razlika u nivou bola u odnosu na stepen kompresije, kao i rizik od parestezija u odnosu na godine starosti. Za ostale ispitivane parametre nije bilo statističke značajnosti između ispitivanih grupa. Viši stepen kompresivne terapije u obema starosnim grupama bolesnika dovodi do porasta stope bolesnika sa parestezijama i većim nivoom osetljivosti na bol. Ova studija je pokazala da ne postoji statistički značajan rizik od površne nekroze i diskoloracije kože koji bi bio povezan sa starošću bolesnika tretiranih kompresivnom terapijom.

*Acta Medica Medianae 2025; 64(2):71–79.****Ključne reči:*** venske ulceracije, kompresivna terapija, parestezija, bol

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ALCOHOL CONSUMPTION AMONG FIRST AND SECOND-YEAR UNIVERSITY STUDENTS IN NOVI SAD

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Alcohol is the most consumed psychoactive substance worldwide. The aim of this study was to assess the prevalence of alcohol consumption among university students in Novi Sad and the association of socioeconomic characteristics and psychoactive substance use with alcohol consumption.

The study was a cross-sectional survey of University of Novi Sad students in 2019, as part of the project "Health behaviours of students: benefits and risks" funded by the Provincial Secretariat for Higher Education and Scientific Research. The research involved 664 students from thirteen faculties. The study instrument was an online questionnaire, and alcohol consumption was classified by daily intake units. The association of socioeconomic characteristics and psychoactive substance use with alcohol use was analyzed using the Chi-square or Fisher's exact test.

Two of five students drank alcohol more than once a month (43.2%), and only 15.5% reported that they had never drunk alcohol. Half of the students (50.2%) reported binge drinking, with a higher prevalence among men than women (64.2% vs. 44.1%) ($p < 0.001$). Approximately 4% (3.8%) of students believed that peer influence led them to drink more alcohol than they otherwise would have, with a higher prevalence among young men than women (7.0% vs. 2.4%) ($p < 0.001$). The prevalence of heavy drinking and binge drinking was higher among smokers and illicit drug users ($p < 0.001$).

The prevalence of alcohol use and binge drinking among university students in Novi Sad is high. Peers have a negative influence on student alcohol use. Heavy drinking and binge drinking were more prevalent among smokers and illicit drug users.

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Key words: alcohol drinking, binge drinking, peer influence, smoking, illicit drugs

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Introduction

Alcohol is the most often consumed psychoactive substance in the world (1). In 2016, according to global statistics, alcohol was the seventh risk factor for both deaths and disability-adjusted life years (DALYs), which emphasizes the seriousness of this public health problem (2). Alcohol consumption among young people represents a serious public health challenge that requires a thorough analysis of risk factors in

order to develop effective prevention strategies (3). According to the data of the European School Survey Project on Alcohol and Other Drugs (ESPAD), in almost all countries, more than half of students reported drinking alcohol at least once in their lives. In ESPAD countries, 47% of students reported alcohol use in the last 30 days, and an average of 13% of students reported being intoxicated in the last 30 days. Based on data from the ESPAD project in Serbia, 87% of students reported consuming alcohol at least once during their lifetime, 56% of students reported alcohol use in the last 30 days, and 12% of students reported being intoxicated in the last 30 days (4). The COVID-19 pandemic significantly influenced global alcohol consumption, with a trend of decreasing alcohol consumption among young people (5). Early onset and intensive alcohol consumption during the period of adolescence can have long-term consequences on brain development, including hypoglycemia in certain parts of the cerebral cortex and increased risk of addiction (6). The binge drinking can reduce cognitive abilities and motor skills, which leads to increased risk of injury, accidents, and risky

sexual behavior (7). The influence of alcohol on the ability to drive is pronounced, especially in young drivers, which leads to an increased risk of severe head injuries and fatal accidents (8). Drivers under the influence of alcohol show a tendency to accidents during parking, which directly depends on the level of alcohol in the blood (9).

In 2019, alcohol was the cause of 156,000 deaths and 10 million lost years of healthy life due to traffic injuries caused by drivers under the influence of alcohol (5). The mortality rate in our country was 1.3 per 100,000 inhabitants due to the same circumstances, according to data from the Road Traffic Agency of the Republic of Serbia in the same year (10, 11). A study conducted in Serbia in 2019 showed that every hundredth driver drove under the influence of alcohol (12).

It is important to understand the factors that affect alcohol consumption among the young to successfully design preventive programs. Special emphasis should be placed on family support, changes in norms in peer groups and community influence on attitudes towards alcohol. Social and cultural influences, as they are family models, social norms, and media influence are key in the formation of attitudes and habits of young people towards alcohol. Family influence is one of the first and most significant factors that shape the perception of young people about alcohol. Peers often function as role models and can exert direct pressure on young people to adhere to certain norms related to alcohol. Increased alcohol consumption is also influenced by individual characteristics (3). Increased alcohol consumption can also be influenced by the fact that students are exposed to elevated levels of stress during their studies (13).

Aim

This study aimed to assess the prevalence of alcohol consumption among first- and second-year university students in Novi Sad and the association of socioeconomic characteristics and the use of other psychoactive substances with alcohol consumption.

Materials and Methods

The study is a part of the project "Health behaviours of students: benefits and risks" financed by the Provincial Secretariat for Higher Education and Scientific Research. This cross-sectional study was conducted among students at the University of Novi Sad in the year 2019. Study included 664 students (456 first-year and 208 second-year students) from the following faculties: Faculty of Medicine (43.1%), Faculty of Technical Sciences (12.5%), Faculty of Law (7.1%), Faculty of Sciences (7.1%), Faculty of Agriculture (6.3%), Faculty of Sport and Physical Education (6.3%), Faculty of Economics (5.9%), Faculty of Education (5.6%), Faculty of Philosophy (1.7%), "Mihajlo

Pupin" Technical Faculty (1.4%), Faculty of Technology (0.9%), Academy of Arts (0.6%) and other faculties (0.2%).

The instrument of the study was an online questionnaire with electronic consent for participation in the study. The link to the questionnaires was given to the first- and second-year students after lectures. The questionnaire included questions on demographics (gender, age, and year of the study), socioeconomic status (self-assessed material status of the family), and questions on lifestyle factors (cigarette smoking, electronic cigarette smoking, water pipe smoking, marijuana use, ecstasy use, amphetamine use, and sedative or sleeping pills use without a prescription). The outcome variables were alcohol consumption and binge drinking. According to answers on alcohol consumption on a typical day (number of alcohol units), students were categorized into three groups: students who never drink, moderate drinking (1-2 alcohol units daily), and heavy drinking (three or more alcohol units daily). Binge drinking was defined as having six or more drinks on a single occasion.

Statistical analysis

Descriptive and inferential statistics methods were used for data analysis. Categorical variables were presented as frequencies and percentages, and numerical variables as means and standard deviations. The Chi-square or Fisher's exact test was used to test the association between socioeconomic characteristics and the use of other psychoactive substances and alcohol use. Statistically significant results were considered p-values less than 0.05. The statistical analyses were done using the IBM Statistical Package for the Social Sciences (IBM SPSS).

Ethical considerations

Participation in the study was anonymous and voluntary. The ethical aspects of the study were approved by the Ethics Committee of the Institute of the Public Health of Vojvodina, Novi Sad (Approval number: 01-340/2).

Results

The sample included 664 students (69.7% females and 30.3% males) of average age 19.8 (SD = 1.43) (min 18 and max 38 years). More than half of the students assessed their material status as average (55.6%), 40.4% as good, and 4.1% as bad. Around 43% of students were from the Faculty of Medicine (Table 1). Other characteristics of the sample are presented in Table 1.

Around 40% of the students drank alcohol once a month or less often (41.3%), one-third drank 2–4 times per month (33.6%), 8.4% drank 2–3 times weekly, and 1.4% drank 4 times or more weekly.

Approximately 44% of the surveyed students consumed three or more alcohol units on a typical day (44.3%), 40.2% drank less, and only 15.5% answered that they had never drunk alcohol. Alcohol consumption was more prevalent in men than women ($p < 0.001$). On the days when students consumed alcohol, their consumption typically exceeded current guidance. Every second young men (53.8%) consumed three or more drinks on a typical day (heavy drinking), and 40.2% of girls consumed three or more drinks. There were no significant differences in alcohol consumption according to age, faculty type, year of the study, and material status of the family (Table 2).

Binge drinking was widespread among students. Approximately half of the students reported binge drinking (50.2%), sometimes multiple times per month. Every third student

binge drank less than once monthly (33.3%), 13.7% binge drank at least once monthly, 2.9% at least once weekly, and 0.5% reported binge drinking almost every day or daily. Binge drinking was significantly more prevalent among young men (64.2%) than girls (44.1%) ($p < 0.001$). There were no significant differences in binge drinking according to age, type of faculty, year of the study, and material status of the family (Table 3). Approximately 4% of students (3.8%) believed that they drank more alcohol than they otherwise would have due to the influence of their friends. This influence was significantly more prevalent among young men (7.0%) than girls (2.4%) ($p < 0.001$) (Table 4).

The higher influence of friends on drinking behaviour was associated with higher alcohol consumption and more prevalent binge drinking among students.

Table 1. Sample characteristics by gender, Novi Sad in 2019

Characteristic	Total	Men	Women	p-value*
	n (%)	n (%)	n (%)	
Age (years)				
18–19	325 (49.3)	91 (45.7)	234 (50.9)	0.444
20–21	300 (45.5)	96 (48.2)	204 (44.3)	
22+	34 (5.2)	12 (6.0)	22 (4.8)	
Faculty				
Medicine	286 (43.1)	78 (38.8)	208 (44.9)	0.168
Other	378 (56.9)	123 (61.2)	255 (55.1)	
Year of the study				
First	456 (68.7)	128 (63.7)	328 (70.8)	0.042
Second	208 (31.3)	73 (36.3)	135 (29.2)	
Material status of family				
Good	268 (40.4)	89 (44.3)	179 (38.7)	0.236
Average	369 (55.6)	102 (50.7)	267 (57.7)	
Bad	27 (4.1)	10 (5.0)	17 (3.7)	
Total	664 (100.0)	201 (30.3)	463 (69.7)	-

Table 2. Prevalence of alcohol consumption according to sociodemographic characteristics of students, Novi Sad in 2019

Characteristic	Total				Men				Women			
	Never drinks	Moderate	Heavy	p-value*	Never drinks	Moderate	Heavy	p-value*	Never drinks	Moderate	Heavy	p-value*
	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Gender												
Men	24 (12.1)	68 (34.2)	107 (53.8)	0.005	-	-	-		-	-	-	-
Women	78 (17.0)	197 (42.9)	185 (40.2)		-	-	-		-	-	-	
Age (years)												
18–19	54 (16.7)	133 (41.2)	136 (42.1)	0.147	8 (8.9)	34 (37.8)	48 (53.3)	0.341	46 (19.7)	99 (42.5)	88 (37.8)	0.075
20–21	41 (13.8)	113 (37.9)	144 (48.3)		15 (15.8)	27 (28.4)	53 (55.8)		26 (12.8)	86 (42.4)	91 (44.8)	
22+	6 (18.2)	18 (54.5)	9 (27.3)		1 (8.3)	6 (50.0)	5 (41.7)		5 (23.8)	12 (57.1)	4 (19.0)	
Faculty												
Medicine	49 (17.4)	116 (41.3)	116 (41.3)	0.306	10 (13.2)	27 (35.5)	39 (51.3)	0.849	39 (19.0)	89 (43.4)	77 (37.6)	0.447
Other	53 (14.0)	149 (39.4)	176 (46.6)		14 (11.4)	41 (33.3)	68 (55.3)		39 (15.3)	108 (42.4)	108 (42.4)	
Year of the study												
First	71 (15.7)	186 (41.1)	196 (43.3)	0.723	12 (9.5)	44 (34.9)	70 (55.6)	0.351	59 (18.0)	142 (43.4)	126 (38.5)	0.432
Second	31 (15.0)	79 (38.3)	96 (46.6)		12 (16.4)	24 (32.9)	37 (50.7)		19 (14.3)	55 (41.4)	59 (44.4)	
Material status of family												
Good	36 (13.6)	97 (36.7)	131 (49.6)	0.261	8 (9.1)	28 (31.8)	52 (59.1)	0.619	28 (15.9)	69 (39.2)	79 (44.9)	0.550
Average	62 (16.8)	157 (42.7)	149 (40.5)		14 (13.9)	37 (36.6)	50 (49.5)		48 (18.0)	120 (44.9)	99 (37.1)	
Bad	4 (14.8)	11 (40.7)	12 (44.4)		2 (20.0)	3 (30.0)	5 (50.0)		2 (11.8)	8 (47.1)	7 (41.2)	
Total	102 (15.5)	265 (40.2)	292 (44.3)	-	24 (12.1)	68 (34.2)	107 (53.8)	-	78 (17.0)	197 (42.9)	185 (40.2)	-

Table 3. Prevalence of binge drinking according to socio-demographic characteristics of students, Novi Sad in 2019

Characteristic	Total			Men			Women		
	No	Yes	p-value*	No	Yes	p-value*	No	Yes	p-value*
	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
Gender									
Men	72 (35.8)	129 (64.2)	< 0.001	-	-		-	-	-
Women	259 (55.9)	204 (44.1)		-	-		-	-	
Age (years)									
18–19	168 (51.7)	157 (48.3)	0.654	30 (33.0)	61 (67.0)	0.734	138 (59.0)	96 (41.0)	0.442
20–21	144 (48.0)	156 (52.0)		36 (37.5)	60 (62.5)		108 (52.9)	96 (47.1)	
22+	18 (50.0)	16 (50.0)		5 (41.7)	7 (58.2)		12 (54.5)	10 (45.5)	
Faculty									
Medicine	152 (53.1)	134 (46.9)	0.158	31 (39.7)	47 (60.3)	0.440	121 (58.2)	87 (41.8)	0.218
Other	179 (47.4)	199 (52.6)		41 (33.3)	82 (66.7)		138 (54.1)	117 (45.9)	
Year of the study									
First	233 (51.1)	223 (48.9)	0.358	42 (32.8)	86 (67.2)	0.305	191 (58.2)	137 (41.8)	0.124
Second	98 (47.1)	110 (52.9)		30 (41.1)	43 (58.9)		68 (50.4)	67 (49.6)	
Material status of family									
Good	130 (48.5)	138 (51.5)	0.832	30 (33.7)	59 (66.3)	0.590	100 (55.9)	79 (44.1)	0.966
Average	187 (50.7)	182 (49.3)		36 (36.3)	65 (63.7)		150 (56.2)	117 (43.8)	
Bad	14 (51.9)	13 (48.1)		5 (50.0)	5 (50.0)		9 (52.9)	8 (47.1)	
Total	331 (49.8)	333 (50.2)	-	72 (35.8)	129 (64.2)	-	259 (55.9)	204 (44.1)	-

*Chi-square test or Fisher's exact test

Table 4. Influence of friends on higher alcohol consumption according to gender, Novi Sad in 2019

Gender	Higher alcohol consumption due to a friend's influence			p-value*
	No	Sometimes	Always	
	n (%)	n (%)	n (%)	
Men	116 (57.7)	71 (35.3)	14 (7.0)	< 0.001
Women	329 (71.1)	123 (26.6)	11 (2.4)	
Total	445 (67.0)	194 (29.2)	25 (3.8)	

*Chi-square test

This association was significant in both genders. Among students who said that they had always drunk more due to the influence of friends, 87.5% reported heavy drinking, and 80% reported binge drinking (Table 5).

Among first- and second-year university students, 22.1% were smokers and 9.2% were former smokers. Electronic cigarettes were used by 2.6% of students and water pipes by 5.9%. Every fifth student consumed marijuana at least once in a lifetime (21.4%). Ecstasy was used by 4.4% of students and amphetamines by 2.0% of students. Every sixth student (17.3%) used sedatives or sleeping pills without a doctor's prescription.

The prevalence of heavy drinking was significantly higher among smokers (62.6%) than among non-smokers (37.8%) ($p < 0.001$). Binge drinking was also more prevalent among smokers (63.9%) than non-smokers (43.2%) ($p < 0.001$). Electronic cigarette smoking was associated with a higher prevalence of heavy drinking, but not with binge drinking. Among students who reported marijuana use, 63.8% were heavy drinkers, compared to 39% of those who had never used marijuana. The use of ecstasy and amphetamines was associated with a higher prevalence of heavy drinking, as well as with binge drinking. The use of sedatives or sleeping pills was not associated with alcohol consumption and binge drinking (Table 6).

Table 5. Influence of friends on higher alcohol consumption and binge drinking, Novi Sad in 2019

Higher alcohol consumption and binge drinking due to friend's influence	Alcohol consumption				Binge drinking		
	Never	Moderate	Heavy	p-value*	No	Yes	p-value*
	n (%)	n (%)	n (%)		n (%)	n (%)	
Total							
No	96 (21.7)	184 (41.6)	162 (36.7)	< 0.001	260 (58.4)	185 (41.6)	< 0.001
Sometimes	6 (3.1)	78 (40.4)	109 (56.5)		66 (34.0)	128 (66.0)	
Always	0 (0.0)	3 (12.5)	21 (87.5)		5 (20.0)	20 (80.0)	
Men							
No	22 (19.1)	45 (39.1)	48 (41.7)	< 0.001	56 (48.3)	60 (51.7)	< 0.001
Sometimes	2 (2.8)	22 (31.0)	47 (66.2)		14 (19.7)	57 (80.3)	
Always	0 (0.0)	1 (7.7)	12 (92.3)		2 (14.3)	12 (85.7)	
Women							
No	74 (22.6)	139 (42.5)	114 (34.9)	< 0.001	204 (62.0)	125 (38.0)	< 0.001
Sometimes	4 (3.3)	56 (45.9)	62 (50.8)		52 (42.3)	71 (57.7)	
Always	0 (0.0)	2 (18.2)	9 (81.8)		3 (27.3)	8 (72.7)	

*Chi-square test

Table 6. Association of cigarette smoking and illicit drug use with alcohol consumption and binge drinking, Novi Sad in 2019

	Alcohol consumption				Binge drinking		
	Never	Moderate	Heavy	p-value*	No	Yes	p-value*
	n (%)	n (%)	n (%)		n (%)	n (%)	
Smoking							
Non-smoker	94 (20.8)	187 (41.4)	171 (37.8)	< 0.001	259 (56.8)	197 (43.2)	< 0.001
Former smoker	2 (3.3)	29 (48.3)	29 (48.3)		19 (31.1)	42 (68.9)	
Smoker	6 (4.1)	49 (33.3)	92 (62.6)		53 (36.1)	94 (63.9)	
Electronic cigarettes							
Non-smoker	102 (16.2)	258 (40.9)	271 (42.9)	0.009	322 (50.6)	314 (49.4)	0.133
Former smoker	0 (0.0)	4 (36.4)	7 (63.6)		3 (27.3)	8 (72.7)	
Smoker	0 (0.0)	3 (17.6)	14 (82.4)		6 (35.3)	11 (64.7)	
Water pipe smoking							
Never	92 (17.6)	221 (42.2)	211 (40.3)	< 0.001	286 (54.1)	243 (45.9)	< 0.001
Yes, before	9 (9.4)	36 (37.5)	51 (53.1)		39 (40.6)	57 (59.4)	
Yes	1 (2.6)	8 (20.5)	30 (76.9)		6 (15.4)	33 (84.6)	
Marijuana							
Never	96 (18.5)	220 (42.5)	202 (39.0)	< 0.001	296 (56.7)	226 (43.3)	< 0.001
At least once	6 (4.3)	45 (31.9)	90 (63.8)		35 (24.7)	107 (75.4)	
Ecstasy							
Never	102 (16.2)	258 (41.0)	270 (42.9)	< 0.001	327 (51.5)	308 (48.5)	< 0.001
At least once	0 (0.0)	7 (24.1)	22 (75.9)		4 (13.8)	25 (86.2)	
Amphetamine							
Never	102 (15.8)	262 (40.6)	282 (43.7)	0.045	330 (50.7)	321 (49.3)	0.003
At least once	0 (0.0)	3 (23.1)	10 (76.9)		1 (7.7)	12 (92.3)	
Sedatives or sleeping pills							
Never	92 (16.9)	218 (40.0)	235 (43.1)	0.080	282 (51.4)	267 (48.6)	0.101
At least once	10 (8.8)	47 (41.2)	57 (50.0)		49 (42.6)	66 (57.4)	

* Chi-square test or Fisher's exact test

Discussion

This study estimated the prevalence of alcohol consumption among university students in Novi Sad and its relationship with socioeconomic characteristics and the use of other psychoactive substances. Two of five students in our study consumed alcohol once a month or less often (41.3%). The average prevalence of alcohol consumption among students in Europe was 47% during the past 30 days, with the highest prevalence of alcohol use in Denmark (74%) (4). There are large variations in alcohol consumption among university students in and outside European countries. Asians tend to have the lowest risk for alcoholism compared to Europeans and African Americans, partly due to genetic variations that cause more intense and unpleasant reactions to alcohol consumption (14). Every sixth student from our research answered that they never drink alcohol. In the Spain study, 7.8% of students did not drink alcohol during the last year (15). According to the results of our questionnaire, men drink alcohol more often than women, which is consistent with other studies (16, 17). Also, in most European countries from the ESPAD project, men drink alcohol more often than women (4).

