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Evaluation of Clinical Response in Patients with Inflammatory Bowel Disease on Biological Therapy

Evaluacija kliničkog odgovora kod pacijenata sa upalnim bolestima crijeva na biološkoj terapiji

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ABSTRACT

Introduction: the etiology of inflammatory bowel diseases is not fully understood, but it is believed to result from a combination of genetic factors, immune dysregulation, environmental influences and dysbiosis of the gut microbiota. These diseases have a chronic and unpredictable clinical course, making treatment complex. The introduction of biological therapy has enabled effective disease control and favorable clinical response in a significant number of patients. Clinical response to treatment is assessed using standardized disease activity scores, such as the Harvey-Bradshaw Index (HBI) for Crohn's disease and the partial Mayo score for ulcerative colitis. Aim: to describe the sociodemographic and clinical characteristics of patients with inflammatory bowel disease. To compare the values of clinical scores - the Mayo score in UC patients and the HBI score in CD patients at weeks 8, 26 and 52 following initiation of biological therapy. Materials and methods: this cross-sectional clinical study was conducted at the Clinic of Gastroenterology of the Clinical Center University of Sarajevo, from January 2007 to March 2025. A total of 150 patients with UC and CD receiving biological therapy were included. The partial Mayo score was calculated for UC patients, and the HBI index for CD patients, at 8, 26 and 52 weeks after initiation of therapy. Results: the average age at diagnosis of IBD was 29 years, with a standard deviation of 11.3 years, and a higher prevalence in males (54%) compared to females (46%). There was no statistically significant difference in Mayo score values between baseline (week 0, treatment initiation) and weeks 8, 26, and 52 in UC patients, nor within patient subgroups treated with specific biological agents. A statistically significant decrease in HBI score values was observed at weeks 8, 26, and 52 compared with baseline in CD patients, including the subgroup treated with infliximab. Conclusion: the incidence of IBD is increasing, representing a growing global health concern. The Mayo score and HBI score are useful non-invasive markers for evaluating disease activity. A statistically significant decrease in HBI index values was observed during biological treatment in CD patients. Disease control without dose or frequency optimization was achieved in 44.2% of UC patients and 59.2% of CD patients. The most common reasons for therapy modification were active disease and the development of anti-drug antibodies.

Keywords: inflammatory bowel disease, biological therapy, colonoscopy, Mayo score, HBI index.

SAŽETAK

Uvod: etiologija upalnih bolesti crijeva (IBD) nije u potpunosti razjašnjena, ali se vjeruje da proizlazi iz kombinacije genetskih faktora, imunološke disregulacije, faktora okoliša i disbioze crijevne mikrobiote. Ove bolesti imaju hroničan i nepredvidiv klinički tok, što liječenje čini složenim. Uvođenje biološke terapije omogućilo je efikasnu kontrolu bolesti i povoljan klinički odgovor kod značajnog broja pacijenata. Klinički odgovor na liječenje procjenjuje se pomoću standardizovanih bodovnih sistema za aktivnost bolesti, kao što su Harvey-Bradshaw indeks (HBI) za Crohnovu bolest i parcijalni Mayo skor za ulcerozni kolitis. Cilj: opisati sociodemografske i kliničke karakteristike pacijenata sa upalnom bolešću crijeva. Uporediti vrijednosti kliničkih skorova - Mayo skora kod pacijenata sa UC i HBI skora kod pacijenata sa CD u 8., 26. i 52. sedmici nakon uvođenja biološke terapije. Materijali i metode: ova klinička studija presjeka provedena je na Klinici za gastroenterologiju, Kliničkog centra Univerziteta u Sarajevu, u periodu od januara 2007 do marta 2025. godine. Uključeno je ukupno 150 pacijenata sa UC i CD koji primaju biološku terapiju. Parcijalni Mayo skor izračunavan je za pacijente sa UC, a HBI indeks za pacijente sa CD, u 8., 26. i 52. sedmici nakon početka terapije. Rezultati: prosječna starost pri postavljanju dijagnoze IBD-a bila je 29 godina, sa standardnom devijacijom od 11,3 godine, uz veću prevalenciju kod muškaraca (54%) u odnosu na žene (46%). Nije zabilježena statistički značajna razlika u vrijednostima Mayo skora između početne tačke (nulta sedmica, početak liječenja) i 8., 26. i 52. sedmice kod pacijenata sa UC, kao ni unutar podgrupa pacijenata liječenih specifičnim biološkim agensima. Statistički značajan pad vrijednosti HBI skora zabilježen je u 8., 26. i 52. sedmici u poređenju sa početnim vrijednostima kod pacijenata sa CD, uključujući i podgrupu liječenu infliksimabom. Zaključak: incidenca IBD-a je u porastu, što predstavlja rastući globalni zdravstveni problem. Mayo skor i HBI skor su korisni neinvazivni markeri za procjenu aktivnosti bolesti. Statistički značajan pad vrijednosti HBI indeksa uočen je tokom biološkog tretmana kod pacijenata sa CD. Kontrola bolesti bez optimizacije doze ili učestalosti postignuta je kod 44,2% pacijenata sa UC i 59,2% pacijenata sa CD. Najčešći razlozi za modifikaciju terapije bili su aktivna bolest i razvoj antitijela na lijek.

Ključne riječi: supalna bolest crijeva, biološka terapija, kolonoskopija, Mayo skor, HBI indeksateroskleroza

INTRODUCTION

Inflammatory bowel diseases (IBD) include patients with ulcerative colitis (UC) and Crohn's disease (CD), while 5–10% of patients present with an indeterminate form of IBD. The diagnosis is established based on medical history, physical examination, laboratory tests, radiological imaging, and endoscopic evaluation. The gold standard for establishing the diagnosis includes endoscopic procedures, mucosal biopsies of the colon and histopathological verification. The incidence and prevalence of IBD have markedly increased during the second half of the 20th century, and since the beginning of the 21st century, IBD has been considered one of the most widespread gastrointestinal diseases (1).