This research paper showed that 44% of students drink heavily on a typical day. More than half of Norwegian students reported drinking five or more alcoholic drinks on a typical day when drinking, which is more compared to our study. Among students, heavy drinking is considered normal, which is certainly worrying (17). Excessive alcohol consumption can lead to many diseases including cancers, liver cirrhosis, heart disease, and an increased risk of HIV transmission, and it can be lethal (5).

Our results showed that half of the students reported binge drinking. More than 30% of students reported binge drinking in Hungary, which is less compared to our study (18). Asian authors have concluded that over time, as students progress through their years of study, the propensity for binge drinking increases (19). University students who prefer binge drinking, especially extreme ones (≥ 10 drinks/occasion), had lower scores on the logical memory subtest (20, 21). Extreme prepartying and drinking games in college can lead to intoxication and negative consequences. The frequency of prepartying and participation in drinking games was linked to an increased risk of extreme drinking among students (22). The prevalence of binge drinking was higher among young men than girls, but according to age, type of faculty, year of the study and material status, there were no significant differences. Similar to the results obtained in the present study, binge drinking is reported more often in male students in other studies (19, 23).

Almost 4% of students in our study reported that they always drank more alcohol when influenced by friends, while less than one-third of students believed that they sometimes consumed more alcohol than usual under the influence of friends. These results are also confirmed in some

other studies. According to the study of Salame et al., peer influence has a more significant effect on the harmful use of alcohol. This study shows that people often neglect all potential dangers related to alcohol due to peer pressure and influence (24). Schaefer et al. recorded that students were seven times more likely to drink alcohol weekly if their friends also drank weekly (25).

Results of the current study indicate that 87.5% of students always drink heavily under the influence of friends. In the study Schaefer et al., around 30% of students reported binge drinking in the past two weeks. Under the influence of peers, students engage in binge drinking 6.8 times more often (25). The influence of friends on higher alcohol consumption and binge drinking is more pronounced in men than in women in our study.

In our research, more than 20% of students were cigarette smokers, while a much smaller percentage of students used electronic cigarettes and water pipes. Cigarette smokers and water pipe smokers were more prone to heavy drinking and binge drinking. Meanwhile, electronic cigarette smokers had a higher prevalence of heavy drinking, but not binge drinking. In a cross-sectional multicentric study conducted in Germany and Hungary, 18% of students were cigarette smokers, and, as in our study, a much smaller percentage of students used electronic cigarettes and water pipes (26). The worrying fact is that every fifth student tried marijuana at least once in their life according to our study. Other types of illicit drugs, like ecstasy and amphetamines, are less frequently used. Higher alcohol consumption was associated with the use of illicit drugs like marijuana and ecstasy. The use of sedatives and sleeping pills was not associated with higher alcohol consumption, which is positive because simultaneous consumption of sedatives or sleeping pills with even a small amount of alcohol can lead to overdose, sleepiness, dizziness, slowed or difficulty breathing and can be potentially fatal (27). At the university, students have easy access to alcohol and illicit drugs and consume these substances for a sense of belonging and interaction with other peers. Students at the university in Spain most often combine alcohol with cannabis (28). According to a Spanish study conducted among university students, every third binge drinker has tried cannabis (29). Students less often use ecstasy and amphetamine according to the study conducted in the United Kingdom, which is the same as in our study (30). According to the authors from Hungary, binge drinking is associated with smoking and illicit drug use (18).

Medical students play a key role in the promotion and display of healthy habits because their knowledge and example can influence their peers to adopt healthy lifestyles (31). Unfortunately, our study didn't find any differences between medical students and others. Appropriate education programs are needed to reduce early and intensive alcohol use among young people. Ridoult et al. suggested introducing messages about safe alcohol use to students on Facebook (32). There is less consumption in

countries that have limitations in terms of shop working hours, licenses for the retail sale of alcohol outside the premises, and legal drinking age provisions (33).

One of the limitations of the study is that a questionnaire was used to collect responses, which may lead to some distortion of the results. However, due to the anonymous nature of the questionnaire, we believe that students were less likely to be dishonest. The second limitation of the study is that it included only first- and second-year university students, while older students were not included.

Conclusion

The prevalence of alcohol use and binge drinking among university students in Novi Sad is high, and it is more prevalent among young men than women. The study shows that peers have a negative influence on student alcohol use. The prevalence of heavy drinking and binge drinking was higher among students who reported that they always drank more due to the influence of friends. Smoking and the use of illegal drugs are linked to heavy drinking and more frequent binge drinking among students.

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**KONZUMIRANJE ALKOHOLA MEĐU STUDENTIMA PRVE
I DRUGE GODINE FAKULTETA U NOVOM SADU**

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Alkohol je psihoaktivna supstanca koja se najčešće konzumira u svetu. Cilj ovog rada bio je da proceni prevalenciju konzumiranja alkohola kod studenata Univerziteta u Novom Sadu i povezanost socioekonomskih karakteristika i korišćenja psihoaktivnih supstanci sa konzumiranjem alkohola.

Studija je sprovedena kao studija preseka među studentima Univerziteta u Novom Sadu 2019. godine, u okviru projekta „Zdravstveno ponašanje studenata: koristi i rizici” koji je finansirao Pokrajinski sekretarijat za visoko obrazovanje i naučnoistraživačku delatnost. U istraživanju su učestvovala 664 studenta sa trinaest fakulteta. Kao instrument istraživanja korišćen je onlajn upitnik. Konzumacija alkohola je klasifikovana prema jedinicama dnevnog unosa. Povezanost socioekonomskih karakteristika i korišćenja psihoaktivnih supstanci sa upotrebom alkohola analizirana je pomoću Hi-kvadrat testa ili Fišerovog testa egzaktnosti.

Dvoje od pet studenata pilo je alkohol više od jednom mesečno (43,3%), a samo je 15,5% njih izjavilo da nikada nije pilo alkohol. Polovina studenata (50,2%) prijavila je opijanje, a prevalencija je bila veća kod muškaraca nego kod žena (64,2% naspram 44,1%; $p < 0,001$). Približno 4% (3,8%) studenata veruje da ih je uticaj vršnjaka naveo da piju više alkohola nego što bi inače pili; prevalencija je bila veća kod mladih muškaraca nego kod žena (7,0% naspram 2,4%; $p < 0,001$). Prevalencija prekomerne konzumacije alkohola i opijanja bila je veća među pušačima i studentima koji koriste ilegalne droge ($p < 0,001$).

Zabeležena je visoka prevalencija konzumiranja alkohola i opijanja među studentima Univerziteta u Novom Sadu. Vršnjaci imaju negativan uticaj kada je reč o upotrebi alkohola među studentima. Prekomerna konzumacija alkohola i opijanje bili su češći kod pušača i kod studenata koji koriste ilegalne droge.

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Ključne reči: konzumiranje alkohola, opijanje, uticaj vršnjaka, pušenje, ilegalne droge

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ENTERAL NUTRITION IN SEPSIS: CAN WE BREAK THE MYTH?

Milena Stojanović

Sepsis is a complex disorder that occurs as a result of the host's inadequate response to infection, and it is associated with acute organ failure and a high mortality rate. Over the past 30 years lot of research has been conducted in this field, which resulted in a faster recognition of the septic patient by using adequate scores and changing the existing ones. Septic shock is defined as hypotension despite adequate fluid resuscitation, therefore requires vasopressor support and is accompanied by circulatory, metabolic and cellular abnormalities.

Sepsis and septic shock are typically associated with catabolic stress, where patients exhibit a systemic inflammatory response associated with complications such as multiorgan dysfunction, morbidity, prolonged hospitalization and death. Malnutrition is common in septic patients, taking into account pronounced catabolism in the early phase. In septic patients, enteral nutrition can be important for covering the energy requirements. Early nutrition therapy, primarily by enteral route, is today seen as a proactive therapeutic strategy with the aim of reducing the severity of the disease, complications, length of hospital stay and positive outcome of the patient.

Enteral nutrition should not be started in patients who are hypotensive (MAP < 50 mmHg), in whom treatment with vasopressors has just started or in whom there is an escalation of vasopressor doses. According to recommendations, enteral nutrition should be withheld until hemodynamic stability is achieved. The risks and benefits of enteral nutrition must be considered for each patient in the state of septic shock. Noradrenaline dose of < 0.3 mcg/kg/min can be considered safe, and such patients can be characterized as hemodynamically stable.

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Key words: sepsis, septic shock, enteral nutrition, vasopressors, noradrenaline

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Introduction

Sepsis is a complex disorder that occurs as a result of the host's inadequate response to infection, and it is associated with acute organ failure and a high mortality rate (1). It is a very important health problem and requires urgent recognition and treatment because any delay reduces the survival rate. Over the past 30 years lot of studies and research have been conducted in this field, which resulted in a faster recognition of the septic patient by using adequate scores and changing the existing ones (2, 3). In 2017, the

World Health Organization declared sepsis a health priority and adopted the Resolution concerning the improvement of prevention, diagnosis and treatment (4).

Starting in 1992, the definition of sepsis underwent many revisions, since it was then defined as an inflammatory response to infection. The clinical diagnosis was based on 2 or more criteria (hypo/hyperthermia, leukocytosis/leucopenia, heart rate > 90/min, respiratory rate > 20/min) of Systemic Inflammatory Response (SIRS) with a suspected or confirmed source of infection (5). At that time, septic shock was defined as persistent hypotension and hyperlactatemia refractory to fluid resuscitation. Considering that these definitions lacked both sensitivity and specificity, in 2001, a criterion for organ dysfunction evaluation (SOFA) score was introduced, which indicated severe sepsis (6).

After about two decades, in 2016, the Third International Consensus on the Definition of Sepsis and Septic Shock defined sepsis as a life-threatening organ dysfunction which occurs as a result of an inadequate host response to infection, and septic shock as sepsis accompanied by circulatory, metabolic and cellular abnormalities (7). Clinically, shock is defined as persistent

hypotension accompanied by MAP < 65 mmHg and increased serum lactates > 2 mmol/L after fluid resuscitation. Also, septic shock is accompanied by a higher mortality rate, of 40%, which is in the case of sepsis about 10% (1).

The importance of nutrition in hospital conditions should not be neglected. Critical illness, including sepsis and septic shock, is typically associated with catabolic stress where patients exhibit a systemic inflammatory response associated with complications such as multiorgan dysfunction, morbidity, prolonged hospitalization and death. Malnutrition is common in septic patients, taking into account pronounced catabolism in the early phase. In this case, enteral nutrition (EN), which delivers nutrients into the digestive system, can be important for covering the energy requirements of these patients (8).

In the last three decades, great progress has been made in understanding the molecular and biological effects of nutrients in maintaining homeostasis in critically ill patients. Traditionally, nutrition support meant an additional form of treatment and patient care with the goal of providing enough energy to maintain lean body mass. Recently, this type of therapy has been called *nutritional therapy*, which implies support in maintaining a normal metabolic response to stress, prevention of oxidative cell damage and positive impact on immune response (9).

Early nutrition therapy, primarily by enteral route (EEN), is today seen as a proactive therapeutic strategy with the aim of reducing the severity of the disease, reducing complications, length of hospital stay and positive outcome of the patient.

Based on experts' opinions, comorbidities, the condition and function of the digestive system, as well as the risk of aspiration, should be taken into account within the nutrition assessment. Traditional serum markers, albumin, prealbumin, transferrin, retinol-binding protein, as indicators of the acute inflammatory phase, are no longer recommended in the Intensive Care Unit (ICU) (10). For determining energy requirements, indirect calorimetry is an option whenever it is available. If it is not available, ESPEN (European Society for Parenteral and Enteral Nutrition), ASPEN (American Society for Parenteral and Enteral Nutrition) and SCCM (Society for Critical Care Medicine) recommend to use predictive equations based on patient's body weight (25–30 kcal/kg/day) (9, 11, 12).

The most important macronutrients are proteins, necessary for wound and anastomoses healing, to support the immune system and maintain the lean body mass. For the majority of critically ill patients, the need for proteins is greater than daily energy requirements, and they often cannot be met with EN only, especially in the first days of illness. Patients with frequent interruption of EN may benefit from protein supplementation. Daily needs of protein are about 1.2–2 gr/kg/day (9, 11, 12).

Recommendations for starting early EN are within 24–48 h of admission in the ICU (9, 11, 12). Enteral nutrition maintains the integrity of the gastrointestinal tract and strong junctions between epithelial cells, promotes blood flow through mucosa, stimulates the trophic factors release (cholecystokinin, gastrin, bombesin and bile salts). It also stimulates the secretion of IgA (13–15).

Patients with septic shock are a special group of critically ill patients and EN in these patients is recommended with great caution. The reason lies in the fact that early EN may lead to additional hemodynamic instability and can be the cause of mesenteric ischemia and necrosis (9). In the early phase of shock, the blood flow is directed from splanchnic circulation to the vital organs, such as the brain, heart and lungs. This can result in intestinal dysfunction, which is considered to be the key in the development of sepsis-mediated multiorgan dysfunction by increasing the permeability of intestinal mucosa, bacterial translocation and systemic inflammation (16). Splanchnic hypoperfusion and inadequate oxygen utilization in cells result in significant pathophysiological disorders in the digestive tract (enterocyte necrosis, overgrowth of pathogenic bacteria and their dislocation into the systemic circulation and disruption of local immune response), which can be additionally enhanced by reperfusion and use of vasopressors (17–19). High doses of vasopressors can increase the already existing high metabolic and energy needs of endothelial cells (20–22). In septic patients, there is a subclinical ischemic/reperfusion damage as a result of intestinal microcirculation disorders. Intestinal ischemia due to the use of EN is very rare but can be unrecognized and fatal (23). Retrospective studies in patients who are on stable doses of vasopressors have shown that early EN reduces length of hospital stay and mortality compared to late EN (24).

Enteral nutrition should not be started in patients who are hypotensive (MAP < 50 mmHg), in whom treatment with vasopressors has just started or in whom there is an escalation of vasopressor doses. In these patients, signs of feeding intolerance should be especially monitored, and if they are present, the dose of EN should be reduced or discontinued (9).

According to recommendations, enteral nutrition should be withheld until hemodynamic stability is achieved (11, 25, 26). It is necessary to define hemodynamically stable and unstable patients, because EN increases cardiac output, decreases systemic vascular resistance and increases mesenteric blood flow. The concept of early EN implies the use of EN within 72 h, and even 48 h of admission. It is reasonable to continue EN in patients who are on stable doses of vasopressors, assuming the patient is hemodynamically stable and the MAP is > 70 mmHg (27). Current evidence and experts' opinions suggest that the shock is not a contraindication for enteral nutrition (9, 27). It is

recommended to start with lower doses of EN in patients with a controlled state of shock and who are on low or moderate doses of vasopressors (27, 28). However, the exact doses of vasopressors are not clearly defined in clinical practice.

Although clinical studies have shown the benefits of EN, the risk and benefit in patients with shock must be rationally evaluated. Enteral compared to parenteral nutrition lowers the risk of infection and length of hospital stay (29, 30). However, the FRANS study showed that providing large amounts of macronutrients during the early phase of ICU stay can have a negative impact (31). Studies NUTRIREA 2 and NUTRIREA 3 provided evidence in terms of reducing the risk of introducing EN in patients with shock, but early EN in hemodynamically unstable patients could have a negative impact on patients' outcome (32, 33). This may be due to the fact that hyperinflammation, oxidative stress and mitochondrial dysfunction are present in the early stage of septic shock, while in the other hand, EN requires an increase of blood flow through gastric mucosa and can worsen an already existing poor visceral perfusion that accompanies the state of shock (34). Therefore, in patients who are hemodynamically stable and without need for vasopressor escalation, EN should be started by providing 20–50% of the estimated energy requirements and gradually increased while monitoring the tolerance of the gastrointestinal tract of each patient.

Enteral nutrition should start with small, trophic doses (10–20 ml/h or 500 kCal/day) and gradually increase the dose by the end of the first week. Trophic nutrition is sufficient to prevent atrophy and apoptosis of enterocytes and maintain the integrity of the gastric mucosa. It is necessary to provide 50–60% of energy needs in order to reduce the permeability of the gastrointestinal mucosa and systemic inflammation. Using gastric residual volume (GRV) as a sign of intolerance is still controversial; generally, it is not recommended. If it is used in some ICUs, the guidelines recommends delaying EN if GRV is above 500 ml/6h (9, 11).

Risk Assessment of Early EN in Patients with Septic Shock

In patients with shock who are on vasopressors, early EN can additionally worsen already existing gastrointestinal dysfunction and even lead to non-occlusive mesenteric ischemia or intestinal necrosis. Use of catecholamines during EN is a risk factor for intolerance (35). Use of sedatives, opiates, hyperglycemia, increased IAP (intra-abdominal pressure) and increased lactate levels are some of the additional risk factors for intolerance (36). These factors should be carefully considered when deciding to initiate EN in patients

on vasopressors. The risk of non-occlusive mesenteric ischemia is greater when vasopressors are combined with inotropes (37–39).

Safe Threshold Doses of Vasopressors for Initiating Early EN

The German Nutrition Association suggests that EN can be harmful when the doses of noradrenaline are greater than 0.5 mcg/kg/min (28). When using the vasopressor score, ASPEN recommends that if it is > 12, only trophic doses should be provided (9, 40). However, this score has not been proven in clinical practice.

Noradrenaline has little effect on intestinal perfusion (41), but it can lead to flow redistribution. In animal models, it has been shown that microcirculation through the superior mesenteric artery, jejunum and pancreas is reduced during the infusion of noradrenaline and adrenaline (42). Adrenaline has also been proven to disrupt gastrointestinal perfusion in patients with shock (43). Otherwise, some authors believe that flow can be increased by increasing the cardiac output (44). The effect of dopamine depends on the dose. Lower doses increase and higher decrease flow through the gastrointestinal mucosa (45, 46). Dobutamine increases (46–49) while vasopressin decreases flow (50, 51).

By searching the data, it is indicated that starting EN is safe if noradrenaline doses are < 0.3 mcg/kg/min (50). Studies NUTRIREA 2 and NUTRIREA 3 suggest a threshold of 0.5 mcg/kg/min (32, 33). For now, the safe doses of vasopressors remain a matter of debate. It is important to emphasize that the state of shock is not a contraindication for starting EN. The risks and benefits of enteral nutrition should be considered for each patient in the state of septic shock. Noradrenaline dose of < 0.3 mcg/kg/min can be considered safe, and such patients can be characterized as hemodynamically stable (9, 27).

Conclusion

Sepsis and septic shock are not contraindications for the initiation of enteral nutrition. This type of nutrition is essential in maintaining homeostasis, integrity and function of the gastrointestinal tract. For now, there is not enough evidence to support clear benefits of early compared to delayed enteral nutrition. The complex effects of vasopressors on the gut must be considered when deciding when and at what doses of nutrients should be started. A norepinephrine dose < 0.3 mcg/kg/min is considered safe for starting EN in patients with septic shock. The risks and benefits of early EN should be weighed carefully for each critically ill patient.

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Pregledni rad

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doi: 10.5633 / amm.2025.0210**ENTERALNA ISHRANA U SEPSI: DA LI MOŽEMO DA
RAZBIJEMO MIT?***Milena Stojanović*

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Sepsa je kompleksan poremećaj koji nastaje kao posledica neadekvatnog odgovora domaćina na infekciju i povezuje se sa akutnom disfunkcijom organa i visokim stepenom smrtnosti. Budući da je velik broj istraživanja u poslednjih trideset godina bio posvećen sepsi, upotrebom adekvatnih skorova i menjanjem postojećih omogućeno je brže prepoznavanje pacijenata sa sepsom. Septički šok se definiše kao perzistentna hipotenzija, refraktorna na nadoknadu tečnosti, koja zahteva upotrebu vazopresora, a praćena je cirkulatornim, metaboličkim i ćelijskim poremećajima.

Sepsa i septički šok povezani su sa kataboličkim odgovorom na stres, koji karakteriše sistemski inflamatorni odgovor praćen komplikacijama poput multiorganske disfunkcije, morbiditeta, produženog bolničkog lečenja i smrti. Malnutricija je, s obzirom na izraženi katabolizam u akutnoj fazi, česta pojava kod pacijenata sa sepsom. Enteralna ishrana je važna za podmirivanje energetske potrebe ovih pacijenata. Rana ishrana, prvenstveno enteralnim putem, danas se posmatra kao proaktivna terapijska strategija koja ima za cilj redukovanje težine bolesti, smanjenje komplikacija i dužine bolničkog lečenja i postizanje pozitivnog ishoda samog lečenja.

Sa enteralnom ishranom ne treba počinjati kod pacijenata sa hipotenzijom (MAP < 50 mmHg), kod kojih je tek započeta upotreba vazopresora ili kod kojih je povećana doza vazopresora. Preporučuje se da se enteralna ishrana zaustavi do hemodinamske stabilizacije. Rizici i benefiti enteralne ishrane moraju se uzeti u obzir kod svakog pojedinačnog pacijenta u stanju septičkog šoka. Noradrenalin u dozi < 0,3 mcg/kg/min smatra se bezbednim, a pacijent kome je data ova doza noradrenalina može se okarakterisati kao hemodinamski stabilan.

*Acta Medica Medianae 2025; 64(2):92–97.***Ključne reči:** sepsa, septički šok, enteralna ishrana, vazopresori, noradrenalin

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DEATH CERTIFICATION ERRORS: PRACTICAL PROBLEMS IN EVERYDAY PRACTICE

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Accurate, precise, current and complete information about national mortality is necessary for planning, determining health priorities, distributing services, allocating budgets and delivering equitable healthcare services. Despite World Health Organisation guidelines, errors in death certificates (DC) have been observed in all regions and are very common. Many studies have pointed to various error types during death certification, focusing on the presence or absence of certain specific entities. Although there are many ways to stratify the errors, they are generally categorised into major and minor. Major errors refer to errors that seriously impact the selection and classification of the underlying cause of death (UCD) (misclassification and definition of UCD, improper sequencing, mechanism of death without data of UCD, multiple and independent causes of death, insufficiently specific cause of death). In contrast, the minor errors have little impact on the classification of UCD (absence of time interval, abbreviations, specifying other significant conditions (comorbidities), more than one diagnosis on a line in Part I of DC, illegible handwriting). Completing the DC is an essential skill that physicians should possess. The key to reducing these errors lies in continuous training based on international guidelines, underlining the importance of ongoing education in this field.

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Key words: death certificate, underlying cause of death, major errors, minor errors

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Introduction

Accurate, precise, current, and complete information about national mortality is imperative. This information is necessary and the foundation for planning, determining health priorities, distributing services, allocating budgets, and delivering equitable healthcare services (1). Any deficiency in the quality of data reported on death certificates (DC) directly impacts the effectiveness of health management and politics. The medical section of DC, regularly completed by the attending physician or coroner, is the source of mortality data, making the accuracy of this information crucial.

The recognised factors in the occurrence of errors are clearly defined in three categories: the ability of the physician to fill in the DC accurately, the skill of the physician to code the condition from the DC accurately following the rules of the World Health Organisation (WHO), the separation of the underlying cause of death (UCD), and the application of clear statistical procedures and standards that convert individual UCD into mortality statistics (2, 3). The physician who determines the death and completes the DC represents the first link in determining the UCD. Corrections will not be needed if this step is done adequately, and the data will be of high quality.

Despite WHO guidelines, errors in the death certification have been observed in all regions and are very common (4–8). Many studies have shown that DC filling is often incomplete and inaccurate (9–14). The quality of mortality data is affected by several factors: the method of filling in and issuing the DC, the education of the physician who fills out the DC and who codes the UCD, the application of different versions of disease classifications, the application of inadequately grouped diagnoses, the application of different formats in which data are collected, poor choice of the International Classification Disease (ICD) 10 codes concerning the nature of the fatal outcome

as well as the application of garbage codes (GC) (1, 3).

Many studies have pointed to various error types, focusing on the presence or absence of certain specific entities. Although there are many ways to stratify the errors, they are generally categorised into major and minor (Table 1) (9, 11, 15–17). The first refers to errors that seriously impact the selection and classification of UCD. In contrast, the second one refers to errors that have little impact on the classification of UCD. However, their absence may help in the more precise determination and classification of the underlying aetiology of the fatal outcome.

II Major errors

III-defined UCD

Incorrect classification and definition of UCD is classified as a group of major errors. Namely, the indication of UCD may be missing or indicated on an inappropriate line in the DC or even in another medical part of the DC (17). From the public health perspective, the most effective strategy in preventing and controlling diseases and injuries is correctly identifying UCD since disease trends are based on that data (18). Therefore, the wrong identification of UCD can lead to incorrect decisions and interventions at the national level. Misidentification is directly related to filling out the DC. When determining the death in a violent manner, doctors very often neglect and do not mention the external circumstances of

the injury that lead to the fatal outcome. The consequences of such mistakes are enormous. The absence of external circumstances of injury leads to masking of the accurate picture of the frequency of certain types of violent death (Table 2). As a result, death of violent origin is masked by some other, very often cardiovascular causes (ICD I00-I99) or undetermined causes of death (ICD R00-R99), which automatically changes the manner of death and the pattern of dying.

Improper sequencing

The death certification delivers the ability to differentiate the time sequence of diseases that may have led to fatal outcomes. The improper sequence in the immediate, intermediate and underlying causes of death belongs to the group of major errors since the correct sequence of events plays a crucial role in correctly selecting different causes. Inadequate sequence of events is the most frequently reported error in the literature (0.7%–94.6%) (5). Studies from India (19, 20) and Iran (21) have shown that inadequate sequencing is the most common mistake made by physicians. An acceptable sequence may be chronic kidney disease, congestive heart failure, and, finally, acute pulmonary oedema (Table 3). An improper sequence is an error that accounts unacceptable sequences, such as acute pulmonary oedema, chronic kidney disease, and congestive heart failure.

Table 1. Classification of the most common errors

MAJOR ERRORS	MINOR ERRORS
Misclassification and definition of UCD	Absence of a time interval
Improper sequencing	Abbreviations
Mechanism of death without data of UCD	Specifying other significant conditions (comorbidities)
Multiple and independent causes of death	More than one diagnosis on a line in Part I of DC
Insufficiently specific cause of death	Illegible handwriting

Table 2. Example of III-defined UCD

Medical data		WRONG	ACCURATE
Part I Cause of death	a) Immediate cause	R40.2 Coma, unspecified	R40.2 Coma, unspecified
	b) Intermediate cause		S06.5 Traumatic subdural haemorrhage
	c) Underlying cause	S06.5 Traumatic subdural haemorrhage	V29.4 The driver injured in a collision with another unmarked motor vehicle in a traffic accident
Part II Other significant conditions contributing to death		E10.9 Type 1 diabetes mellitus without complications	E10.9 Type 1 diabetes mellitus without complications

Table 3. Example of improper sequencing

Medical data		WRONG	ACCURATE
Part I Cause of death	a) Immediate cause	I50.0 Congestive heart failure	J81.0 Acute pulmonary oedema
	b) Intermediate cause	N18.5 Chronic kidney disease	I50.0 Congestive heart failure
	c) Underlying cause	J81.0 Acute pulmonary oedema	N18.5 Chronic kidney disease
Part II Other significant conditions contributing to death		E66. 9 Obesity	E66.9 Obesity

Mechanism of death without data of UCD

The mechanism of death is not part of the disease process and should not be considered the UCD (9). Mechanism of death was listed as the fifth most common error in a meta-analysis (22). It belongs to garbage diagnosis with no analytical value, and many diagnoses fall into the first severity level in public health decision-making. For this reason, the mechanisms are not usable in practice, and the UCD cannot be analysed based on them, nor can adequate prevention measures be applied. Many physicians identify cardiovascular events, most commonly cardiac arrest (I46.9), syncope (R05.4), as well as unspecified respiratory insufficiency (J96.0), and asphyxia (R09), as the UCD without understanding that it is a pathophysiological process that represents the terminal outcome. The Framingham study found that cardiovascular

causes of death were overestimated in at least 24% of death certificates. (23) At the same time, Behrendt et al. concluded that nearly two-thirds of physicians used nonspecific cardiovascular mechanisms to code OUS (24).

Multiple and independent causes of death

This type of error refers to the existence of two or more causally unrelated, etiologically specific causes of death, listed in Part I of the DC (25). This error also occurs when several etiologically different diagnoses of the cause of death are written on one line. An example of such an error would be when entering on line a) a diagnosis of lung cancer (C34.1), on line b) thyrotoxicosis with goitre (E05.0), and on line c) atrial fibrillation and flutter (I48). In that case, a diagnosis should be determined according to the ICD rules, representing the UCD, which would be lung cancer (C34.1).

Table 4. Example of mechanism of a death without data of UCD

Medical data		WRONG	ACCURATE
Part I Cause of death	a) Immediate cause	I46.9 Cardiac arrest, cause unspecified	I21.9 ST elevation (STEMI) myocardial infarction of anterior wall
	b) Intermediate cause		
	c) Underlying cause	I46.9 Cardiac arrest, cause unspecified	I21.9 ST elevation (STEMI) myocardial infarction of anterior wall
Part II Other significant conditions contributing to death		I21.9 ST elevation (STEMI) myocardial infarction of the anterior wall I70.9 Other and unspecified atherosclerosis	I70.9 Other and unspecified atherosclerosis

Table 5. Example of multiple and independent causes of death

Medical data		WRONG	ACCURATE
Part I Cause of death	a) Immediate cause	C34.1 Lung cancer	C34.1 Lung cancer
	b) Intermediate cause	E05.0 Thyrotoxicosis with diffuse goitre	
	c) Underlying cause	I48 Atrial fibrillation and flutter	C34.1 Lung cancer
Part II Other significant conditions contributing to death			E05.0 Thyrotoxicosis with diffuse goitre I48 Atrial fibrillation and flutter

Insufficiently specific cause of death

WHO emphasises that specific causes should be used instead of general (non-specific) conditions (26). General and ill-defined conditions should not be listed as UCD since they have little value for public health analysis (27). Therefore, if there are more specific diagnoses, avoiding vaguely defined and general diagnoses is recommended. An example would be the diagnosis of an unspecified type of diabetes (E14.0–E14.9) or malignant neoplasm without specification of the site (C80.0). This error also occurs when entering three-digit instead of four-digit ICD codes if they exist in the codebook (e.g. J45 instead of J45.0).

II Minor errors

The absence of a time interval

The most common error reported in meta-analysis is the absence of a time interval, with a frequency ranging from 22% to 100% (22). Although the absence of a time interval falls into the category of minor errors, it makes it difficult to determine the UCD accurately (28). This information is mainly used to assist the nosologist, who codes the causes of death, ensuring they are listed correctly: the newest terms first, then the older ones on each subsequent row below in Part I DC. The Serbian version of DC has no field for specifying the time interval when entering diagnoses of the underlying, previous, and immediate cause of death. This deficiency in the national DC can be one of the more critical prerequisites for reporting errors in the coding of UCD.

Abbreviations

The use of abbreviations is not so rare in DC (22). However, abbreviations are not permissible due to their ambiguity. Their use may lead to wrong interpretations and conclusions since DC is used for different purposes, such as forensic medical evidence for family members by public health researchers. The most commonly used abbreviations are AMI for acute myocardial

infarction, DM for diabetes mellitus, CKD for chronic kidney disease and MS for multiple sclerosis (Table 6).

Specifying other significant conditions (comorbidities)

Although most studies count comorbidities instead of UCD among minor errors, some classify this type as a MAJOR error (29). According to Alipur and Payandeh's research (22), there is a listing of other significant conditions (comorbidities) when filling out the DC. Comorbidities are entered in Part II of the DC and represent conditions and diseases that affect the development of events but do not directly lead to death. For this reason, comorbidities should not be coded instead of the UCD. Although other significant conditions do not directly affect the chain of events leading to death, analysing their association with UCD is of considerable value for policymaking and plans to reduce mortality.

Say a person who appears to have died from a combination of cryptococcal myocarditis and pneumocystis pneumonia, which occur as complications of human immunodeficiency virus (HIV) infection (Table 7). The instructions tell us not to list two states on the same line in part I. Furthermore, cryptococcal myocarditis and pneumocystis pneumonia should not be listed on separate lines in Part I because one did not cause or lead to the other. Since HIV infection causes both diseases, it should be written on the c) line as a UCD, and one of the mentioned diseases should be put in Part II.

More than one diagnosis on a line in Part I of DC

More than one diagnosis per line in the first part of the DC results in multiple sequences of events leading to death and makes the choice of UCD difficult. Therefore, this type of error should be avoided as much as possible. In Table 8, Osteoporosis, Acute gastric ulcer with haemorrhage, and Benign neoplasm of the parotid gland are written in line c). According to the WHO rules, only Acute gastric ulcers with bleeding can be UCD, and the other two will be written in Part II.

Table 6. Example of using abbreviations

Medical data		WRONG	ACCURATE
Part I Cause of death	a) Immediate cause		
	b) Intermediate cause		
	c) Underlying cause	CKD	Chronic kidney disease
Part II Other significant conditions contributing to death		DM, AMI, MS	Diabetes mellitus, Acute myocardial infarction, Multiple sclerosis

Table 7. Example of specifying other significant conditions (comorbidities)

Medical data		WRONG	ACCURATE
Part I Cause of death	a) Immediate cause	Cryptococcal myocarditis	Cryptococcal myocarditis
	b) Intermediate cause		
	c) Underlying cause	Pneumocystis pneumonia	Human immunodeficiency virus infection
Part II Other significant conditions contributing to death		Human immunodeficiency virus infection	Pneumocystis pneumonia

Table 8. Example of more than one diagnosis on a line in Part I of DC

Medical data		WRONG	ACCURATE
Part I Cause of death	a) Immediate cause		K25.0 Acute gastric ulcer with haemorrhage
	b) Intermediate cause		
	c) Underlying cause	K25.0 Acute gastric ulcer with haemorrhage M81 Osteoporosis D11.0 Benign neoplasm of parotid gland	K25.0 Acute gastric ulcer with haemorrhage
Part II Other significant conditions contributing to death			D11.0 Benign neoplasm of parotid gland M81 Osteoporosis

Conclusion

Illegible handwriting

This type of error is specific to paper-based death certification systems. The illegibility of information recorded in DC significantly influences the interpretation of the cause of death determination of the UCD and cause coding death. Computerising the death certificate form and planning the system prevents the registration of abbreviations instead of the causes of death, and the mandatory completion of the necessary fields can be a crucial step in reducing the number of death certificate completion errors in countries that continue to use the paper-based death certificate form.

Errors in filling out death certificates are common in both undeveloped and developed countries. Classification errors into major and minor groups helps prioritise issues related to DC completion and planning to improve the quality of cause-of-death documentation. Continuous training of physicians based on international guidelines, providing standard instructions for DC filling, implementing quality control mechanisms, providing feedback, and correcting existing errors can potentially reduce death certification errors.