It is estimated that IBD affects 2.6 million people in Europe and 1.2 million in North America. The disease often begins in adolescence, and 25% of patients with IBD are younger than 20 years (2). The exact cause of inflammatory bowel diseases remains unknown. These conditions belong to the group of autoimmune diseases, characterized by an abnormal immune response to specific luminal antigens in the intestine. Infections with certain pathogens (viruses, E. coli, mycobacteria, Clostridium), as well as stressful lifestyle factors may contribute to the development of IBD. Changes in the intestinal microbiota and dysbiosis are closely associated with the onset and progression of IBD, although it remains unclear whether dysbiosis is a primary or secondary event. Fetal ischemia during childbirth, duration of breastfeeding, antibiotic exposure during childhood and adolescence are also considered potential risk factors for the development of IBD. Air pollution, a consequence of progressive environmental contamination represents another factor associated with IBD, as pollutants influence mucosal defense mechanisms and stimulate immune activation (3,4). In recent decades, research has focused on determining genetic predisposition in patients with IBD. Advances in sequencing technologies have enabled detailed genomic screening. To date, 242 genetic determinants associated with the development of IBD have been identified (5).

A large number of biological agents are now available for the treatment of IBD; however, many patients still fail to respond adequately. Therefore, selecting the appropriate first-line therapy is essential. The choice is based on assessments of clinical severity, serological markers, and endoscopic findings. Equally important is monitoring clinical response after initiating biological therapy, for which the use of serological and non-invasive parameters has become increasingly emphasized. Assessment of the effectiveness of these parameters in monitoring disease activity and timely therapy optimization has been the subject of numerous studies (6).

Over the past several decades, multiple biological agents and small molecules with different mechanisms of action have been approved for the treatment of moderate to severe UC and CD. In Bosnia and Herzegovina, three groups of medications are approved:

- anti-TNF agents (infliximab and adalimumab),
- anti-integrin therapy (vedolizumab), and
- JAK inhibitor (tofacitinib).

The most common reasons for switching biological therapy include inadequate therapeutic response despite optimization (increasing the dose or shortening the interval), development of anti-drug antibodies or the occurrence of adverse effects. The rate of anti-drug antibody formation is low, approximately 5% within the first six years of treatment.

Anti-TNF agents are typically used as first-line biological therapy in clinical practice, and nearly all key induction trials have included patients both with and without prior exposure to anti-TNF therapy. Studies have shown that adalimumab and vedolizumab achieve lower rates of clinical remission as second-line agents (after infliximab therapy) in patients with UC and CD when compared to tofacitinib, ustekinumab, and upadacitinib.

Conversely, prior vedolizumab therapy does not significantly reduce the effectiveness of anti-TNF treatment, suggesting that vedolizumab may be a suitable option as a first-line biological agent. Since the incidence of adverse events remains low across all treatment classes, the harm resulting from untreated or poorly managed disease far outweighs the risks associated with any single therapy. Regardless of treatment sequencing, careful monitoring of treatment response and timely assessment of the need to modify biological therapy are essential for effective management of IBD (7).

Use of Clinical Scores to Measure Treatment Response

For patients with UC, the Mayo score was developed as a disease activity index for use in clinical trials. It includes components reported by patients stool frequency and rectal bleeding and components assessed by clinicians endoscopic appearance of the mucosa and physician's global assessment (8).

The partial Mayo score (pMS) is used to assess disease activity in ulcerative colitis. This score focuses on clinical symptoms and does not include the endoscopic mucosal assessment

Table 1 **Partial Mayo score.**

Category	0 point	1 point	2 points	3 points
Frequency	Normal or decreased	1-2 stool/day more than normal	3-4 stool/day more than normal	>5 stool/day more than normal
Rectal bleeding	none	Visible blood with stool less than half the time	Visible blood with stool half of the time or more	Passing blood alone
General condition of patient	Normal	Mild disease	Moderate disease	Severe d isease

0 - 1 score remission; 2 - 4 scores mild illness; 5 – 7 scores moderate illness; 8 - 9 scores severe illness

The Harvey-Bradshaw Index (HBI) for patients with Crohn's disease consists of several questions that allow physicians to quickly categorize the severity of Crohn's disease and determine remission (9).

Table 2 **Harvey-Bradshaw Index (HBI).**

Criteria	Scores
General condition of patient	0 (very well), 1 (slightly below average), 2 (poor), 3 (very poor), 4 (terrible)
Abdominal pain (frequency and intensity)	0 (none), 1 (mild), 2 (moderate), 3 (severe)
Number of liquid stools in last 24h	Number of liquid stools (4 stools = 4 points)
Palpable abdominal mass	0 (no), 1 (dubious), 2 (definite), 3 (definite with tenderness)
Complications of Crohn´s disease (1 point for every complication)	0 (none) 1 (1 point for every complication -arthralgia, uveitis, erythema nodosum, pyoderma gangrenosum,aphthous ulcers,anal fissure, fistula, abscesses

HBI < 4 points clinical remission (disease is inactive); 4 - 7 points mild disease activity; 8 - 16 points moderate disease activity; > 16 points severe disease activity.

MATERIALSAND METHODS

The study was conducted as a cross-sectional clinical study at the Clinic of Gastroenterology and Hepatology of the Clinical Center University of Sarajevo (CCUS).The study included 150 patients over 18 years of age, diagnosed with ulcerative colitis (UC) or Crohn's disease (CD), who underwent biological therapy in the last 17 years.

Inclusion criteria: patients older than 18 years, diagnosed with UC or CD, and receiving biological therapy for 12 months or longer from the start of the study.

Exclusion criteria: patients under 18 years at the time of the study, IBD patients not receiving biological therapy, and IBD patients who were initially prescribed biological therapy but discontinued it within less than 12 months due to inadequate response or adverse effects.

Data from electronic health records (BIS) were organized into a tabular database using Microsoft Excel (Microsoft Corporation, Redmond, USA) and analyzed using the statistical software Jamovi (The Jamovi Project, jamovi.org).