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**GREŠKE U POTVRDI O SMRTI: PRAKTIČNI PROBLEMI
U SVAKODNEVNOJ PRAKSI**

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Tačne, precizne, aktuelne i potpune informacije o nacionalnim podacima u vezi sa mortalitetom neophodne su za planiranje ciljeva, određivanje zdravstvenih prioriteta, raspodelu budžeta i pružanje adekvatne zdravstvene zaštite. Uprkos smernicama Svetske zdravstvene organizacije, greške u popunjavanju Potvrde o smrti (POS) primećuju se u svim regionima sveta i veoma su česte. Brojne studije ukazale su na različite vrste grešaka prilikom izdavanja POS-a, fokusirajući se pritom na prisustvo ili odsustvo određenih specifičnih pojava. Mada postoje različiti načini za stratifikaciju grešaka, one se generalno razvrstavaju na velike i male greške. Velike greške odnose se na greške koje ozbiljno utiču na izbor i klasifikaciju osnovnog uzroka smrti (OUS) i u njih spadaju: pogrešna klasifikacija i definicija OUS-a, nepravilan redosled dijagnoza, mehanizam smrti bez podataka o OUS-u, višestruki i nezavisni uzroci smrti, nedovoljno specifičan uzrok smrti. Nasuprot tome, male greške imaju neznatan uticaj na klasifikaciju OUS-a; takve su, na primer, odsustvo vremenskog intervala, skraćenice, navođenje drugih značajnih stanja (komorbiditeta), više od jedne dijagnoze na liniji u delu I POS-a i nečitak rukopis. Popunjavanje POS-a predstavlja važnu veštinu koju lekar koji se bavi utvrđivanjem smrtnog ishoda treba da ima. Ključ za smanjenje ovih grešaka leži u kontinuiranoj obuci lekara zasnovanoj na primeni međunarodnih smernica. Osim toga, treba naglasiti i da je stalna edukacija u ovoj oblasti od izuzetnog značaja.

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Ključne reči: *Potvrda o smrti, osnovni uzrok smrti, velike greške, male greške*

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DYSPHAGIA LUSORIA: A CASE REPORT OF A PATIENT WITH ABERRANT RIGHT SUBCLAVIAN ARTERY AND TRUNCUS BICAROTICUS

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Aberrant right subclavian artery (ARSA) is a rare vascular anomaly. It is even more rarely associated with dysphagia and common carotid artery trunk—truncus bicaroticus (TB). Bayford described ARSA in 1794 and called it arteria lusoria, and this type of dysphagia—dysphagia lusoria. Infundibular extension to deviate ARSA was first described by Kommerell in 1936, therefore, it is called a Kommerell's diverticulum.

A 69-year-old patient, MD, suffered from dysphagia and hoarseness, showing a weight loss of 9.5 kg, two months before the examination. Esophagoscopy revealed external compression of the posterior wall of the middle third of the esophagus. MSCT examination of the chest, as the most reliable non-invasive diagnostic tool, showed ARSA and Kommerell diverticulum with TB.

The presence of ARSA is usually asymptomatic and accidentally located in examinations and autopsies. Dysphagia with a loss of body weight, if present, originate from the outer compression of ARSA to esophagus, while hoarseness occurs within Ortner cardiovocal syndrome. The patient was advised to undergo surgical treatment in case of deterioration of dysphagic disorder, and until then more frequent food consumption during the day, in small bites, and the use of proton pump inhibitor.

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Key words: *dysphagia lusoria, arteria lusoria, aberrant right subclavian artery, Kommerell's diverticulum*

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Introduction

Aberrant right subclavian artery (ARSA) is a rare vascular anomaly (0.2–2.5% of people) (1–4). Even more rarely is it associated with dysphagia and a common carotid arterial trunk that deviates from the aortic arch—truncus bicaroticus (TB). Bayford described it in 1794 and named it arteria lusoria, and this type of dysphagia, dysphagia lusoria (5). Infundibular extension in the deviation ARSA was first described in 1936 by Kommerell, and consequently it was named Kommerell's diverticulum (KD) (6).

Usually, the right subclavian artery (SA) stems from the truncus brahiocephalicus while ARSA stems directly from the aortic arch behind

deviated left SA. Truncus bicaroticus accompanied with ARSA is present even more rarely (7). In most cases, ARSA is asymptomatic but there may occur dysphagia, dyspnea, stridor, hoarseness, loss of body weight and pain in the chest (7–9). In the presence of ARSA, certain problems may occur requiring cannulation of the right subclavian artery in the interventional cardiology and cardiovascular surgeries. Unrecognized ARSA represents the risk of major bleeding during transhiatal esophagectomy and operations on the aortic arch and the descending aorta.

Case Presentation

A 69-year-old patient, MD, complained of difficulty swallowing solid food and hoarseness for a month, or a month and a half. During that period he lost around 9.5 kg. For two years now, the patient has not been consuming alcohol, which he previously used in large quantities. He reported no other diseases or previous surgeries. Suspecting the presence of pseudoachalasia, or motility disorders of the esophagus, esophagoscopy was done during which a neoplastic process was not found, but the impression was created of the external compression in the middle third of the esophagus

at the back. Since the lungs' Rtg P-A findings were correct, differential diagnosis assumed the presence of vascular abnormalities in the mediastinum. A chest multi-slice computed tomography (MSCT) was performed, which revealed ARSA resulting from a descending aorta KD, located below the left SA deviation. ARSA moves back and to the right, passing behind the trachea and esophagus, and in front of the spinal column. In the course of its aberration, it puts pressure on

the esophagus in its middle third. Both carotid arteries are derived from TB, which is another rare variety of blood vessels of the aortic arch branching (Figures 1–5).

The patient was advised to undergo surgical treatment in case of dysphagic disorder deterioration, and until then, eat more often during the day, in small bites, if necessary, purée foods, and use proton pump inhibitors (PPI).



Figure 1. MSCT chest shows sagittal image of arteria lusoria



Figure 2. MSCT chest shows coronal image of arteria lusoria



Figure 3. MSCT chest shows (thick arrow), compressed esophagus (thin arrow)



Figure 4. MSCT chest shows arteria lusoria (arrow)



Figure 5. MSCT chest shows truncus bicaroticus (black arrow), Kommerell's diverticulum (thicker black arrow), compressed esophagus (white arrow)

Discussion

Aberrant right subclavian artery is a rare anomaly, but also the most common anomaly of the aortic arch (1, 10). First description of this arterial abnormality was cited by Hunauld in 1735 (11). A term "dysphagia lusoria" was first used in 1794 by Bayford, who described this type of dysphagia in an older woman who died of starvation due to the consequent esophagus obstruction (2, 5, 12, 13). On that occasion, ARSA was described as a "freak of nature" (*L. lusus nature*) and named *arteria lusoria* (5).

Arteria lusoria occurs due to disturbances during embryogenesis, when it comes to the involution of the fourth aortic arch on the right side along with the cranial part of the right dorsal aorta, whereby the seventh intersegmental artery remains annexed to the descending aorta (12). If there is no involution of the right dorsal aorta (in about 60% of cases) there occurs aneurysmal, infundibular expansion at the origin of ARSA—Kommerell's diverticulum (according to B. Kommerell, who first described it in 1936) (6, 12). ARSA can be accompanied by other anomalies of the heart and blood vessels, usually in the tetralogy of Fallot (14). Aberrant left subclavian artery and right-sided aortic arch branching are far less common (7).

The classification of ARSA types is based on the ways of anomalous branching from the aortic arch (13). Adachi–Williams classification of the anomalous ARSA branching describes four basic types of branching: 1) Type G-1, ARSA branching from distal aortic arch as the last branch, other branches of the aortic arch are normally separated; 2) Type CG-1, branching as in G-1, with a deviation of the left vertebral artery directly from the aortic arch; 3) Type H-1, ARSA separates as G-1, and the left and right common carotid arteries branch off from TB, which deviates from the aortic arch. This is found in 0.16% of cases and is usually detected as an incidental finding (15). It is even more rare in patients with dysphagia associated with ARSA (as in the presented case); 4) Type N-1 of branching is the mirror image of type G, is rarely encountered and represents right aortic arch and the left subclavian artery (LSA) which deviates beyond the right subclavian artery (15).

In the case of the presence of ARSA right *n. laryngeus recurrens* separates directly from the ipsilateral *n. vagus* and does not make a loop or a return arch around ARSA, but in the shortest route enters the laryngeal area (16). This must be taken into account when operating on the thyroid gland and trachea.

The presence of ARSA is usually asymptomatic and/or is found in autopsy as incidental findings. Rarely, the presence of ARSA creates difficulties with different degree dysphagia, dyspnea, stridor and hoarseness, usually after a long asymptomatic period and in old age. The appearance of symptoms in the elderly is explained by ARSA stretching around the

esophagus, the appearance of atherosclerotic changes with increasing rigidity of the ARSA or its aneurysms or the aorta arch (7). Hoarseness as a sign occurs within Ortner's cardiovascular syndrome, similar to the dilation of the left atrium in mitral estuary stenosis (17, 18). Other signs and symptoms that occur less frequently include: retrosternal pain, cough, loss of body weight, asymmetrical pulse on the hands, trophic changes on the right hand, cyanosis, arterial insufficiency and erosion of thoracic vertebrae (11, 19). Problems related to the presence of ARSA in the showcased patient can be partly explained by the fact that due to the previous excessive alcohol consumption, a development of alcoholic polyneuropathy occurred, which has engulfed the submucosus plexus and plexus myentericus, thus causing a weakening of the esophagus wall resistance to already existing ARSA. Since alcohol is a good source of energy, the question is how well and how often the patient ate, and what quality the patient's food intake was in the previous period, and whether he was noticing and attributing any significance to the dysphagia occurrence.

The diagnosis of this anomaly is performed by esophagoscopy, barium contrast esofagography, MSCT, MRI and angiography (20, 21). ARSA diagnosis (with non-invasive methods) is set with high probability using MSCT (21). For diagnosing the existence of ARSA, it is essential to suspect its presence. The patient had previously visited many doctors because of the symptoms related to difficulty swallowing—otolaryngologists, neurologists, cardiologists, endocrinologists, hematologists and gastroenterologists. Interestingly, the first association of our team on the endoscopic findings of the extramural compression on the esophagus was the existence of ARSA, then descending aorta aneurysm and mediastinal/lung tumor. A performed MSCT of the thorax has confirmed the presence of ARSA.

Surgical therapy, endovascular and hybrid procedures in dysphagia lusoriae are considered a treatment option when difficulties are present and get worse over time (22, 23). The risk of complications and side events during the operative treatment of these patients does exist, since the surgical procedures are non-standardized, and are rarely performed in a small number of centers in the world.

Knowledge of the anatomy and anatomical anomalies of the blood vessels of the aortic arch and the timely detection of ARSA when planning operations on the esophagus, aortic arch, trachea and thyroid are essential for the prevention of possible complications during the surgery itself. One of the most serious complications is the injury of ARSA itself and the abundant intraoperative bleeding that is difficult to treat surgically (24). As one of the chronic complications of the transhiatal esophagectomy and the presence of the unrecognized ARSA arterio-esophageal fistula is described (11, 25).

If the complaints are mild and do not get worse, the changes in the hygienic-dietary regimen of life and a diet with the use of PPI are recommended (22). For this patient, we opted for a combined PPI therapy, also applying hygienic and dietary life regime, recommending frequent meals with small bites of food, use of mixed and liquid food, and the outcome was: a loss of body weight stopped, stable general condition and acceptable troubles for the time being. The patient was advised that, in case of symptoms worsening, the possibility of surgical, endovascular and/or hybrid treatment is available. Gastrostomy as a form of treatment was dismissed because the oral food intake is one of the major "sources of pleasure", and the patient is not acutely life threatened by dysphagia.

Conclusion

Aberrant right subclavian artery with truncus bicaroticus is a rare clinical entity. It should be suspected in the occurrence of dysphagia accompanied by hoarseness and loss of body weight. MSCT proved to be the most reliable non-invasive diagnostic tool. Asymptomatic patients should be monitored and their hygienic-dietary regimen of life modified, and the PPI should be used. Active surgical, endovascular and/or hybrid treatment should be performed in symptomatic patients whose ailments worsen.

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Prikaz bolesnika

UDC: 616.329:616.133/.135-008
doi: 10.5633/amm.2025.0212**DYSPHAGIA LUSORIA: PRIKAZ PACIJENTA SA
ABERANTNOM DESNOM POTKLJUČNOM ARTERIJOM I
ZAJEDNIČKIM STABLOM KAROTIDNIH ARTERIJA***Milorad Pavlović¹, Bojan Ilić¹, Miloš Milojković¹, Miloš Stanković¹, Aneta Jovanović¹, Aleksandar Bogdanović¹, Dimitrije Đorđević²*¹Univerzitetski klinički centar Niš, Klinika za grudnu hirurgiju, Niš, Srbija²Univerzitetski klinički centar Niš, Klinika za plastičnu i rekonstruktivnu hirurgiju, Niš, SrbijaKontakt: Milorad Pavlović
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Aberantna desna arterija subklavija (ADAS) predstavlja retku vaskularnu anomaliju. Još se ređe dešava da bude udružena sa disfagijom i zajedničkim stablom karotidnih arterija – *truncus bicaroticus* (TB). Bejford je 1794. godine opisao ADAS i nazvao je tu pojavu *arteria lusoria*, a ovaj tip disfagije *dysphagia lusoria*. Infundibularno proširenje na odstupu ADAS-a prvi je opisao Komerel 1936. godine, pa se naziva Komerelov divertikulum.

Kod šezdesetdevetogodišnjeg pacijenta M. D. potvrđene su disfagija i promuklost. Dva meseca pre pregleda došlo je do gubitka težine (9,5 kg). Ezofagoskopski je uočena spoljna kompresija na zadnjem zidu srednje trećine jednjaka. Pregled grudnog koša urađen pomoću višeslojne kompjuterizovane tomografije (engl. *Multi-slice Computed Tomography*), kao najpouzdanijeg neinvazivnog dijagnostičkog sredstva, ukazao je na ADAS i Komerelov divertikulum sa TB-om.

Prisustvo ADAS-a je najčešće asimptomatsko i slučajno se otkriva prilikom pregleda i obdukcija. Disfagične tegobe praćene gubitkom telesne težine (ako su prisutne) potiču od spoljne kompresije ADAS-a na ezofagus, dok se promuklost javlja u okviru Ortnerovog kardiovokalnog sindroma. Bolesniku je dat savet da se uputi na operativno lečenje ako dođe do pogoršanja disfagičnih tegoba. Preporučeno mu je da dotad jede češće u toku dana, da zalogaji budu mali, kao i da koristi inhibitore protonске pumpe.

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Cljučne reči: *dysphagia lusoria, arteria lusoria, aberantna desna arterija subklavija, Komerelov divertikulum*

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DIPEPTIDYL PEPTIDASE-4 INHIBITORS INDUCED BULLOUS PEMPHIGOID: A REVIEW OF CURRENT CONCEPTS

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Bullous pemphigoid (BP) is a chronic, autoimmune, subepidermal blistering disease observed primarily in the elderly. Over the past two decades, there has been a substantial increase in BP incidence and prevalence in several countries worldwide. The reasons driving these increases include an increase in average life expectancy, better diagnostic methods, recognition of atypical variants of BP, and increased use of certain medications.

Drug-associated BP (DABP) is a term used to describe cases of BP demonstrating clinical, histological, or immunopathological features identical or similar to those of the idiopathic form of BP, but associated with the systemic ingestion or topical application of particular drugs. Since the first report of DABP in 1970, more than 90 individual drugs have been reported associated with BP so far. The strongest evidence of an association with DABP is found in dipeptidyl peptidase-4 inhibitors, also known as gliptins, a relatively new class of oral antidiabetics.

DABP should be considered a possible diagnosis in patients who have recently changed or added a new drug to their standard therapy. The temporal relationship between drug administration and the onset of BP is critical, and the culprit drug's withdrawal is the most crucial step toward clinical improvement.

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Key words: pemphigoid, bullous, dipeptidyl-peptidase 4 inhibitors, vildagliptin, diabetes mellitus

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Introduction

Bullous pemphigoid (BP) is a chronic, autoimmune, subepidermal blistering disease observed primarily in the elderly (1). Several clinical variants have been described, including classic (bullous), localized, nodular, vegetating, erythrodermic, erosive, childhood, and drug-induced forms. Autoantibodies target the BP230 and BP180 antigens, located in the hemidesmosome complex of the skin basement membrane zone. Subsequent complement activation recruits chemical and cellular immune

mediators to the skin, ultimately resulting in blister formation. Both autoantibodies and complement may be detected by various immunofluorescent, immune electron microscopy, and molecular biology techniques (2).

BP usually affects patients over 70 years of age, with an average age of onset between 66 and 83 years. The incidence of bullous pemphigoid ranges between 2.4 and 21.7 new cases per million inhabitants in different populations worldwide. In the United Kingdom, the annual incidence is as high as 42.8 cases per million inhabitants (3). There is a continuous increase in BP incidence, with a 1.9- to 4.3-fold increase in the last two decades. Gender distribution shows a predominance of females in most studies, with a female-to-male ratio between 1.04 and 5.1. Still, after 75 years of age, the incidence is higher in males. Despite the marked increase in incidence, the prevalence is still < 5 per 100,000 population (3).

The reasons driving these increases include an increase in average life expectancy, better diagnostic methods, recognition of atypical variants of BP, and increased use of certain medications (2, 3).

Drug-associated BP (DABP) is a term used to describe cases of BP demonstrating clinical, histological, or immunopathological features

identical or similar to those of the idiopathic form of bullous pemphigoid, but associated with the systemic ingestion or topical application of particular drugs (4).

The purpose of this article is to present a review of the current literature for DABP with a focus on dipeptidyl peptidase 4 (DPP-4) inhibitors.

Drugs associated with bullous pemphigoid

Since the first report of DABP in 1970, in an 11-year-old boy treated with sulfasalazine for ulcerative colitis (5), more than 90 individual drugs were found to be associated with BP (6). As presented in Table 1, these drugs mainly belong to one of the following classes: NSAIDs, salicylates, diuretics, cardiovascular drugs, antibiotics, antidiabetics, neurologic/psychotropics, anti-rheumatics, biologics, and vaccines (6). Systemic psoralens, followed by exposure to ultraviolet rays

and topical PUVA, have also been reported to be risk factors for BP.

The association between certain neuroleptics, aldosterone antagonists, and loop diuretics, and BP is well established (4, 7). The use of DPP-4 inhibitors has recently been shown to increase the risk for BP markedly.

Without an accurate medication history, we cannot identify potential DABP. Such a record should consist of a list of all medicines (prescribed and purchased) that a patient was taking before the onset of cutaneous lesions, as well as recent short courses of antimicrobials or NSAID. Although some patients may not consider these as medicines, the use of herbal remedies, supplements, topical drugs and topical remedies should also be noted. The information should be documented to facilitate the making of valid conclusions.