Categorical variables were expressed as absolute and relative frequencies, while numerical variables were summarized as mean and standard deviation, and when appropriate, as range, median and interquartile range.

For comparison of categorical variables, the x^2 test was applied. For comparison of longitudinal measurements that did not follow the assumptions of normal distribution, the Friedman test for repeated measures was used. In cases where the Friedman test for repeated measures was statistically significant, a post-hoc analysis using the Durbin-Conover test was performed to determine differences between time points.

The statistical significance level was set at $\alpha = 0.05$ and p-values below this threshold were considered statistically significant.

RESULTS

The study included 150 participants treated in the period between January 2007 and March 2025. Out of the total of 150 participants included in the study, 98 (65.3%) were patients with Crohn's disease, and 52 (34.7%) were patients with ulcerative colitis. The mean age at diagnosis was 29 years, with a standard deviation of 11.3 years. This value was 28.5 years in patients with Crohn's disease and 29.9 years in patients with ulcerative colitis.

Table 3 **Age distribution of patients.**

Diagnosis	Average	Median	SD	Min	Max
Crohn`s disease	28.5	26.0	10.6	10	60
Ulcerative colitis	29.9	28.0	12.4	13	68
Total	29.0	27.0	11.3	10	68

The study included a total of 81 (54%) male participants and 69 (46%) female participant. Among patients with ulcerative colitis, the most frequently used drug in the initial biological therapy was infliximab (29 patients, 55.8%), with the most commonly administered dose being 400 mg (25.0%). This was followed by adalimumab and vedolizumab, each used in 9 patients (17.3%). Tofacitinib was administered to 5 patients (9.6%), while infliximab at a dose of 200 mg was used in only one patient (1.9%) (Table 4a).

Table 4a **Initial biological therapy in patients with ulcerative colitis- drug type, dose, and frequency.**

Drug	Frequency	Dose (mg)	Number of patients	Relative frequency
Adalimumab	2 weeks	40	9	17.3%
Infliximab	8 weeks	200	1	1.9%
		300	11	21.2%
		400	13	25.0%
		500	4	7.7%
Vedolizumab	8 weeks	300	9	17.3%
Tofacitinib	2x/day	2x5	5	9.6%

Among patients with Crohn's disease, adalimumab was the most frequently administered drug (45 patients, 45.9%). Infliximab was administered to a total of 45 patients (45.9%), with doses ranging from 200 mg to 800 mg, most commonly 300 mg and 400 mg (15 patients each, 15.3%). Vedolizumab was initially administered to 8 patients (8.2%) (Table 4b).

Table 4b **Initial biologic therapy in patients with Crohn's disease- drug type, dose, and frequency.**

Drug	Frequency	Dosage (mg)	Number of patients	Relative frequency
Adalimumab	2 weeks	40	45	45.9%
Infliximab	8 weeks	200	1	1.0%
		300	15	15.3%
		400	15	15.3%
		500	7	7.1%
		600	1	1.0%
		700	5	5.1%
		800	1	1.0%
Vedolizumab	8 weeks	300	8	8.2%

At the beginning of biological therapy, the mean Mayo score was 2.28 (SD 2.76), which decreased to 1.69 (SD 2.19) after 8 weeks (Table 5a). By week 26, the Mayo score further decreased to 1.66 (SD 2.06), while at week 52, the mean Mayo score was 1.22 (SD 1.24). By the end of follow-up (week 52), the highest recorded Mayo score was 4.

Table 5a **Mayo score values in patients with ulcerative colitis from the initiation of biological therapy.**

Time since the start of treatment	N	Without control *	Average	95% CI	SD	Min	Max
Mayo score (0 week)	50	2	2.28	1.50-3.06	2.76	0	9
Mayo s core (8 weeks)	48	4	1.69	1.05-2.32	2.19	0	9
Mayo score (26 weeks)**	44	8	1.66	1.03-2.28	2.06	0	9
Mayo s core (52 weeks)**	36	16	1.22	0.80-1.64	1.24	0	4

*The Mayo score was not assessed due to loss to follow-up or due to a change from the initial treatment to another drug.
**Mayo score assessments include patients who underwent dose optimization.

There was no statistically significant difference in Mayo score values between week 0 (treatment initiation) and weeks 8, 26, and 52 (Friedman test, $x^2 = 4.72$, df = 3, p = 0.193).

The Harvey-Bradshaw Index (HBI) values in patients with Crohn's disease are presented in Table 5b. At the start of therapy (week 0), the mean HBI was 2.18 (SD 2.33), decreasing to 1.70 (SD 1.91) after 8 weeks. At week 26, a slight increase in the mean HBI to 1.95 (SD 2.96) was observed, followed by a reduction to 1.50 (SD 1.93) at week 52. The range of HBI values was widest at the start of therapy (0–14) and at week 26 (0–15), while at week 8 no HBI value greater than 7 was recorded in any participant.

Table 5b **Harvey-Bradshaw Index values in patients with Crohn's disease from the initiation of biological therapy.**

Time since the start of treatment	N	Without control *	Average	95% CI	SD	Min	Max
HBI (0 week)	96	2	2.18	1.71-2.65	2.33	0	14
HBI (8 weeks)	94	4	1.70	1.31-2.09	1.91	0	7
HBI (26 weeks)**	86	12	1.95	1.32-2.59	2.96	0	15
HBI (52 weeks)**	72	26	1.50	1.05-1.95	1.93	0	9

*The HBI was not assessed due to loss to follow-up or due to a change from the initial treatment to another drug.
**HBI assessments include patients who underwent dose optimization.

Table 6 **Post-hoc test (Durbin-Conover) for comparison of HBI values between different measurements.**

Time since the start of treatment	0 week		8th week		26th week	
	D-C test	p	D-C test	p	D-C test	p
52nd week	2.97	**0.004**	0.086	0.931	0.90	0.367
26th week	3.78	**<0.001**	0.81	0.414		
8th week	2.97	**0.003**				

The analysis revealed a statistically significant reduction in HBI at weeks 8, 26, and 52 compared to baseline (week 0) (p = 0.003, p < 0.001, and p = 0.004, respectively), indicating a rapid and sustained positive response to therapy. However, there were no statistically significant differences in HBI values between weeks 8, 26, and 52 (all p-values > 0.05). Following the initial improvement, clinical disease activity remained stable at a lower level throughout the follow-up period.