Table 1. Drugs associated with bullous pemphigoid. Adapted from Verheyden et al. (6)

GROUP	DRUGS ASSOCIATED WITH BULLOUS PEMPHIGOID
NSAIDs	Azapropazone, Celecoxib, Diclofenac, Ibuprofen, Mefenamic acid, Mesalazine, Phenacetin
Salicylates	Aspirin, Sulfasalazine, Salicylazosulfapyride
Cardiovascular	Ace inhibitors: Captopril, Enalapril, Lisinopril Calcium channel blockers: Amlodipine, Nifedipine Statins: Rosuvastatin Angiotensin II agonists: Losartan, Valsartan β-blockers: Metoprolol Anticoagulants: Enoxaparin
Diuretics	Bumetanide, Furosemide, Hydrochlorothiazide, Spironolactone
Antibiotics	Actinomycin D, Amoxicillin, Chloroquine, Flupenthixol, Ciprofloxacin, Griseofulvin, Levofloxacin, Metronidazole, Novoscabin (benzylbenzoate), Penicillin, Rifampicin, Terbinafine
Anti-rheumatics	D-penicillamine, Tiobutaryl
Neurologic/Psychotropics	Amantadine, Doxepin, Escitalopram, Fluoxetine, Flupenthixol, Gabapentin, Galantamine hydrobromide, Levetiracetam, Risperidone
Vaccines	Hepatitis B, Herpes zoster virus, Hexavalent combined vaccines, Influenza, Rotavirus, Swine flu, Tetanus
Anti-diabetics	Alogliptin, Anagliptin, Linagliptin, Sitagliptin, Tenoeligliptin, Vildagliptin
Biologics	Anti-TNFα: Adalimumab, Efalizumab, Etanercept, Infliximab PD-1/PD-L1 inhibitors: Atezolizumab, Durvalumab, Nivolumab, Pembrolizumab m-TOR inhibitor: Everolimus, Sirolimus Interleukins/IL-antagonists: Aldesleukin (IL-2), Ustekinumab Tyrosine Kinase Inhibitors: Erlotinib CTLA-4 inhibitors: Ipilimumab BRAF inhibitors: Dabrafenib

Characteristics of drug-induced bullous pemphigoid

The diagnosis can be a clinical challenge since DABP often does not differ from idiopathic BP. However, some clinical features may favor the drug as a trigger factor: I) younger age of onset, II) heterogeneous cutaneous manifestations, III) improvement of the lesions after the withdrawal of the suspected drug (7).

The classic form is characterized by large, tense, serohemorrhagic blisters on an erythematous base. In contrast, in DABP, lesions usually appear on normal-looking skin and may resemble other entities such as erythema multiforme or pemphigus. Patients typically complain of severe pruritus, and Nikolsky's sign may be positive (8). Unlike the classic BP's predominant sites, the DABP may involve the palms, soles, and face (9). Mucosal involvement can occur, although it is quite rare in the classical form of the condition.

It was suggested that some histologic features might assist in diagnosing DABP, including marked eosinophilic infiltrate, intraepidermal vesicles, necrotic keratinocytes, and thrombus formation. Direct and indirect immunofluorescence reveals a similar profile to idiopathic BP, and no specific antigens for DABP have been identified (9).

From the laboratory analyses, a significantly increased number of eosinophils is in favor of DABP.

Other features that support the drug as a trigger factor are resolution following the withdrawal of the supposed drug and the low rates of recurrence.

Mechanisms involved in DABP

The pathogenetic mechanism by which drugs lead to BP is still unknown. It is known that, in those individuals with a genetic predisposition, they lead to an alteration of the immune response (10). One presumed mechanism is by altering the antigenic properties of the basement membrane (BM) by binding to molecules in the basement membrane zone (BMZ), acting as neoantigens, and causing the formation of anti-BMZ antibodies (5, 11). They can also structurally alter molecules and detect previously hidden epitopes, which also stimulate the immune response (8, 12).

Also, an induced reaction from one drug may lead to a cross-reaction with another drug in the same group (13). Through interaction with sulfur groups in BMZ, sulfur-containing drugs in their structure can lead to direct damage without involving the immune system (10, 14).

Dipeptidyl peptidase-4 inhibitors

There is a growing body of evidence of an association between dipeptidyl peptidase-4 inhibitors (DPP-4i) and the risk of developing BP (2–7).

DPP-4i or gliptins are a class of oral hypoglycaemic drugs that are used for the treatment of diabetes mellitus type 2, especially in the adult population.

Dipeptidyl peptidase-4 (DPP4), also called CD26, is a protein found on the surface of most cells. It plays a key role in immune system regulation, cell signaling, and programmed cell death (apoptosis). DPP-4 is an enzyme serine exopeptidase that cleaves X-proline and X alanine dipeptides from the N-terminus of polypeptides (15).

DPP4 participates in glucose metabolism through inactivation of incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP). Incretins are metabolic hormones that are released after eating. On one hand, they increase insulin secretion, while on the other hand, they decrease the release of GLP-1, two mechanisms that lead to decreased glycemia. Both GLP-1 and GIP are immediately inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4), so DPP4 inhibitors prolong the physiological function of incretins (16).

Sitagliptin, the first DPP-4i was approved by the FDA in 2006, followed by dozens of others, including saxagliptin, linagliptin, and alogliptin (FDA) (17), vildagliptin (EU) (18), anagliptin (19) and teneligliptin (Japan).

DPP4i associated bullous pemphigoid

Since the first five reported cases of DPP4i-BP in 2011 (20), the association of gliptins as inducers of BP development has been reported in numerous case studies and case series. The most common inductors are vildagliptin (20–32), sitagliptin (20, 28, 32), linagliptin (23, 28, 32), anagliptin (33), and alogliptin (28). This association has been further confirmed in large-scale studies of several national drug adverse reactions databases in France (34), European Pharmacovigilance (24), Japan (35), Finland (36), and in controlled observational studies in France and Switzerland (37), Israel (38), South Korea (39) and France (40).

Clinical, immunological and pathological features of gliptin-associated BP have been reported to be distinct, compared to classic BP. Patients with DPP-4 inhibitor-induced BP may present either an inflammatory or a noninflammatory phenotype of BP (41), more severe blistering, and erosive lesions (42). A non-inflammatory phenotype with less erythema, fewer urticarial lesions and fewer eosinophils in skin lesions has also been reported in Japanese populations (43). Patients with DPP-4i-associated BP may be presented with an extensive mucosal involvement (38).

IgG antibody response against other BP180 regions different from the NC16A domain (41, 43), and a case with anti BP230 antibody DPP-4i induced BP were reported (30). Patients receiving DPP-4i treatment may show different DIF patterns than DIF in classic BP (44). The association between gliptin-associated BP and HLA-

DQB1*03:01 was reported by Japanese patients (45), but no such association was found in the Finnish study (46).

Although significant progress has been made, the precise pathogenesis of the association between DPP4i and BP remains unclear. There are several presumed pathogenetic mechanisms such as the expression of antigens on the surface of many different cell types in the human body, including T lymphocytes, as well as different cell types in the skin and subcutaneous tissue (47).

One presumed pathogenetic mechanism is the association between DPP-4 inhibitors and BP via the eotaxin CCL11/CCR3 axis. Namely, among other cytokine substrates of this enzyme under physiological conditions, its substrate is also eotaxin (CCL11), which through its protease activity inactivates the enzyme and consequently this inactivation leads to activation and infiltration of eosinophils in the skin, tissue damage and possible formation of blisters (48). Another possible pathogenetic mechanism is the plasmin activity on NC16A from BP180. DPP4 is a plasminogen receptor expressed on the cell surface, which activates the plasminogen with subsequent plasmin formation (49). Plasmin leads to cleavage of the NC16A immunodominant domain from BP180 to 120 kD and 97 kD ectodomains, i.e. new epitopes for DPP4i-BP autoantibodies (50).

DPP-4i may influence keratinocytes in an epithelial-mesenchymal transition (EMT)-dependent manner. An EMT is a biological process that allows a polarized epithelial cell, which normally interacts with the basement membrane via its basal surface, to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype (51). EMT may exert an effect on various basement membrane proteins including BP180.

In the presence of gliptins modifications in the activity of allied proteases, like the highly homologous seprase (52) with marked gelatinase activity, might affect the epidermal basement membrane zone (53).

DPP4i-BP is a newly recognized autoimmune blistering disease. While DPP4 inhibitors (DPP4i) are known to act as a trigger, the underlying mechanisms of the disease remain unclear and require further investigation.

Conclusion

BP is the most common autoimmune bullous dermatosis, especially in elderly patients, with a growing incidence in the last two decades. There is an ongoing list of medications that have been incriminated to trigger BP formation. DPP4i are of particular importance, not only because they are mostly used in the risk groups for BP, but because it has been proven that they cause DABP. Hence, in a patient with BP, practitioners should always rule out any medication trigger factors and hold back with introductions of new drugs, especially ones that are proven to cause DABP. Obtaining an accurate medication history is the first step in the diagnosis of DABP. DABP must always be considered as a possible diagnosis in elderly patients who have recently changed or added a new drug to their regular medication regimen, because it usually resolves after withdrawal of the suspected medication, with very good therapeutic response, and fewer or no recurrences.

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Pregledni rad

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**BULOZNI PEMFIGOID INDUKOVAN INHIBITORIMA
DIEPTIDIL PEPTIDAZE-4: PREGLED TRENUTNIH
SAZNANJA**

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Bulozni pemfigoid (BP) predstavlja hroničnu autoimunu bolest koja podrazumeva stvaranje mehurova ispod epidermisa, a pretežno se javlja kod starijih ljudi. U poslednje dve decenije zabeležen je značajan porast incidencije i prevalencije BP-a u većem broju zemalja širom sveta. Kao uzroci tog porasta mogli bi se navesti produženje očekivanog životnog veka, unapređene dijagnostičke metode, prepoznavanje atipičnih varijanti BP-a i povećana upotreba određenih lekova.

Lekovima izazvan BP (engl. *drug-associated bullous pemphigoid* – DABP) jeste termin koji se koristi za opisivanje slučajeva BP-a koji pokazuju kliničke, histološke ili imunopatološke karakteristike koje su identične sa idiopatskim oblikom BP-a ili su mu slične, a povezani su sa sistematičnom primenom ili lokalnom aplikacijom određenih lekova. Od prvog izveštaja o DABP-u iz 1970. godine do sada, sa BP-om je povezano više od 90 pojedinačnih lekova. Najsnažniji dokazi o povezanosti sa DABP-om zabeleženi su kod inhibitora dipeptidil peptidaze-4, poznatih i kao gliptini, koji predstavljaju relativno novu klasu oralnih antidijabetika.

Na DABP treba posumnjati kod bolesnika kojima je nedavno promenjena ili dodata nova terapija. Vremenska povezanost između primene leka i pojave BP-a od ključnog je značaja, a povlačenje sumnjivog leka predstavlja najvažniji korak ka kliničkom poboljšanju.

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Ključne reči: pemfigoid, bulozni, inhibitori dipeptidil peptidaze-4, vildagliptin, dijabetes melitus

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MAGNETIC RESONANCE IMAGING DIAGNOSIS OF AN OPEN SPINA BIFIDA DEFECT

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Spinal neural tube defects are a group of disorders that allude to spina bifida, anencephaly, interspinal lipoma, congenital dermal sinus, simple tethered cord and other diseases that can cause neurological problems such as motor and/or sensory disorders and urinary and/or fecal incontinence. Usually, they can be diagnosed with a combined interpretation of alpha fetoprotein and ultrasound imaging. However, sometimes the ultrasound image quality is poor and necessitates the use of a higher resolution imaging technique – magnetic resonance imaging.

A woman in her 18th week of pregnancy was scheduled for a routine prenatal check-up. Upon examination, a mild, yet, suspicious lemon sign of the fetus's cranium had been observed. The sagittal view of the fetus's spine was normal, however the transversal view of the spine could not be assessed. The complete ultrasound examination was of lower quality due to fetal and maternal factors. Thus, a decision for a fetal MRI had been made. The MRI scan confirmed an open spina bifida.

Since MRI scanning is a greater tool than ultrasound, whenever it isn't possible to conclusively diagnose a neural tube defect via ultrasound for any reason, an obstetrician should opt for an MRI scan to confirm the condition.

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Key words: *magnetic resonance imaging, neural tube, defect, diagnosis*

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Introduction

Spinal neural tube defects are a group of disorders that allude to spina bifida, anencephaly, interspinal lipoma, congenital dermal sinus, simple tethered cord and other diseases that can cause neurological problems such as motor and/or sensory disorders and urinary and/or fecal incontinence (1–3).

The incidence rate of spinal neural tube defects is 0.5–2 %, and the incidence of spina bifida is around 3,63 % in the US and 18.6 % worldwide (4, 5).

Spina bifida itself is a condition where the spinal column fails to close and remains split-bifid. This is a result of a neural tube failing to close during the fourth week of embryonic development.

Spina bifida can be presented as a closed defect – *occulta*, or a more common and more severe open defect – *aperta* (6, 7).

People born with spina bifida have lifelong consequences (8).

This makes early diagnosis and prevention crucial in reducing morbidity and for optimizing outcomes.

In recent times ultrasonography combined with alpha fetoprotein analyses is integral to prenatal diagnosis of spina bifida (9, 10).

However, AFP can be elevated in other conditions in pregnant women such as gastroschisis, omphalocele, osteogenesis imperfecta etc. (11). Ultrasound also has its shortcomings. Low resolution, factors that can affect image quality or visibility of structures (12, 13).

In cases where ultrasound findings aren't conclusive, a physician can opt for a higher quality imaging method - an Magnetic Resonance Imaging.

Since its beginnings (MRI) Magnetic Resonance Imaging has evolved into a highly sophisticated imaging method particularly suitable for visualizing soft tissues. Modern MRIs have

reduced artifacts caused by fetal movements, which makes them reliable in visualizing in utero disorders (14).

Case report

A pregnant woman at 18 weeks of gestation was scheduled for routine prenatal care.

Ultrasound examination showed an inconclusive lemon sign. The posterior cranial fossa had not been accessible for visualization.

The sagittal view of the spine seemed normal, but the transversal view could not be performed.

Transabdominal ultrasound findings were indicative for a neural tube defect. However, the tissue resolutions, maternal obesity, fetal

malposition, an anterior placenta, along with the inability to view the spine transversally, caused the impossibility of definitive conclusion.

Thus, a decision had been made for the patient to undergo magnetic resonance imaging in order to confirm the ultrasound findings.

The MRI findings are presented in Figure 1.

The MRI images were interpreted by a radiologist and concluded that the scans were positive for neural tube defect – specifically spina bifida aperta.

Afterwards, on an ethical committee meeting, a decision had been made to approve an elective abortion due to medical reasons.

The medical abortion had been performed and the extracted fetus had an open neural tube defect – spina bifida aperta (Figure 2).

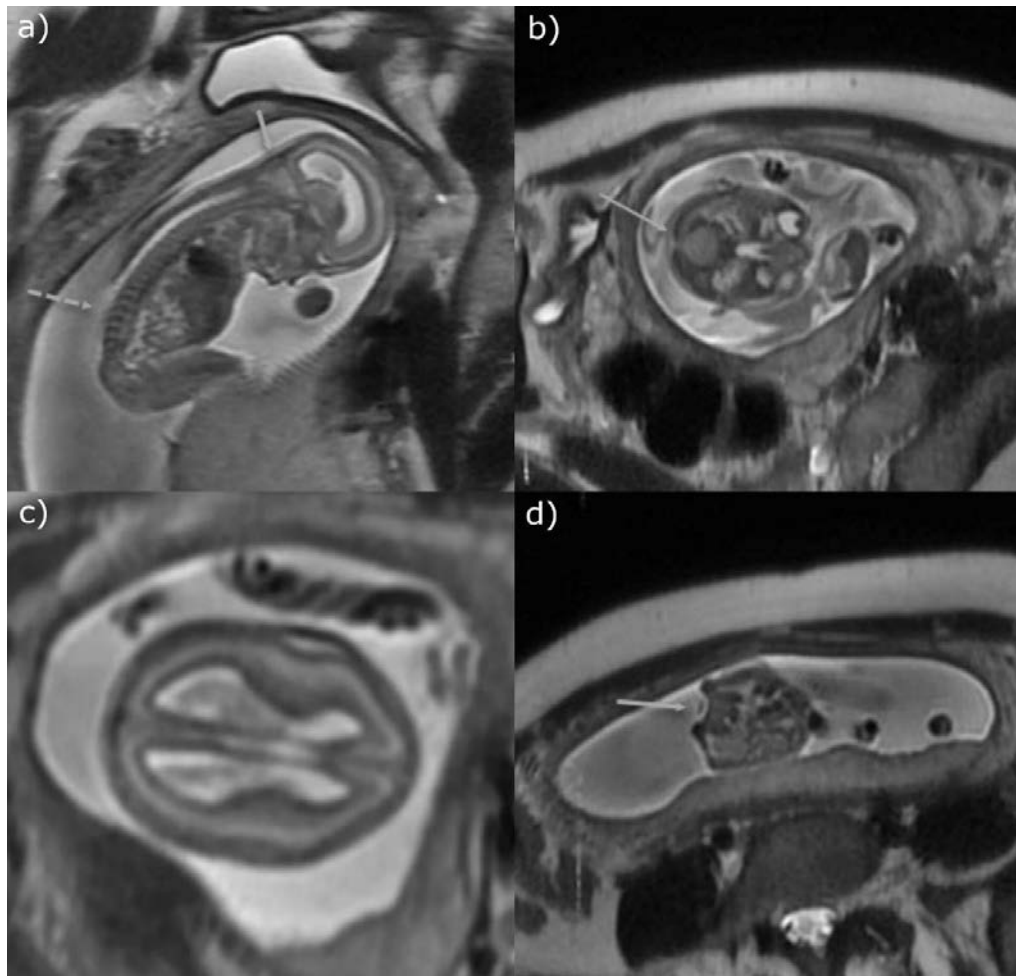


Figure 1. a) Sagittal T2W SSFSE tomogram. Posterior fossa is small. Cerebellar tonsils are pointed, compressed, protruding through foramen magnum (arrow). IV ventricle is compressed. Cisterna magna is effaced. Discontinuity of the skin and subcutaneous tissue on a long segment involving lower thoracic, complete lumbar and part of the sacral region is seen (dotted arrow). b) Axial T2W SSFSE tomogram just below the level of foramen magnum. Cerebellar tonsils are wrapped around medulla, filling the foramen magnum. c) Axial T2W SSFSE tomogram through endocranium at the level of lateral ventricles. Lateral ventricles are on the upper limit of the normal size, with maximum width of 10 mm. d) Axial T2W SSFSE tomogram through abdomen below the level of kidneys. Posterior vertebral elements are lacking. The neural tissue forming the placode is at the level of the adjacent skin surface and exposed to the amniotic fluid. No expansion of subarachnoid space is seen. Findings are consistent with myelocoele.



Figure 2. The fetus with an apparent neural tube defect – open spina bifida

Discussion

Spina bifida is not an uncommon birth defect and it carries with itself potentially lifelong consequences (8).

Because of this, prenatal diagnosis and adequate assessment are needed to optimize the outcomes.

The optimal period for ultrasound scanning of neural tube defects is 11–14 weeks of gestation (9).

Even though ultrasound has come a long way, it is by no means a perfect diagnostic tool.

The problems of ultrasound imaging such as tissue resolutions, maternal obesity, fetal malposition, an anterior placenta, late pregnancy,

oligohydramnion, abdominal scar in pregnant women can all lead to poor image quality and inability to adequately diagnose spina bifida (12, 13).

This makes MRI imaging the next step in diagnostics since it is a superior visualization method compared to ultrasound (15).