Table 7 **Mayo score by type of initial biological therapy and time of measurement after initiation.**

	0 week	8th week	26th week	52th week	p
Adalimumab	2.55 (0.79 -4.32)	2.44 (0.95-3.94)	0.86 (0.27-1.98)	1.14 (0.21-2.50)	0.26
Infliximab	2.38 (1.29-3.49)	1.63 (0.80-2.45)	2.08 (1.30-2.45)	1.43 (0.92-1.94)	0.58
Vedolizumab	2.38 (0.34-5.09)	1.75 (0.84-4.38)	1.75 (0.84-4.38)	0.5 (0.78-1.79)	0.39
Tofacitinib	0.75 (0.16-3.14)	0.25 (0.05-1.05)	0.25 (0.05-1.05)	1.5 (0.12-2.18)	0.38

*Values are presented as mean (95% CI)

Table 7 presents the mean values and confidence intervals at each measurement, stratified by type of biological therapy. Statistical testing using the Friedman test for repeated measures revealed no statistically significant differences between the groups over time (p = 0.26; p = 0.58; p = 0.39; p = 0.38).

Table 8 **Harvey-Bradshaw Index according to the type of initial biological therapy and measurement time after initiation.**

	0 week	8th week	26th week	52nd week	p
Adalimumab	2.14 (1.47-2.81)	1.56 (0.99-2.13)	2.26 (0.97-3.54)	1.66 (0.89-2.42)	0.135
Infliximab	2.29 (1.50-3.08)	1.82 (1.19-2.45)	1.81 (1.10-2.53)	1.35 (0.69-2.01)	**0.002**
Vedolizumab	1.75 (1.01-2.49)	1.75 (0.43-3.07)	1.38 (0.46-3.21)	1.67 (0.40-2.94)	0.528

*Values are presented as mean (95% CI)

In the group of patients with Crohn's disease, those treated with infliximab showed a statistically significant reduction in HBI between baseline and measurements at weeks 8, 26, and 52 ($p = 0.002$), indicating sustained clinical improvement. Changes in HBI for adalimumab ($p = 0.135$) and vedolizumab ($p = 0.528$) were not statistically significant.

DISCUSSION

Our study shows that out of the total of 150 included participants, 98 (65.3%) were patients with Crohn's disease, and 52 (34.7%) were patients with ulcerative colitis. The ratio of patients receiving biological therapy who met the inclusion criteria was therefore approximately 2:1 in favor of Crohn's disease. The mean age at diagnosis was 29 years, with a standard deviation of 11.3 years. This value was 28.5 years in patients with Crohn's disease and 29.9 years in patients with ulcerative colitis. The youngest age at diagnosis was 10 years in a patient with Crohn's disease and 13 years in a patient with ulcerative colitis. The oldest age at diagnosis was 60 years in patients with Crohn's disease and 68 years in patients with ulcerative colitis. The study included a total of 81 (54%) male participants and 69 (46%) female participants. There were 69 (46%) female participants, with no statistically significant difference in the Crohn's disease group (45.9% women and 54.1% men), as well as in the ulcerative colitis group (46.2% women and 53.8% men). A study by Zaltman C, et al. examined the sociodemographic characteristics of patients with IBD in Brazil and found that of the total 407 patients included, 264 had CD and 143 had UC, with a CD:UC ratio of 2:1, consistent with the results of our study. A slightly higher percentage of female patients was observed (54.2% CD, 56.6% UC) compared to our study, but without a statistically significant difference. The mean age was 45.9 ± 13.8 years (CD) and 42.9 ± 13.0 years (UC), with average disease duration at the time of the cross-sectional study of 10 years (10).

Our study demonstrates a rising incidence of IBD patients in the last decade, in line with a study conducted in 2019th, showing a global increase in IBD not only in Europe but also in North America, Asian countries, and Africa. Once considered a disease of Western civilizations, IBD is now a global health issue due to early disease onset, low mortality, and the high cost of biological therapy (11). Monitoring prevalence trends is important to ensure timely preparation for healthcare system burden, considering an aging population with comorbidities and longer disease duration.

Given the high prevalence of IBD, early recognition of inflammatory activity and rapid intervention, together with monitoring clinical response, form the foundation of a treat-to-target approach in ulcerative colitis. The STRIDE-II consensus highlighted that achieving histologic mucosal healing of the rectum and colon is a long-term goal for patients with ulcerative colitis. Total colonoscopy provides comprehensive information on the extent and severity of inflammatory activity in these patients. In recent decades, efforts have been made to identify an ideal non-invasive score that correlates with endoscopic and histologic findings, reducing the need for frequent invasive colonoscopies (12,13). The Mayo score has been the most widely used. A study conducted during 2012th, evaluating the efficacy of partial Mayo score in assessing clinical remission and it´s correlation with the total Mayo score, aimed at avoiding invasive endoscopic procedures, demonstrated that the total Mayo score can be accurately predicted from the partial Mayo score, allowing it to replace the total score in future clinical trials (14). Conversely, a study by Fluxá D, et al. showed that the partial and total Mayo scores correlate well with each other but only moderately correlate with histopathological results indicating histologic remission (15).

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A study by Kumei S, et al. investigated clinical outcomes in patients with refractory ulcerative colitis treated with adalimumab, who had previously shown inadequate response to conventional therapies or other anti-TNF agents. Clinical response was assessed using the Mayo score at weeks 8 and 52. The results showed that a lower partial Mayo score at week 8 was a significant predictor of clinical response at week 52 (16). The retrospective cohort study by Ferreira SC, et al. included 297 patients with UC, evaluating primary endpoints - clinical remission as partial Mayo score ≤2, endoscopic remission (Mayo sub-score=0), and steroid-free clinical remission at week 52 (17). Based on these studies, our study assessed Mayo score values in UC patients at weeks 8, 26, and 52.

In our study at the start of biological therapy, the mean Mayo score was 2.28 (SD 2.76), decreasing to 1.69 (SD 2.19) after 8 weeks. By week 26, the Mayo score further decreased to 1.66 (SD 2.06), and at week 52, the mean score was 1.22 (SD 1.24). By the end of follow-up (week 52), the highest recorded Mayo score was 4. There were no statistically significant differences in Mayo score values between week 0 (treatment initiation) and weeks 8, 26, and 52, nor when scores were analyzed individually according to the type of biological therapy.

The HBI score, analogous to the Mayo score in UC, is a reliable tool for monitoring clinical and therapeutic response in patients with Crohn's disease (18). A study by Ollech JE, et al. monitored HBI as a reference parameter of clinical response in patients who underwent interval shortening of ustekinumab therapy after standard regimens showed insufficient efficacy (19). Study conducted by Husman J, et al. investigated response to subcutaneous infliximab treatment of Crohn's disease patients with previous immunogenic failure of intravenous infliximab following values of HBI and fecal calprotectin (20).

In our study, a significant reduction in HBI was observed at weeks 8, 26, and 52 in patients with Crohn's disease compared to baseline (week 0) ($p = 0.003$, $p < 0.001$, and $p = 0.004$, respectively), indicating a rapid and sustained positive response to therapy. At the start of therapy (week 0), the mean HBI was 2.18 (SD 2.33), decreasing to 1.70 (SD 1.91) after 8 weeks. At week 26, a slight increase in the mean HBI to 1.95 (SD 2.96) was observed, followed by a reduction to 1.50 (SD 1.93) at week 52. The range of HBI values was widest at baseline (0–14) and at week 26 (0–15), while at week 8 no HBI value greater than 7 was recorded in any participant. No statistically significant differences in HBI values were observed between weeks 8, 26, and 52 (all p-values > 0.05). Following the initial improvement, clinical disease activity remained stable at a lower level throughout the follow-up period. When analyzed according to the type of biological therapy, patients with Crohn's disease treated with infliximab showed a statistically significant reduction in HBI between baseline and weeks 8, 26, and 52 ($p = 0.002$), indicating sustained clinical improvement. Changes in HBI for adalimumab ($p = 0.135$) and vedolizumab ($p = 0.528$) were not statistically significant.

In patients with ulcerative colitis, disease control without dose or frequency optimization was achieved in 44.2% of cases, whereas in Crohn's disease this proportion was 59.2%

CONCLUSION

Monitoring patients with inflammatory bowel disease (IBD) using non-invasive clinical scores, such as the partial Mayo score for ulcerative colitis and the Harvey-Bradshaw Index for Crohn's disease, is highly valuable. These tools allow physicians to regularly evaluate current disease activity in a simple and rapid manner. Regular assessment is particularly critical for patients receiving biological therapy. Continuous monitoring enables early detection of signs of impending relapse or identification of patients who do not respond adequately to the biological agent. Rapid detection of these conditions is essential for timely intervention, therapy adjustment and optimization of long-term treatment outcomes.

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Double Immunoreactive Trypsinogen as a Newborn Cystic Fibrosis Screening Method - Results of a One-Year Pilot Study at the Clinical Centre University of Sarajevo

Dvostruki imunoreaktivni tripsinogen kao metoda probira za cističnu fibrozu novorođenčadi - rezultati jednogodišnje pilot studije u Kliničkom centru Univerziteta u Sarajevu

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ABSTRACT

Introduction: cystic fibrosis (CF) is one of the most frequent life-limiting autosomal recessive diseases among Caucasians. Early detection through newborn screening (NBS) allows timely intervention, improved nutrition, and prolonged survival. Aim: to evaluate the analytical performance of the immunoreactive trypsinogen - immunoreactive trypsinogen (IRT-IRT) protocol implemented at the Clinical Center University of Sarajevo (CCUS) and to define local reference values as a basis for future integration of the immunoreactive trypsinogen - pancreatitis associated protein - DNA (IRT-PAP-DNA) model in accordance with European guidelines. Materials and methods: CF NBS (Cystic Fibrosis Newborn Screening) was performed using a two-tier immunoreactive trypsinogen protocol (IRT–IRT). In this method, immunoreactive trypsinogen (IRT) was measured twice from dried blood spots. Persistent elevation indicated increased risk for CF and required confirmatory sweat chloride testing and/or CF Transmembrane Conductance Regulator (CFTR) genotyping. Cut-off values were defined as IRT I ≥70 ng/mL and IRT II ≥40 ng/mL. Infants with sweat chloride >30 mmol/L underwent CFTR genetic testing. Statistical analysis followed ECFS-NSWG standards. Results: in the period from 14 October 2024 to 13 October 2025, 4,567 newborns (51.3% male), from the Sarajevo Canton were screened using the IRT-IRT protocol. Among 4,567 screened newborns, 58 (1.27%) had elevated IRT I, and 23 (0.50%) had elevated IRT II. Ten infants had borderline sweat chloride (30-60 mmol/L), all genetically negative. Mean IRT I was 88.96 ng/mL (SD 15.64) and mean IRT II 33.58 ng/mL (SD 18.18). The specificity of the first IRT measurement was 98.8%, and 99.5% for the second-tier IRT test. No confirmed CF cases were identified; therefore, sensitivity and PPV could not be determined. The recall rate of 0.5% corresponds to the European CF screening standard (<1%). Conclusion: the pilot program demonstrated feasibility and analytical consistency of CF NBS within the regional organization model. Positive predictive value (PPV) could not be calculated due to absence of confirmed cases. Future implementation of the IRT-PAP-DNA algorithm is recommended to enhance specificity and public health impact.