Conclusion

Since MRI scanning is a greater tool than ultrasound, whenever it isn't possible to conclusively diagnose a neural tube defect via ultrasound for any reason, an obstetrician should opt for an MRI scan to confirm the condition.

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Prikaz slučaja

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POSTAVLJANJE DIJAGNOZE OTVORENOG DEFEKTA NEURALNE CEVI UZ POMOĆ MAGNETNE REZONANCE

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Defekti neuralne cevi predstavljaju grupu poremećaja koja obuhvata spinu bifidu, anencefaliju, interspinalni lipom, urođeni dermalni sinus, jednostavnu vezanu vrpču i druge bolesti koje mogu izazvati neurološke probleme kao što su motorni i/ili senzorni poremećaji i urinarna i/ili fekalna inkontinencija. Ovi poremećaji obično se mogu dijagnostikovati kombinovanom interpretacijom alfa fetoproteina i ultrazvučnog snimanja. Međutim, ponekad je kvalitet slike sa ultrazvuka loš i zahteva upotrebu tehnike snimanja veće rezolucije – magnetnu rezonancu (engl. *magnetic resonance imaging* – MRI).

Žena u 18. nedelji trudnoće imala je zakazan rutinski prenatalni pregled. Nakon pregleda je uočen blag, ali sumnjiv znak limuna na lobanji fetusa. Sagitalni pogled na kičmu fetusa dao je rezultate u okrivu normalnih vrednosti, ali se transversalni presek kičme nije mogao sagledati na adekvatan način. Kvalitet kompletnog ultrazvučnog snimanja bio je lošiji zbog fetalnih i majčinskih faktora. Stoga, doneta je odluka da se obavi MRI fetusa, kojim je potvrđena otvorena spina bifida.

Budući da je MRI bolji alat od ultrazvuka, u svim slučajevima u kojima nije moguće konačno dijagnostikovati defekt neuralne cevi ultrazvukom (bez obzira na razlog) akušer treba da se odluči za MRI da bi se to stanje potvrdilo.

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Ključne reči: magnetna rezonanca, neuralna cev, defekt, dijagnoza

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ACUTE ASCENDING MEDIASTITIS AND NECK PHLEGMON CAUSED BY PANCREATIC PSEUDOCYST: CASE REPORT

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Miloš Stanković¹, Aneta Jovanović¹, Marko Stojanović²

Acute mediastinitis is one of the most severely known infections with high mortality rate. Mediastinitis occurs most commonly as a descending infection, spreading from oral cavity or pharynx. The most common thoracic complications of pancreatic disease are reactive pleural effusion and pneumonia, while rare complications include pancreaticopleural fistula with massive pleural effusion or hemothorax and extension of pseudocyst into the mediastinum. Only 50 cases of mediastinal extension of pancreatic pseudocyst have been published in the last 75 years with only one case of neck involvement reported. We report a case of acute respiratory distress syndrome followed by toxic enzymatic hydropneumothorax, mediastinitis and neck phlegmon caused by pancreatic pseudocyst fistula in 45 years old man. After initial diagnostics emergency operation was performed by multidisciplinary team. Ascending mediastinitis caused by pancreatic pseudocyst required a prompt surgical intervention. Early detection and an aggressive approach are crucial. Treatment should include surgical debridement and broad-spectrum antibiotics followed by adequate supportive care.

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Key words: mediastinitis, pancreatic pseudocyst, neck phlegmon

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published since 1951. Mediastinitis as a complication of pancreatic pseudocyst fistula is very rare and only one case of neck involvement has been reported (3).

Here, we report a case of acute respiratory distress syndrome followed by toxic enzymatic hydropneumothorax, mediastinitis and neck phlegmon caused by pancreatic pseudocyst fistula.

Case study

A 45-year-old man was admitted to emergency department in the state of severe septic shock, hypotensive (TA-95/58 mmHg, HR 105bpm) with redness and swelling of the neck, high fever (39.2°C), already on a mechanical ventilation. He had medical history of acute pancreatitis three years ago, followed by bilateral pleural effusion. Otherwise, patient was healthy man with no history of alcohol abuse or cholelithiasis. Deep neck infection was suspected. Lab analyses showed elevated blood amylase levels (4690 U/L, ref: 0 – 490) urine amylase level (17360 U/L, ref: 25–125), high leukocyte count ($28.1 \cdot 10^9/L$, ref: 4.0–9.0), increased level of C- reactive protein (261.4 mg/L, ref: 0.0–5.0), Cholesterol (4.2 mmol/L, ref: 3.90 – 5.20), Triglyceride (0,75 mmol/L, ref: 0,70 – 1.70), BMI (21,2 ref: 18,5–24.9).

Computed tomography (CT) scan of the neck, chest and abdomen was performed showing fluid accumulation in the posterior and visceral

Introduction

Acute mediastinitis is one of the most severely known infections with high mortality rate ranging between 14–42% (1). Most commonly, mediastinitis occurs as a descending process arising from oral cavity or pharyngeal infection spreading across vascular sheaths or prevertebral fascia to visceral or anterior mediastinum. The most common thoracic complications of pancreatic disease are reactive pleural effusion and pneumonia, while rare complications include thoracic conditions, such as pancreaticopleural fistula with massive pleural effusion or hemothorax and extension of pseudocyst into the mediastinum (2). Only 50 cases of mediastinal extension of pancreatic pseudocyst have been

mediastinum, in both pleural spaces and in front of the prevertebral fascia of the neck (Figure 1, 2 and 3). Pancreas was inflamed with two pseudocyst located in the body, extending to

posterior mediastinum. Size of pseudocysts was 76x47 mm and 123x96 mm respectively, both accumulating opaque dense fluid (Figure 4).

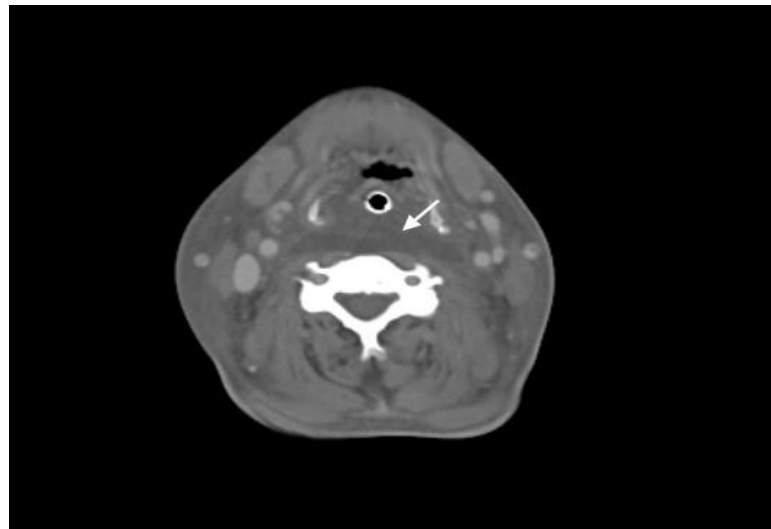


Figure 1. Multislice Computed Tomography presentation of fluid collection in retropharyngeal space of the neck in front of prevertebral fascia (marked with an arrow)

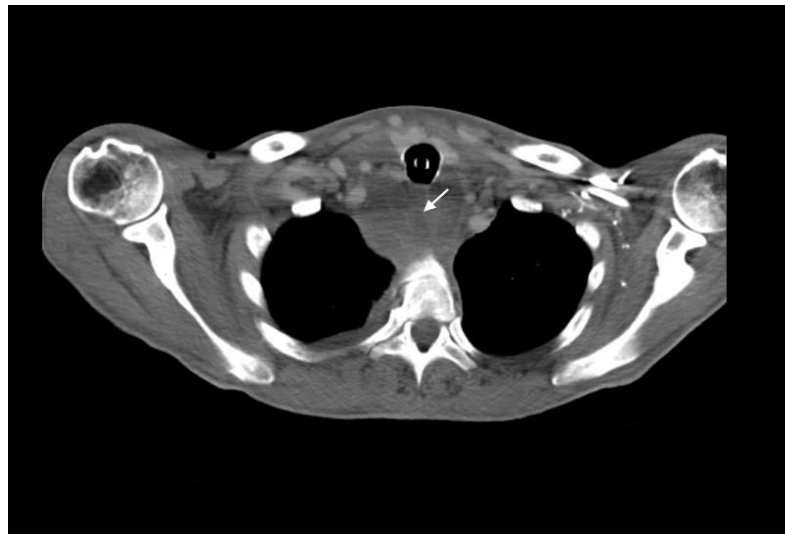


Figure 2. Multislice Computed Tomography presentation of fluid collection in visceral mediastinum (marked with an arrow)

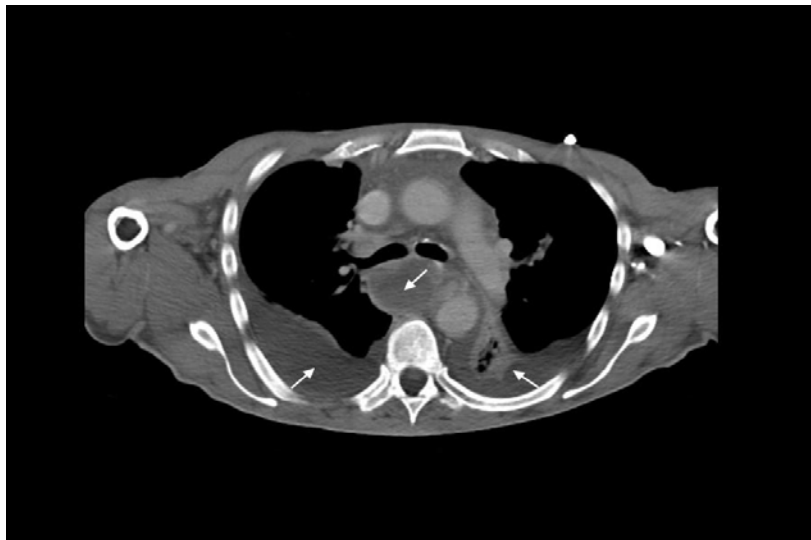


Figure 3. Multislice Computed Tomography presentation of acute mediastinitis followed by bilateral pleural effusion (marked with arrows)



Figure 4. Multislice Computed Tomography presentation of pancreatic pseudocyst (marked with an arrow)

Emergency operation was performed within one hour by a team of maxillofacial, thoracic and abdominal surgeon. The first step of the operation considered cervicectomy and drainage of the prevertebral space all the way to the thoracic inlet. The prevertebral space was full of thick, dark fluid which later showed elevated levels of pancreatic amylase (1339 U/L). A tracheostomy was performed. The next step was the right anterior thoracotomy. Around 1000 ml of opaque fluid was evacuated from the pleural cavity and afterwards mediastinal pleura was widely opened. Debridement and drainage of mediastinum were performed with the evacuation of the necrotic tissue and thick, dark fluid which later showed elevated levels of pancreatic amylase (18999 U/L). The operation was continued with upper median

laparotomy which revealed pancreatic cysts extending to the posterior mediastinum. Simple surgical drainage of pancreatic cysts was performed followed by nutritive gastrostomy which was temporarily placed due to neck phlegmon.

The patient was transferred to the intensive care unit after the operation. The immediate postoperative recovery went slow with the patient on mechanical ventilation. He was extubated on the 5th postoperative day with lungs showing full reexpansion and the absence of pleural complications (Figure 5). Parenteral nutrition combined with somatostatin treatment and gastrostomy rehydration was introduced. Neck, mediastinal, pleural and abdominal drains were removed in the following days. Lab results showed normalized levels of inflammation markers. The

control CT scan showed no sign of neck and mediastinal infection (Figure 6). The patient was transferred to the standard care unit for further treatment with prolonged hospitalization due to impaired pancreatic function which was manifested by oscillations of blood glucose levels from severe hypoglycemia to mediate hyperglycemia.

Unfortunately, four months after the operation the patient had a new relapse of acute pancreatitis with severe multiple organ failure and absence of mediastinal complications. Lab analysis showed complete endocrine and exocrine pancreatic insufficiency. The outcome was fatal.



Figure 5. Postoperative X-ray showing reexpansion of both lungs and absence of pleural effusion



Figure 6. Multislice Computed Tomography showing no sign of mediastinal infection

Discussion

Infections of the mediastinum are serious and associated with high morbidity and mortality. They may result from adjacent disease with direct extension, hematogenous spread, or direct introduction into the mediastinal space (4). Common causes of acute mediastinitis include surgical infection, esophageal rupture, and descending infection from the pharynx. Ascending infection from an intra-abdominal focus to the mediastinum is very rare. The clinical situation of ascending mediastinitis due to pancreatic pseudocyst is similar to the fulminant course of descending necrotizing mediastinitis (5). In this case chronic recurrent pancreatitis lead to the formation of two pseudocysts with their extension to posterior mediastinum forming an active fistula. This subsequently lead to enzymatic mediastinitis with the enzymes extending to the retropharyngeal space. This is only the second case reported in the literature with a pancreatic fistula extending to deep fascial layers of the neck. Pancreaticopleural fistula should be suspected in patients with intense abdominal and chest pain followed by cough and hemoptysis. Opaque and dark fluid found in thoracocentesis should raise a suspicion of enzymatic pleuritic which can easily be confirmed on fluid analysis showing a high level of amylase. CT scan of chest and abdomen is mandatory and prompt surgical intervention is required.

Less invasive techniques, such as VATS, have become effective options for the treatment of mediastinitis by pleuromediastinal drainage with or without cervical drainage (6). Since our patient was in septic shock and due to massive neck phlegmon we opted for an open surgery approach. Regarding pancreatic cysts CT, US or EUS-guided percutaneous catheter drainage and endoscopic drainage, have gained popularity (7–9). When pancreatitis with pseudocyst is a result of pancreatic duct rupture, stent placement via transpapillary approach is considered (10).

Supportive treatment following surgery, with parenteral nutrition combined with or without somatostatin or its analogue, octreotide, has been shown to improve outcomes in patients with mediastinal pancreatic fluid accumulation (11). We introduced somatostatin on the first postoperative day which was continued until the resolution of pancreatitis.

Our case showed the importance of a multidisciplinary surgical approach. Cervicotomy and adequate necrectomy, debridement, lavage and drainage of the mediastinum, use of wide spectrum antibiotics and adequate supportive therapy in the setting of ICU, solved the severe and possibly fatal acute infection and the patient did not die due to this complication of pancreatitis. Drainage of pseudocysts stopped the mediastinal infection recurrence in the new onset of acute pancreatic infection.

However, pancreatitis is an inflammatory disease that results in a spectrum of mortality related to the severity of the disease (12). Inflammatory cascade leads to the systemic manifestations of acute pancreatitis. It ultimately produces capillary permeability and endothelium damage with microvascular thrombosis that gives rise to multiple organ dysfunction syndrome (MODS) as the main cause of morbidity and mortality in the setting of acute pancreatitis (13).

Our patient was an otherwise healthy man with no history of alcohol abuse or cholelithiasis. Some individuals have a genetic predisposition for pancreatitis and suffer from recurrent acute pancreatitis, progressing to chronic pancreatitis. Not surprisingly, the associated genes are involved in activating trypsinogen to trypsin. Many genes play a role in recurrent pancreatitis, e.g., cystic fibrosis transmembrane conductance regulator (CFTR) gene, cationic trypsinogen gene (PRSS1) gain of function mutations, mutations of trypsin degrading enzyme (CTCR) chymotrypsin C and (SPINK1) pancreatic secretory trypsin inhibitor. Furthermore, they are also involved in the increasingly acknowledged spectrum of diseases, from acute to chronic pancreatitis (14–16).

Conclusion

Ascending mediastinitis caused by pancreatic pseudocyst requires a prompt surgical intervention. Although very rare, infection can spread all the way to deep fascial layers of the neck. Early detection and an aggressive approach are crucial in reducing the high mortality rate of infection. Treatment should include surgical debridement and broad-spectrum antibiotics followed by adequate supportive care. Acute pancreatitis is a severe disorder that still carries a high mortality rate due to multiorgan failure.

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Prikaz slučaja

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**AKUTNI ASCEDENTNI MEDIJASTINITIS I FLEGMONA
VRATA UZROKOVANI PSEUDOCISTOM PANKREASA:
PRIKAZ SLUČAJA**

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Akutni medijastinitis spada u grupu najtežih infekcija sa visokom stopom mortaliteta. U najvećem broju slučajeva nastaje kao posledica descendentnog širenja infekcije iz usne duplje ili farinksa. Kao najčešće torakalne komplikacije bolesti pankreasa javljaju se reaktivni izlivi ili pneumonije. Nasuprot tome, komplikacije poput pankreasno-pleuralne fistule praćene masivnim izlivom ili hemotoraksom i ekstenzijom pseudociste u medijastinum retko se sreću. U poslednjih sedamdeset pet godina u literaturi je zabeleženo samo pedeset slučajeva širenja pseudociste u medijastinum; samo je u jednom slučaju došlo do širenja u predeo vrata. Prikazan je slučaj četrdesetpetogodišnjeg bolesnika kod kojeg je potvrđen akutni respiratorni distress sindrom sa razvojem enzimskog hidropneumotoraksa, medijastinitisom i flegmonom vrata uzrokovan pseudocistom pankreasa. Bolesnik je posle inicijalne dijagnostike podvrgnut urgentnoj operaciji u kojoj je učestvovao multidisciplinarni tim. Ascendentni medijastinitis uzrokovan pseudocistom pankreasa zahteva hitno hirurško lečenje, a rana detekcija i agresivni pristup u lečenju od presudnog su značaja. Terapija podrazumeva kombinaciju hirurškog debridmana sa antibioticima širokog spektra i adekvatnom suportivnom terapijom.

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Ključne reči: medijastinitis, pseudocista pankreasa, flegmona vrata

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COMPLEXITY OF REHABILITATION AFTER SIMULTANEOUS BILATERAL INSTALLATION OF ENDOPROTHESIS IN A PATIENT WITH DEVELOPMENTAL HIP DYSPLASIA: CASE REPORT

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Božidar Janković¹, Bratislav Cvetković¹, Mirjana Kocić^{1,2}

The gold standard for the treatment of advanced coxarthrosis is the installation of a total hip endoprosthesis. Degenerative hip disease occurs bilaterally in about 42%. The operation is usually performed first on one hip, and after at least 6 months on the other hip. Rarely, this operation is performed simultaneously on both hips, which represents a more demanding operation as well as rehabilitation. This paper is a case report of a patient with good results of functional recovery after bilateral simultaneous hip arthroplasty, who was followed prospectively from the 5th postoperative day until the end of the 3rd month after surgery. The patient suffered from secondary bilateral coxarthrosis, which is the result of a developmental disorder of the hips. The good recovery results of this patient with simultaneous bilateral hip arthroplasty can be explained by the selection of a good patient selection for simultaneous bilateral arthroplasty, well-chosen endoprostheses, well-done surgery and timely and adequate rehabilitation.

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Key words: hip arthroplasty, coxarthrosis, developmental hip disorder

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young people, without existing comorbidities, with adequately preserved musculature of the lower extremities may have an advantage over surgery in two acts.

Case report

This case report is a prospective study that followed the patient A.R. from the 5th postoperative day to the end of the 3rd month after the operation. This is a 28-year-old male patient with simultaneous bilateral hip arthroplasty (short-stem endoprosthesis used) due to secondary bilateral coxarthrosis, which is a consequence of a developmental disorder of the hips as part of achondroplasia, which was diagnosed at birth. The patient was operated at the Clinic for Academician prof. dr Milorad Mitković of the University Clinical Center (UKC) Niš, where the acute phase of rehabilitation began. On the 5th postoperative day, he was transferred to the Clinic for Physical Medicine and Rehabilitation of the University Clinical Center Niš, where the inpatient post-acute phase of rehabilitation was carried out, which lasted 25 days, until the 30th postoperative day. The first symptoms of the disease began in early childhood in the form of difficulty moving and pain in the groin area. In the last couple of years, there has been a significant worsening of symptoms. Bilateral advanced coxarthrosis was recorded on the roentgenogram of the hips, and after a detailed examination, the installation of

Introduction

Coxarthrosis belongs to a heterogeneous group of diseases with different etiologies, but similar biological, morphological and clinical characteristics (1). Advanced coxarthrosis significantly affects the functional capacity and quality of life of affected persons. The gold standard in the treatment of advanced coxarthrosis is the installation of a total hip endoprosthesis. Degenerative hip disease occurs bilaterally in about 42% of people and it is estimated that about 25% of people with bilateral coxarthrosis require bilateral installation of hip endoprostheses. This operation is usually performed first on one hip, and after at least 6 months on the other hip. Less often, this operation is performed simultaneously on both hips, which represents a more demanding operation, as well as rehabilitation (2). This type of intervention in

bilateral hip endoprotheses was indicated. Simultaneous bilateral installation of total cementless hip endoprotheses was performed with an antero-lateral (Watson Jones) approach on April 8, 2024. at Clinical for ortopedic (Figure 1). Immediately after the operation, the patient's physiatrist recorded a lesion of the *n. femoralis* of the right leg, and postoperatively there was an inability to actively extend the lower leg in the knee joint, but the patient was able to perform a palpable static contraction of the quadriceps, and the gross muscle strength (GMS) of the quadriceps was rated 1 according to the Manual Muscle Test (MMT). Immediately after the operation, acute rehabilitation at the Clinical for ortopedic Surgery and Traumatology was started. On the 5th postoperative day, the patient was transferred to the Clinic, where post-acute rehabilitation continued. On admission, swelling of both upper legs was recorded, more pronounced on the left by 2 cm. Mobility in the hip joints was limited, with absent active extension in the right knee joint and decreased sensation in the front of the right upper leg. Active movements in the right hip: Flexion with the knee extended 0°, Flexion with the knee bent 0°, Abduction 10°, Extension 0°. Active movements in the left hip: Flexion with the knee extended 0°, Flexion with the knee bent 0°, Abduction 10°, Extension 0° (Table 1). Mobility in other joint segments of the lower extremities was functional. Gross muscle strength (GMS) for flexors of both hips was 1+, GMS for m. quadriceps of the right leg was 1 per MMT (Table 2). The transfer to the vertical position was possible with the generous help of 2 physiotherapists, after which walking was possible in the pilot hole with support on the forearms, a few steps with full support on the left and partial support on the right leg (Figure 2, Figure 5). According to the Numerical Pain Rating Scale (NPRS), the pain was 3 (Table 3). The Harris hip score (HHS) on admission was 20. Inpatient rehabilitation at the Clinic for Physical Medicine and Rehabilitation included kinesitherapy, occupational therapy, electrotherapy, and magnetic therapy. Kinesitherapy therapy included breathing exercises, exercises to strengthen the GMS of the upper extremities, active relief exercises for both hips in order to increase the range of motion, and passive exercises to extend the right hamstring. The walk was first performed in the pilot walker, with support on both legs (more on the left), and after the 10th

postoperative day, the patient started walking with the help of underarm crutches (Figure 3). Exercises were performed to the limit of pain, 3 times a day. Also, occupational therapy was carried out in bed. Of the electrical procedures, interference currents were applied to the hip area, as well as a low-frequency pulsed magnetic field for the hip area according to the program for conditions after endoprosthesis installation. At discharge, a significant improvement in the patient's condition was noted. The patient moved using axillary crutches with support on both legs. The swelling of the upper legs was reduced by 2 cm on the left leg and 1 cm on the right compared to the reception, mobility was improved in both hip joints, and extension in the right knee joint was improved. Active movements in the left hip: Flexion with extended knee 55°, Flexion with bent knee 80°, Abduction 25°, Extension 0°. Active movements of the left knee: Flexion 100°, Extension normal. Active movements in the right hip: Flexion with the knee extended 35°, Flexion with the knee bent 40°, Abduction 20°, Extension 0°. Active movements in the right knee: Flexion 80°, Extension normal. There was a subjective improvement of sensibility on the front of the right thigh. The gross muscle strength of the pelvic femoral musculature was strengthened and was rated as 3 on the MMT in both legs. The Harris hip score at discharge was 41. According to the NPRS scale, the pain was 2 at discharge. At the check up that was 3 months after the operation, the continuous progress of the patient was recorded. The patient moved without aid (Figure 4, Figure 6). There was no swelling, but there is still evidence of hypertrophy of the musculature of both legs as a whole. In addition, the mobility in both hips as well as in the right knee has improved. Active movements in the left hip: Flexion with extended knee 75°, Flexion with bent knee 90°, Abduction 50°, Extension 20°. Active movements in the right hip: Flexion with extended knee 55°, Flexion with bent knee 85°, Abduction 45°, Extension 20°. Active movements in the right knee: Flexion 100°, Extension normal. Gross muscle strength of the pelvic femoral musculature was strengthened and was rated 4 for the left leg and 4 for the right leg. The gross muscle strength (GMS) of the right m. quadriceps was 5. The Harris hip score 3 months after discharge was 81. According to the NPRS scale, the pain was 0.3 months after the operation.



Figure 1. RTG post operation

Table 1. Hip mobility on admission, discharge, 3 months after surgery

	F(O) L	F(O) D	F(S) L	F(S)-D	E-L	E-D	ABD-L	ABD-D
On admission	0°	0°	0°	0°	0°	0°	10°	10°
Discharge	55°	35°	80°	40°	0°	0°	25°	20°
3 months post op	75°	55°	90°	85°	20°	20°	50°	45°

F(O) Flexion with knee extended, F(S) Flexion with knee bent, ABD abduction, E extension

Table 2. GMS of the m. quadriceps of the right leg

	GMS m. quadriceps right leg
On admission	1
Discharge	2+
3 months post operation	4



Figure 2. Walk in the pilot hole with the help of 2 therapists at the reception



Figure 5. Standing up at the reception with the help of 2 therapists

Table 3. Pain assessed on the basis of the numerical pain scale (NRPS 0–10)

NPRS	On admission	Discharge	3 months post operation
Rating	3	2	0

**Figure 3.** Gait with axillary crutches at discharge**Figure 4.** Walking without aid 3 months after the operation**Figure 6.** Getting up from a chair 3 months after the operation

Discussion

Our paper is a case report of a patient with good functional recovery results after bilateral simultaneous hip arthroplasty, who we followed prospectively from the 5th postoperative day until the end of 3 months after the operation due to secondary bilateral coxarthrosis, which is a consequence of a developmental disorder of the hips. In the literature, it is pointed out that patients who are proposed to have both hips replaced with artificial ones must meet certain conditions in terms of adequate health status, and preservation of the musculature, which is why the

selection of good candidates is very important. Some works deal with the comparison of recovery, complications, and economic costs between the simultaneous bilateral replacement of both hips and the classic approach with 2 operations with a time interval of no less than 6 months. Simultaneous bilateral hip arthroplasty represents the replacement of both hips with a total hip endoprosthesis during one hospitalization, under one anesthesia (3). According to a study from 2016, there are benefits in favor of simultaneous replacement of both hips due to a lower frequency of deep vein thrombosis (DVT) and other major systemic complications compared to the classic approach (4). Systemic studies from 2019 (5)

have similar conclusions, the results of which also speak in favor of a lower frequency of complications (DVT, respiratory complications, pulmonary thrombosis) with simultaneous replacement. A good selection of patients for surgery probably influenced the good results of these studies.

However, there are studies whose results do not agree with the above-mentioned claims, but claim that there is no statistically significant difference between these two approaches in the functional sense and in the number of complications (6).

There are studies that claim the opposite, that simultaneous bilateral total hip arthroplasty is associated with a greater number of complications. The complications listed are pulmonary embolism, kidney failure, increased need for blood transfusions. In the conclusion of this cohort study, it is stated that the most important thing is the good selection of patients, which is the conclusion of this paper (7).

Conclusion

Good results of functional recovery of a patient with simultaneous bilateral hip arthroplasty were presented, which can be explained by good patient selection for simultaneous bilateral arthroplasty, well-chosen endoprosthesis with a short stem that preserves bone tissue, well-done surgery and timely and adequate rehabilitation. It is important to point out that not every patient with bilateral coxarthrosis is a good candidate for this type of surgery, but mostly younger and healthier patients. A very important influence on the final results of this complex operation was timely and adequate rehabilitation, with emphasis on early verticalization and walking with the help of adequate aid.

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KOMPLEKSNOST REHABILITACIJE NAKON ISTOVREMENE OBOSTRANE UGRADNJE ENDOPROTEZA KOD PACIJENTA SA RAZVOJNOM DISPLAZIJOM KUKOVA: PRIKAZ SLUČAJA

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Zlatni standard u lečenju uznapredovale koksartroze predstavlja ugradnja totalne endoproteze kuka. Degenerativno oboljenje kuka javlja se bilateralno u oko 42% slučajeva. Operacija se najčešće obavlja najpre na jednom kuku, a posle najmanje šest meseci i na drugom kuku. Ređe se ova operacija obavlja i na jednom i na drugom kuku u isto vreme; tada su i operacija i rehabilitacija komplikovanije. U ovom radu se predstavlja slučaj pacijenta kod kojeg su rezultati funkcionalnog oporavka posle bilateralne simultano urađene artroplastike kukova bili dobri. Pacijent je praćen prospektivno od petog dana posle operacije do kraja trećeg meseca posle operacije. Pacijent je bolovao od sekundarne bilateralne koksartroze, koja predstavlja posledicu razvojnog poremećaja kukova. Dobri rezultati postignuti u oporavku ovog pacijenta sa simultano urađenom bilateralnom artroplastikom kukova mogu se objasniti dobrom selekcijom pacijenta za simultanu bilateralnu artroplastiku, dobro odabranim endoprotezama, uspešno obavljenom operacijom i pravovremenom i adekvatnom rehabilitacijom.

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Ključne reči: artroplastika kukova, koksartroza, razvojni poremećaj kukova

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PERIPHERAL NEUROPATHIES IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Patients with chronic kidney disease often suffer from peripheral neuropathies, which have a significant impact on morbidity and mortality. Therefore, it is important to understand their pathophysiological mechanism to enable an effective management strategy for these neurological complications. This review aimed to highlight the importance of detecting clinical parameters that enable the diagnosis and early treatment of peripheral neuropathy (MRI).

To achieve the goal of this review paper, we used PubMed and MEDLINE databases. The information presented in this work is gathered from the literature with the keywords peripheral neuropathy, chronic renal failure, hemodialysis, clinical picture, and treatment. At the beginning of 2024, we conducted research in the PubMed and Medline databases.

Based on the established criteria for searching the literature related to peripheral neuropathy in patients with end-stage renal disease, in the last ten years, as well as some earlier works that were of interest to the issue of peripheral neuropathy, out of a total of 22 works that met the inclusion criteria, there were 8 review works, 9 original works, 4 case reports, and 1 monograph.

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Key words: *peripheral neuropathy, chronic renal failure, hemodialysis, clinical picture, treatment*

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Introduction

The prevalence of chronic kidney disease is increasing worldwide. About 850 million people in the world have kidney failure, which is currently the third leading cause of death. Neurological complications associated with renal insufficiency significantly increase morbidity, mortality and decrease the quality of life of patients with renal insufficiency, and among them, peripheral neuropathy occupies a significant place. Peripheral neuropathy, one of the commonest neurological

complications of chronic kidney disease, its frequency among the population of patients with chronic renal failure, in the terminal stage, is between 60–100% (1, 2).

Methodology

At the beginning of 2024, we conducted research in the PubMed and Medline databases. The criteria for including studies in the research were keywords: peripheral neuropathy, chronic renal failure, hemodialysis, clinical picture, and treatment.

The literature search covered the period of the last ten years, except for some works that we included in the reference literature from an earlier period, which were significant for the assessment of the problem of peripheral neuropathy in hemodialysis patients (Figure 1).

All works with these keywords were reviewed and selected to remove duplicates. Selected publications are organized and summarized for each form of peripheral neuropathies separately with a general description of study characteristics and findings organized by subtopics derived from the data. Of the selected publications, the largest number were original and review works, a smaller part was monographs. Most of the selected papers were written in

English, except the monograph, which was written in Serbian.

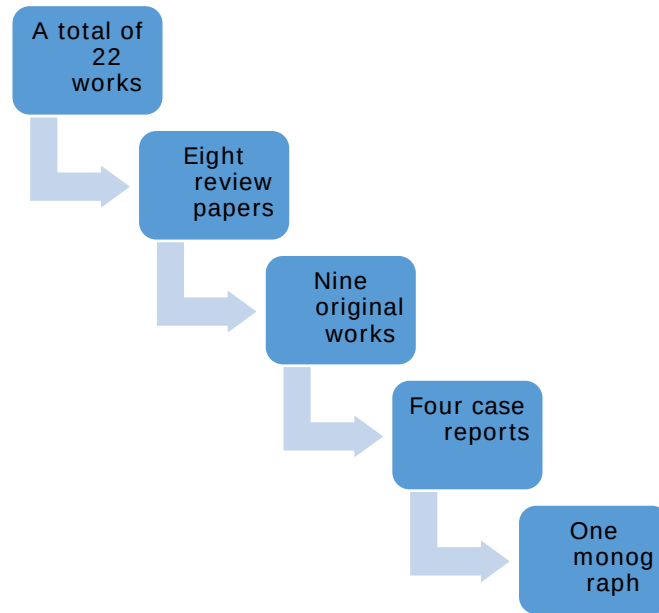


Figure 1. Selection of articles included in the work

Results

Based on the established criteria for searching the literature related to peripheral neuropathy in patients with end-stage renal disease, in the last ten years, as well as some earlier works that were of interest to the issue of peripheral neuropathy, out of a total of 22 works that met the inclusion criteria, there were 8 review works, 9 original works, 4 case reports, and 1 monograph.

Peripheral neuropathies

Current data indicate that the prevalence of neuropathy in patients with end-stage renal failure is between 60% and 100% and that it is clinically manifested when the glomerular filtration rate falls below 12 ml/min. The systemic effects of uremia on the peripheral nerves are emphasized by the generalized nature of the slowing of nerve conduction in the extremities and sensorimotor nerves. Structural changes that occur in peripheral neuropathy depend on the duration of uremia, which in itself can have a universal neurotoxic effect. Creatinine, urea, methylguanidine, phenols, guanidine, uric acid, aromatic hydroxy acids, oxalic acid, guanidosuccinic acid, myoinositol, indican, amines, "intermediate molecules", parathormone, β -2 microglobulin have shown their negative influence on peripheral nerve endings, most often on median, ulnar and femoral nerves. In the pre-dialysis period, that is, in the pre-terminal phase of chronic renal failure, the nerve endings are in a depolarized state, but the membrane potential is normalized after dialysis. It must be emphasized that the degree of

depolarization is correlated with the concentration of potassium, which indicates that hyperkalemic depolarization has a significant role in the development of nerve dysfunction (3).

Peripheral neuropathy caused by chronic renal failure results from various mechanisms. Intracellular calcium accumulation due to hyperkalemia in chronic renal failure and reversal of the K/Ca pump can cause axonal damage and significant axonal depolarization in the early stages of neuropathy. Other factors causing neuropathy in patients with chronic renal failure include the neurotoxic effects of small molecules (eg, myoinositol and methyl guanidine) that accumulate in body fluids, elevated parathyroid hormone levels, and vitamin B1 deficiency. Likewise, many drugs used to treat chronic renal failure can cause peripheral neuropathy (4).

In the early stages of uremic neuropathy, there is a distal loss of needle prick sensation for fistula puncture, as well as a reduction or absence of tendon reflexes in the lower limbs. As the disease progresses, this sensory loss spreads proximally in the lower limb, and then when the motor nerves are damaged, weakness and atrophy may occur in the distal muscles of the lower limb. In addition to the damage to large motor and sensory fibers in uremic neuropathy, neuropathy of small fibers also occurs. In patients with diabetic nephropathy, small fiber neuropathy can lead to clinical manifestations of burning and sharp pain, as well as changes in temperature perception. Advanced uremic neuropathy can be clinically presented by atrophy of the musculature of the distal part of the lower extremities with the appearance of ulcerations and consequent limb amputations (5).

Polyneuropathy, in patients with chronic renal failure, can be a consequence of glomerulonephritis, diabetes, hypertension, as well as multisystem accompanying disorders. Signs and symptoms of uremic polyneuropathy begin distally in the feet, slowly progress along the legs and are symmetrical, also sensory disturbances manifesting as cramps, pain and burning, and restless legs syndrome, while a smaller number of patients report discomfort, pain and hyperalgesia in the feet. Uremic polyneuropathy continues to significantly limit the quality of life of patients with uremia. It must be emphasized that the symptoms of damage to small-diameter nerve fibers are manifested by pain, hyperalgesia, and dysesthesia, while damage to large-diameter fibers is clinically manifested by paresthesias and numbness (6, 7).

The accumulation of uremic toxins associated with oxidative stress-related free radical activity causes motor, sensory and autonomic nerve damage which leads to peripheral neuropathy. Although this exact mechanism remains unknown, hypotheses are supporting the role of electrolytes in this process. Hyperkalemia and hyperphosphatemia cause chronic uremic depolarization of nerves, contributing to the development of peripheral neuropathy. This occurs because potassium disrupts the normal ionic gradient and therefore activates calcium-mediated processes leading to axonal death (5, 8).

Clinical diagnosis of peripheral neuropathies, in patients with chronic kidney diseases, implies, in addition to eliminating other causes of neuropathy (glucose metabolism disorder and connective tissue disease), involves a neurological examination and a nerve conduction test, by which it is determined conduction velocity and amplitude and confirms axonal neuropathy. It should be known that in the initial stages of the disease, axonal neuropathy is characterized by reduced sensory, and in the later stages of the disease, motor amplitudes are reduced, while conduction velocity is minimally impaired (5).