Keywords: cystic fibrosis, newborn screening, IRT–IRT protocol validation, PAP biomarker

SAŽETAK

Uvod: cistična fibroza (CF) je jedna od najčešćih autosomno recesivnih bolesti koje ograničavaju život kod bijele rase. Rano otkrivanje putem neonatalnog skrininga (eng. newborn screening, NBS) omogućava pravovremenu intervenciju, poboljšanu ishranu i produženo preživljavanje. Cilj: procijeniti učinkovitost imunoreaktivni tripsinogen - imunoreaktivni tripsinogen (IRT-IRT) protokola tokom prve godine implementacije na Kliničkom Centru Univerziteta u Sarajevu (KCUS) i definirati lokalne referentne vrijednosti kao osnovu za buduću integracijumodela imunoreaktivni tripsinogen - pankreatitis asocirani protein - DNA (IRT-PAP-DNA) u skladu s evropskim smjernicama. Materijali i metode: skrining novorođenčadi na cističnu fibrozu je proveden korištenjem dvostepenog imunoreaktivnog tripsinogenskog protokola (IRT-IRT). Kod ove metode, imunoreaktivni tripsinogen (IRT) se mjeri dva puta iz osušenih kapljica krvi. Perzistentno povišenje ukazuje na povećan rizik za CF i zahtijeva potvrdno testiranje hlorida u znoju i/ili genotipizaciju CF transmembranskog regulatora provodljivosti (CFTR). Granične vrijednosti su definirane kao IRT I ≥70 ng/mL i IRT II ≥40 ng/mL. Dojenčad sa hloridom u znoju >30 mmol/L podvrgnuta je genetskom testiranju na CFTR. Statistička analiza je uslijedila u skladu sa standardima ECFS-NSWG. Rezultati: u periodu od 14. oktobra 2024. do 13. oktobra 2025. godine, 4.567 novorođenčadi (51,3% muških) iz Kantona Sarajevo su pregledani korištenjem IRT-IRT protokola. Od 4.567 pregledanih novorođenčadi, 58 (1,27%) imalo je povišen IRT 1, a 23 (0,50%) povišen IRT 2. Desetero dojenčadi imalo je granični nivo hlorida u znoju (30–60 mmol/L), svi su imali negativan genetski test na cističnu fibrozu. Prosječni IRT 1 bio je 88,96 ng/mL (SD 15,64), a prosječni IRT 2 bio je 33,58 ng/mL (SD 18,18). Specifičnost prvog mjerenja IRT-a bila je 98,8%, a 99,5% za IRT test drugog nivoa. S obzirom da nisu identificirani potvrđeni slučajevi cistične fibroze; osjetljivost i pozitivna prediktivna vrijednost (PPV) testa se nisu mogli utvrditi. Stopa ponavljanja IRT testa od 0,5% odgovara evropskom standardu za skrining cistične fibroze (<1%). Zaključak: IRT–IRT protokol pokazao je tehničku izvodljivost i usklađenost s evropskim standardima. PPV nije bilo moguće izračunati zbog odsustva potvrđenih slučajeva. Planirana je nadogradnja protokola uvođenjem PAP biomarkera radi povećanja specifičnosti, smanjenja lažno pozitivnih nalaza i unaprjeđenja javnozdravstvenog značaja skrininga.

Ključne riječi: cistična fibroza, novorođenački probir, validacija IRT-IRT protokola, PAP biomarker

INTRODUCTION

Newborn screening (NBS) represents a major achievement of modern preventive medicine and public health. It enables early detection of congenital and metabolic disorders, preventing irreversible damage and reducing neonatal mortality (1,2).

In Bosnia and Herzegovina, NBS is regionally organized with three coordinating centers: Sarajevo, Tuzla, and Banja Luka. The established national program currently includes congenital hypothyroidism and phenylketonuria, while this project introduced cystic fibrosis screening for the first time in the Sarajevo Canton.

Cystic fibrosis (CF) is caused by pathogenic variants in the CF Transmembrane Conductance Regulator (CFTR) gene and affects multiple organ systems. The incidence in Europe ranges from 1:2,000 to 1:10,000 live births (3,4). Early diagnosis leads to improved pulmonary outcomes, nutritional status, and survival (5,6). The European Cystic Fibrosis Society Neonatal Screening Working Group (ECFS-NSWG) has established harmonized protocols and outcome indicators for all European countries (7).

The two-tier immunoreactive trypsinogen (IRT-IRT) protocol is the most commonly used biochemical approach for cystic fibrosis (CF) newborn screening in Europe. It is based on sequential measurement of immunoreactive trypsinogen (IRT) from dried blood spots, first at 48-72 hours of life and again after 2-3 weeks in infants with an initially elevated value. Persistent elevation suggests increased likelihood of CF and warrants confirmatory diagnostic procedures, most commonly sweat chloride testing and CFTR mutation analysis (1,3,7).

AIM

The aim of this study was to evaluate the analytical and clinical performance of the IRT-IRT protocol during its first year of implementation at the CCUS, establish local reference ranges, and identify prospects for future integration of the IRT–PAP–DNA model consistent with European practice (8-10).

MATERIALS AND METHODS

Study Design and Population

In the period from 14 October 2024 to 13 October 2025, 4,567 newborns from Sarajevo Canton were screened. Blood samples were collected 48-72 hours postpartum using dried blood spots and analyzed by DELFIA time-resolved fluorometric immunoassay (PerkinElmer) (6,11).

Screening Algorithm and Cut-Off Values

The two-step IRT–IRT screening algorithm was applied:

- IRT I ≥70 ng/mL → positive result → IRT II
- IRT 2 ≥40 ng/mL → persistent elevation → sweat chloride testing
- Sweat chloride >30 mmol/L → borderline → CFTR genetics
- Sweat chloride ≥60 mmol/L → diagnostic for CF [1]

Follow-Up and Genetic Testing

Infants with borderline sweat chloride (30-60 mmol/L) underwent CFTR gene analysis. None carried pathogenic variants, confirming all as false-positive biochemical results.

Statistical Analysis

Descriptive and comparative statistics were performed using ECFS reference intervals (7). Data were expressed as mean, SD, median, range, and frequency distribution.

Ethical approval

The Ethical Committee of the CCUS gave approval for the study (Approval no. 45-1-46208/2024/2024).

RESULTS

A total of 4,567 newborns were screened (2,342 male (51.28%), 2,225 female (48.72%)). Among 4,567 screened newborns, 58 (1.27%) had elevated IRT 1, and 23 (0.50%) had elevated IRT 2. Ten infants had borderline sweat chloride (30-60 mmol/L), all genetically negative (Table 1.)

Table 1 **Summary of screening results.**

Parameter	Number	% of total (n=4567)
IRT 1 ≥70 ng/mL	58	1.27%
IRT 2 ≥40 ng/mL	23	0.50%
Sweat chloride 30 –60 mmol/L	10	0.22%
Confirmed CF cases	0	0%

Distribution of IRT 1 and IRT 2 values

IRT 1 ranged from 70.8-144 ng/mL (mean 88.96 ±15.64 SD, median 85). The distribution was right-skewed: 67% in the 70-99 ng/mL interval, 24% in 100-129, and 9% ≥130.

IRT 2 ranged from 12.6-120 ng/mL (mean 33.58 ±18.18 SD, median 28.6). 61% were <35 ng/mL, and only three infants exceeded 100 ng/mL.

Table 2 Descriptive statistics of IRT 1 and IRT 2.

Parameter	Mean	Min	Max	SD	Median
IRT 1 (ng/mL)	88.96	70.80	144.00	15.64	85.00
IRT 2 (ng/mL)	33.58	12.60	120.00	18.18	28.60

IThe IRT I and IRT II test results comparison is presented in Figure 1.

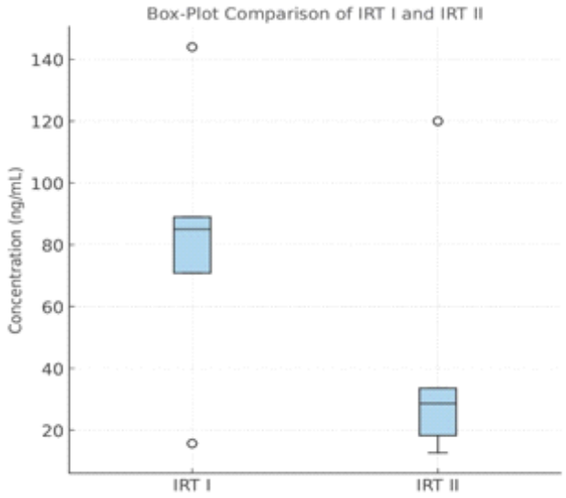

Figure 1 **Box-plot comparison of IRT 1 and IRT 2.**

The average value of IRT 1 (88.96 ng/mL) was significantly higher than IRT 2 value (33.58 ng/mL), which was consistent with the known postnatal decline of immunoreactive trypsinogen and supports the biological validity of the two-tier IRT-IRT protocol (Figure 2).

Two infants (8.7% of those recalled) had IRT 2 > IRT 1, which was consistent with known assay and biological variability. The mean inter-tier difference (55.4 ng/mL) reflected expected physiological decline in IRT concentration over time (9,12).

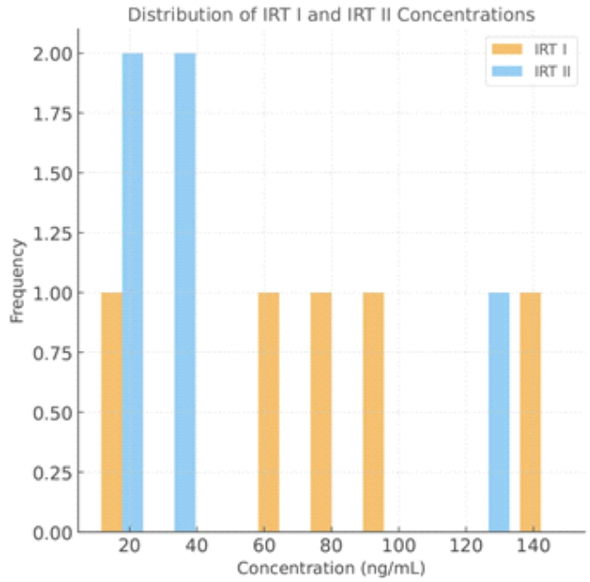

Figure 2 **Distribution of IRT 1 and IRT 2 concentrations.**

Sweat Chloride and Genetic Findings

Ten infants (0.22%) had borderline sweat chloride (30-60 mmol/L), all genetically negative. No infants had sweat chloride ≥60 mmol/L (Figure 3.)

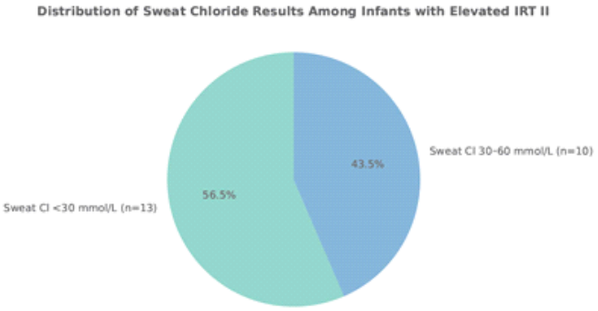

Figure 3 **Sweat chloride test results in infants with elevated IRT 2.**

Analytic Performance

The specificity of the first IRT measurement was 98.8%, and 99.5% for the second-tier IRT test. Sensitivity and positive predictive value could not be calculated due to the absence of true positive cases. The retesting rate was 0.5%, aligning with the ECFS-NSWG recommendations, which suggest an optimal recall rate below 1% in well-performing national programs.

These results confirm that our cut-offs (IRT 1 ≥70 ng/mL, IRT 2 ≥40 ng/mL) were appropriate and aligned with ECFS reference standards (1,7).

DISCUSSION

This first-year evaluation confirmed the feasibility, analytical reliability, and regional applicability of CF NBS within a decentralized system. The observed positivity rate (1.27%) and recall rate (0.50%) match European averages (7,9,13).