Hemodiafiltration, as a dialysis procedure in the treatment of patients with end-stage kidney diseases, can have a positive effect on nerve function. Nevertheless, there is ample evidence that the most effective treatment for peripheral neuropathy in patients with end-stage kidney disease is kidney transplantation. Unfortunately, some drugs used after a kidney transplant, such as calcineurin inhibitors, can contribute to nerve damage. Likewise, it should be emphasized that maintaining normal serum potassium homeostasis can have a positive effect on the prevention of peripheral nerve damage, which can be a potential strategy for the prevention and treatment of neuropathy in chronic kidney disease. Interesting are the findings of recent studies that confirmed the potential benefit of limiting potassium intake in preventing the progression of neuropathy in stage 3–4 chronic renal failure. Drugs used against neuropathic pain belong to the group of tricyclic

antidepressants and anticonvulsants and they are avoided due to numerous side effects, although they belong to the first line of drugs, of which amitriptyline is the best known. Also, extra care is needed when using anticonvulsant drugs such as pregabalin or gabapentin, because the doses are limited and they are dependent on creatinine clearance. There is some research that emphasizes the importance of an exercise program for improving neuromuscular function. Despite the significant share and breadth of the therapeutic range, apart from the control of potential risk factors, unfortunately, there is no specific treatment for uremic myopathy (5, 9, 10).

Ischemic neuropathy

Ischemic neuropathy is the most common clinical manifestation among diabetics who have serious damage to peripheral arteries, especially when the brachial artery is used to create an arteriovenous fistula immediately after arteriovenous anastomosis (usually within a few hours). Clinically, it is manifested by severe pain, weakness in the hands and paresthesia. A neurological examination shows weakness of the distal muscles innervated by the median nerve. The incidence of ischemic neuropathy ranges between 1 and 10% (11). The mechanism of ischemic neuropathy is not clear enough, but it is known that it is a form of the phenomenon of blood theft after the creation of an arteriovenous anastomosis, which contributes to the loss of axons in the distal part of the limb. Ischemic monomyelopathy in the lower extremities is more sensitive to ischemic monomyelopathy due to greater loss of axons, compared to the ulnar nerve. In these circumstances of arteriovenous anastomosis, there is an inversion of a small amount of blood from the distal blood vessels into the arteriovenous fistula or arteriovenous graft, which is sufficient for the consequent ischemia and the appearance of ischemic neuropathy, most likely due to the lack of collateral blood flow (12, 13). Ischemic monomyelopathy involves dysfunction of multiple peripheral nerves of the upper extremities. Symptoms are usually immediate, predominantly neurological, and often without significant ischemia of the hand. There is also paresthesia and numbness in the area of innervation of all three nerves of the forearm, as well as diffuse motor weakness or paralysis. The main diagnostic feature of ischemic monomyelopathy is the lack of findings indicating reduction or redirection of arterial flow. One of the factors that creates a dilemma in the setting of diagnostic criteria is the high percentage of diabetics in the population of patients with chronic kidney disease (14, 15).

When the neurological examination is not enough to make a diagnosis, an electrodiagnostic test can be of clinical importance. Dominant, clinically significant signs of ischemic monomyelopathy are sensorimotor symptoms in

the area of the hands or feet, in the sense of unpleasant, deep and burning pain, which lasts continuously for months. The expressiveness of the sensory, in relation to the physical findings, can introduce confusion and lead the clinician to think about a psychogenic or radiculopathic etiology. Clinical manifestations of motor symptoms manifest as weakness or paralysis of the muscles innervated by the three forearm nerves and include impairment of wrist and finger extension (radial nerve), ulnar abduction and adduction of extended fingers (ulnar nerve), and thumb opposition, flexion, and abduction (medial nerve). Ischemic monomyelopathy is a consequence of reduced blood flow in the nerve trunk, and due to the extreme sensitivity of nerve tissue to ischemia, there may be a significant risk of permanent damage. It has been described in the literature that, regardless of the establishment of radial pulses, when the blood flow is reduced 6 weeks after the creation of an arteriovenous fistula, there is no neurological improvement. From the meager literature data, it can be concluded that ischemic neuropathy can be considered a severe, focal neurological consequence of arterial theft syndrome. Electromyographic nerve conduction studies indicate the loss of motor and sensory nerve axons in the distal part of the limb and a decrease in the amplitude of nerve conduction, especially of sensory fibers in the forearm and hand (15, 16).

The therapeutic approach to the treatment of ischemic monomyelopathy involves reducing the flow in the vascular access, which will consequently lead to an increase in perfusion in the extremities and thus reduce the symptoms of neuropathy. Reduction of blood flow or complete shutdown improves blood flow to the extremities, which can lead to recovery, but unfortunately without convincing results in practice, because the majority of patients will remain with neurological damage. Further treatment is mostly symptomatic and includes drugs for pain control (anticonvulsants, antidepressants and narcotics) (13).

However, the most important treatment for ischemic neuropathy is immediate closure of the access, which increases the probability of recovery and leads to partial or full restoration of the sensory and motor function. That's why it would better education and awareness on the part of the surgeon and nephrologist should lead to an early diagnosis and the proper management of ischemic neuropathy (17).

Uremic and diabetic polyneuropathy

Uremic and diabetic polyneuropathy is also a type of peripheral neurological dysfunction, characterized by acute onset and bilateral symmetry. It worsens with the duration of the primary condition that caused it, and distal sensory symmetry is a common denominator for both diabetic and uremic etiology. If they occur

simultaneously, the deterioration of the clinical condition can be even more progressive, with gradual progression as time goes on, creating even greater disability, making patients unable to stand or walk. The effect of uremic or diabetic peripheral polyneuropathy on the manifestation of symptoms is without significant differences, except that the clinical symptoms of diabetic neuropathy are most often the result of the influence of symptomatic autonomic neuropathy, and the severity of symptoms is positively correlated with the duration of diabetes and chronic renal insufficiency that is dependent on dialysis (16).

Compressive neuropathy

Carpal tunnel syndrome occurs in about 10% of the uremic population with a prevalence of about 3% and is the most common form of compressive neuropathy. It occurs as a result of damage to the median nerve in the area of the carpal tunnel of the hand joint, and all as a result of ischemia and compression of this nerve. Carpal tunnel syndrome can occur acutely, after a wrist fracture, but most often occurs as a result of chronic accumulation of multiple elements that increase pressure in the carpal tunnel, edema occurs, fibrosis, collagen proliferation, amyloid deposition, and thickening of blood vessel walls. However, in addition to the usual risk factors, genetic predisposition, obesity, hypothyroidism, and inflammatory arthropathies, such as rheumatoid arthritis and diabetes, should also be considered. The presence of an arteriovenous fistula can cause symptoms of an already existing asymptomatic lesion of the carpal tunnel through additional microischemia, as well as an independent increase of inflammatory deposits inside the carpal tunnel. There is a thought that the development of carpal tunnel syndrome, after the formation of an arteriovenous fistula, is a consequence of venous hypertension, due to compression of the median nerve. On the other hand, the deposition of β 2-microglobulin in the carpal tunnel is a consequence of amyloidosis-induced carpal tunnel syndrome, which represents a serious complication of hemodialysis, independent of the influence of arteriovenous fistula. In dialysis patients, compared to the general population, compressive nerve entrapment syndrome occurs more often. Ischemic monomyelopathy and complex regional pain syndrome represent complications due to nerve damage during the surgical procedure of creating vascular access. The etiology of compressive neuropathy is diverse and knowledge of the anatomy of the vascular and nerve supply is essential for diagnosis and intervention to prevent complications. The anatomical proximity of the brachial artery and the median nerve may be the reason for potential compression due to the development of an eventual (pseudo)aneurysm or hematoma that occurs after an unsuccessful

puncture of the fistula. Clinical manifestations of median nerve entrapment reduce wrist flexion, and pronation and make fine hand movements difficult, which significantly affects the patient's functional ability. Cubital tunnel syndrome is a phenomenon that occurs as a result of pinching of the ulnar nerve in the cubital fossa, it is also called Guyon's syndrome, it is manifested by pain in the hand, paresthesia and dominant weakness of the muscles of the hand, where created arteriovenous fistula. This manifestation indicates a gradual compression syndrome of the medial and ulnar nerve. The differential diagnosis in relation to ischemic syndrome due to distal hypoperfusion is the absence of a temperature difference between the arms, as well as good pulses of the radial artery. There are conflicting views in the literature on whether an arteriovenous fistula increases the risk of developing carpal tunnel syndrome (3, 7, 18–22).

Carpal tunnel syndrome, as a compressive neuropathy, is clinically manifested by numbness, and tingling pain in the distribution region of the median nerve, most often as a result of compression of the median nerve in the area of the carpal tunnel, it can be progressive due to various sensory and motor disorders. It occurs in 2-4% of the adult population and women are more often affected than men. The incidence of carpal tunnel syndrome in the general population is approximately one to five cases per 1000 people per year, more often in the dominant hand (18–22).

The incidence of carpal tunnel syndrome in patients with end-stage renal disease ranges between 8% and 31%, with a tendency to increase in relation to the duration of hemodialysis. Some data indicate that carpal tunnel syndrome occurs in up to 30.5% of the extremities where the arteriovenous fistula was created, while the frequency in the opposite extremity is 12.2%. Therefore, arteriovenous fistula, due to the diversion of blood from the distal limbs, is cited as one of the causative factors contributing to the occurrence of carpal tunnel syndrome, but still without clear evidence. Perhaps one of the reasons is that there are no studies that used electrophysiological tests to confirm the diagnosis (3, 7, 18–22).

In the general population, carpal tunnel syndrome usually occurs in the dominant hand, while in patients with arteriovenous fistula, it occurs in the hand with the fistula (usually the non-dominant limb). In patients with arteriovenous fistulas, swelling of the forearm and hand may occur, most likely as a result of destruction of the valve of the superficial veins distal to the arteriovenous anastomosis. The swelling is more pronounced in post-puncture fistula vein compression for mechanical hemostasis when venous pressure can reach the level of systemic arterial pressure. Amyloid deposits of β 2-microglobulin are another proposed etiological factor that, by provoking inflammation,

leads to the formation of adhesions and edema, thus compressing the median nerve, regardless of the type of vascular access, thus accumulating β 2-microglobulin in the synovium and tendon area, which can be the reason for the establishment of carpal tunnel symptoms in patients who do not have arteriovenous fistulas (19).

Median nerve entrapment is clinically manifested in the area of innervation of the medial nerve, that is, in the palmar region, with pain, numbness and a burning sensation, mostly during dialysis and at night. Muscular changes are manifested by weakness and atrophy of the thenar muscles, which is clinically reflected in the patient's inability to join the thumb and index finger. When in the later period, motor dysfunction occurs, the recovery is prolonged and uncertain. In the general population without chronic kidney disease approximately 15% of patients have relevant complaints, but only 2 to 3% of them have a confirmed diagnosis of median nerve entrapment and that, unfortunately, is only based on the clinical picture. The research results indicate a significant increase in the incidence median nerve entrapment (up to ten times), with the dominance of men, with an uncertain outcome, and frequent recurrences in the postoperative period. Symptoms of compressive neuropathy most often appear after 6 to 8 years of hemodialysis treatment, which supports the view that compressive neuropathy increases with longer time spent on dialysis (16).

The diagnosis of carpal tunnel syndrome is usually based on clinical findings, but recently ultrasonography has been proposed as a valuable test for the diagnosis and evaluation of carpal tunnel syndrome, that is, the measurement of the cross-sectional area of the median nerve (15). Many comorbidities and various conditions contribute to the severity of the clinical picture of carpal tunnel syndrome, such as diabetes, pregnancy, gout, acromegaly, rheumatoid arthritis, amyloidosis, hypothyroidism, use of corticosteroids and estrogen, etc. This form of compressive peripheral neuropathy is more common in some occupations that require more activity of the hand joints, such as construction workers and typists. It is believed that the etiology of carpal tunnel syndrome, in hemodialysis patients, is the deposition of amyloid protein in the area of the carpal tunnel, elevated venous pressure distal to the site, nerve ischemia, edema, damage to the median nerve by the uremic toxin, as well as some still insufficiently known reasons. It is still not clear enough which is the predominant causative factor, whether mechanical (due to the thickening of surrounding tissues) or vascular (due to arteriovenous fistula). Some studies, however, favor compression of the median nerve as a consequence of amyloid protein deposition. When there is a strong suspicion of carpal tunnel syndrome and electrodiagnostic tests do not confirm the diagnosis, the so-called provocative tests, namely Phalen's and Tinel's

tests, of which Phalen's test showed more reliable results. Therefore, the validity of electrodiagnostic results is increasingly being questioned, especially in patients with arteriovenous fistula who are treated with chronic hemodialysis. In patients with chronic kidney disease, distal motor and sensory polyneuropathy is caused by segmental demyelination and degeneration of axons, on the other hand, the presence of central-peripheral axonopathy is indicated by a reduced conduction velocity of sensory and motor nerves, which is why doubts have been expressed about the reliability of electrodiagnostic tests in patients on hemodialysis (16).

The treatment strategy of compressive neuropathy recommends decompressive operations in the region of the median nerve. This type of intervention, in the general population, relieves pain in more than 70% of patients, and they are completely satisfied with the intervention. However, the return of muscle strength occurs gradually, months later. In hemodialysis patients, the results are less satisfactory. Nevertheless, improvement was reported by 76% of those operated on, but it turned out that patients with preoperative symptoms longer than 2 years had the least improvement, especially motor functions. Operative intervention involves resection of the calcified medial artery, however, if a tourniquet is used for postpunctal hemostasis, it must be

released to assess whether the resection leads to circulatory disturbances in the hand. If the artery is thrombosed, resection is uncomplicated and necessary to allow adequate tissue perfusion of the index and middle fingers (18).

It is clear that the pathophysiology of carpal tunnel syndrome in patients dialyzing with arteriovenous fistulas is currently unknown, and there is controversy as to whether arteriovenous fistula increases the risk of carpal tunnel syndrome developing. Correct and timely interventions are required for these different conditions and hence, the need to review the known literature to identify an evidence-based approach (22).

Conclusion

Due to the high incidence of peripheral neuropathies in patients with chronic kidney disease and the significant impact on the morbidity and mortality of these patients, early recognition of clinical symptoms and signs of peripheral neuropathy is extremely important. It is also important to understand the pathophysiological mechanisms of peripheral neuropathies in order to approach an effective strategy of diagnostic and therapeutic planning for these neurological complications.

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Pregledni rad

UDC: 616.833:616.61-008.6
doi: 10.5633 / amm.2025.0217**PERIFERNE NEUROPATIJE KOD BOLESNIKA SA
HRONIČNOM BUBREŽNOM INSUFICIJENCIJOM**Vekoslav Mitrović¹, Radojica Stolić², Vladimir Mitrović³¹Univerzitet u Istočnom Sarajevu, Medicinski fakultet Foča, Katedra za neurologiju, Foča, Republika Srpska, Bosna i Hercegovina²Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Katedra za internu medicinu, Kragujevac, Srbija³Univerzitet u Prištini sa privremenim sedištem u Kosovskoj Mitrovici, Medicinski fakultet, Kosovska Mitrovica, Srbija

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Pacijenti sa hroničnom bolešću bubrega često pate od perifernih neuropatija, koje značajno utiču na morbiditet i mortalitet. Dakle, važno je razumeti patofiziološki mehanizam ovih neuroloških komplikacija da bi se mogla osmisliti efikasna strategija upravljanja ovim poremećajima. Cilj ovog preglednog rada bio je da ukaže na važnost otkrivanja kliničkih parametara koji omogućavaju dijagnozu i rano lečenje periferne neuropatije.

U te svrhe korišćene su baze podataka *PubMed* i *MEDLINE*. Podaci predstavljeni u ovom radu prikupljeni su iz literature na koju je uputila pretraga sledećih ključnih reči: periferna neuropatija, hronična bubrežna insuficijencija, hemodijaliza, klinička slika, lečenje.

Pronađeno je ukupno dvadeset radova koji su ispunili pomenute kriterijume – osam preglednih radova, sedam originalnih radova, četiri prikaza slučaja i jedna monografija.

Budući da kliničke manifestacije periferne neuropatije nisu specifične, veoma je važno da se rano otkriju kako bi se sprečilo njihovo napredovanje i postigao uspeh u njihovom lečenju.

Acta Medica Medianae 2025; 64(2):137–144.

Ključne reči: periferna neuropatija, hronična bubrežna insuficijencija, hemodijaliza, klinička slika, lečenje

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**PERCUTANEOUS NEPHROSTOMY IN OBSTRUCTIVE UROPATHY:
COMPLICATIONS AND FEASIBILITY ANALYSIS OF OUTPATIENT
SURGERY UNDER LOCAL ANESTHESIA (Vol. 64, No. 1, p. 92–98,
2025)**

Editorial Board of the journal Acta Medica Medianae

Abstract

This erratum is issued because there was an oversight, and the type of the article was wrongly stated. First author, Bojan Vučković, noticed this mistake. The mistake occurred during the application process, when the author incorrectly selected the type of paper. We are very grateful to him for noticing this mistake, and we sincerely apologise for this error.

Acta Medica Medianae 2025; 64(2):145–146.

In the article "Percutaneous Nephrostomy in Obstructive Uropathy: Complications and Feasibility Analysis of Outpatient Surgery Under Local Anesthesia", authored by Bojan Vučković, with contributions of co-authors Bratislav Vasiljević, and Petar Vesović, published in Volume 64, Issue 1 (March issue), pages 92–98, the type of paper was wrongly stated. The article was issued as a review article by mistake. The article is an original and should be noted as such.

DOI of the original article: 10.5633/amm.2025.0112

Erratum DOI: 10.5633/amm.2025.0218

**PERKUTANA NEFROSTOMIJA U OPSTRUKTIVNOJ UROPATIJU:
ANALIZA IZVODLJIVOSTI OPERACIJE I KOMPLIKACIJA U
JEDNODNEVNOJ HIRURGIJI I LOKALNOJ ANESTEZIJI
(Vol. 64, br. 1, str. 92–98, 2025)**

Uređivački odbor časopisa Acta Medica Medianae

Apstrakt

Izdajemo ovaj erratum zbog greške koja je nastala u procesu tehničkog uređivanja časopisa koja se odnosi na vrstu rada. Naime, autor je pogrešno zaveo tip dokumenta prilikom prijave rada. Grešku je primetio prvi autor rada, Bojan Vučković, na čemu smo mu jako zahvalni i ovom prilikom izražavamo žaljenje zbog propusta.

Acta Medica Medianae 2025; 64(2):145–146.

U radu *Perkutana nefrostomija u opstruktivnoj uropatiji: analiza izvodljivosti operacije i komplikacija u jednodnevnoj hirurgiji i lokalnoj anesteziji* autora Bojana Vučkovića i koautora Bratislava Vasiljevića i Petra Vesovića objavljenog u časopisu *Acta Medica Medianae*, Volumen 64, broj 1 (martovski broj), strane 92–98, pogrešno je zavedena vrsta dokumenta. U radu stoji da je reč o preglednom radu, a vrsta dokumenta koja bi trebalo da bude jeste originalni rad.

DOI originalno rada: 10.5633/amm.2025.0112

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