Neonatal CF screening is implemented in 26 European countries; with four of them using regional organization models similar to Bosnia and Herzegovina (1,8). Regional coordination enhances logistic continuity and enables centralized data analysis. The Sarajevo pilot was the first systematic evaluation of CF NBS performance in the Western Balkans.

Analytical performance of the DELFIA method proved excellent, with minimal inter-assay variation and reproducible calibration curves. The difference between IRT 1 and IRT 2 reflects physiological normalization of pancreatic enzyme secretion after the early neonatal period (10). A right-skewed IRT 1 distribution and more symmetrical IRT 2 distribution correspond to postnatal trypsinogen kinetics described in European cohort studies (9,14).

Specificity and sensitivity

High specificity is critical for minimizing unnecessary recalls and parental anxiety (5,9). The absence of confirmed cases limited the ability to calculate the positive predictive value (PPV). However, PPV depends strongly on prevalence and test architecture rather than procedural accuracy (2).

PPV, sensitivity, and specificity were defined as follows:

- Sensitivity = TP / (TP + FN)
- Specificity = TN / (TN + FP)
- PPV = TP / (TP + FP) (1,3,9).

The analytical specificity achieved in this cohort (98.8% for IRT 1 and 99.5% for IRT 2) is consistent with published European benchmarks, where specificity values range between 98–99% depending on the algorithm used (3,9,11). The observed retesting rate of 0.5% corresponds exactly to ECFS-NSWG recommendations (<1%), confirming adequate cut-off selection and analytical performance within international standards.

Comparison with European experience

Countries using IRT-IRT-DNA or IRT-PAP–DNA protocols report PPV between 5-10% (11,14). Integration of PAP, a pancreatic inflammation marker, can enhance specificity without reducing sensitivity (14). We plan to introduce PAP as a second-tier biomarker, aiming to transition to IRT-PAP-DNA screening.

Limitations of the study

This study has several limitations that should be considered when interpreting the results.

First, the study represents a one-year pilot program conducted in a single regional center. Although the Sarajevo Canton includes a large proportion of births in Bosnia and Herzegovina, the findings may not be fully applicable to the entire national population, particularly regions with different ethnic composition, birth rates, or healthcare access.

Second, no confirmed cystic fibrosis cases were identified during the study period. While this reflects the low prevalence of CF and is consistent with expectations for the screened population size, it precluded calculation of sensitivity and positive predictive value. As a result, the clinical performance of the screening algorithm could only be partially assessed, focusing mainly on specificity, recall rate, and analytical validity.

Third, the study relied exclusively on a biochemical IRT–IRT screening algorithm without incorporation of PAP or DNA analysis. Although this approach aligns with initial ECFS recommendations for pilot implementation, biochemical-only protocols are inherently limited by lower PPV compared to multi-tier strategies. The absence of PAP measurement may have contributed to borderline sweat chloride results and limits comparison with contemporary IRT–PAP–DNA protocols used in several European programs.

Fourth, CFTR genetic testing was restricted to infants with borderline sweat chloride values, and comprehensive genotyping was not performed in the entire recalled cohort. Therefore, the presence of rare CFTR variants with variable penetrance or CFTR-related metabolic syndrome (CFSPID) cannot be entirely excluded. Fifth, the study did not include long-term clinical follow-up of screen-negative infants. Consequently, late-presenting or atypical CF cases could not be evaluated, and false-negative rates could not be assessed.

Finally, pre-analytical variables such as timing of sample collection, transport conditions of dried blood spots, and postnatal factors influencing IRT levels (e.g., prematurity, perinatal stress) were not analyzed separately, although they may influence IRT distribution and screening outcomes.

Despite these limitations, the study provides the first structured evaluation of CF newborn screening in Bosnia and Herzegovina and establishes a robust foundation for national program expansion and future integration of PAP and DNA-based algorithms.

Recommendations

- Extend CF NBS to Tuzla and Banja Luka centers for national coverage.
- Introduce PAP measurement as a second-tier test to improve PPV.
- Establish centralized registry and continuous ECFS-NSWG collaboration.

CONCLUSION

The CCUS pilot program successfully demonstrated the feasibility, analytical robustness, and regional scalability of CF newborn screening. The findings validate the use of IRT-IRT thresholds and confirm the logistical readiness of the regional model. Although no cases were confirmed, the experience built laboratory capacity, trained staff, and established local quality indicators consistent with ECFS benchmarks. Future integration of the IRT-PAP-DNA model will enhance diagnostic precision and public health efficiency, ensuring early detection and equal access for all newborns nationwide.

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Analysis of Human Resource Management Practices and Managerial Competencies in Pharmaceutical Organizations in Montenegro

Analiza praksi upravljanja ljudskim resursima i menadžerskih kompetencija u farmaceutskim organizacijama Crne Gore

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ABSTRACT

Introduction: human resource management in contemporary pharmaceutical organizations is a key factor in ensuring the quality of healthcare services, professional development of employees, and overall organizational efficiency. The development of managerial competencies is particularly important, as it directly influences motivation, job satisfaction, and job performance of healthcare professionals. The aim of this study was to analyze human resource management practices and the level of managerial competencies in pharmaceutical organizations in Montenegro. Materials and methods: a descriptive, quantitative study was conducted among 82 employees working in ten pharmaceutical organizations. Data were collected using a structured questionnaire, and statistical analysis was performed using IBM SPSS Statistics version 26.0. Results: The majority of respondents were female (72%). The highest mean scores were recorded for customer orientation (4.46) and technological preparedness (4.29), while the lowest scores were related to employee reward systems (3.65). A statistically significant correlation was found between motivation and job satisfaction ($r = 0.71$; $p = 0.001$), as well as between the level of education and the perception of managerial competencies ($p = 0.022$). Conclusion: the findings indicate the importance of professional development and adequate motivational systems in strengthening managerial competencies and improving employee satisfaction in pharmaceutical organizations.

Keywords: human resource management, managerial competencies, pharmaceutical organizations, motivation, job satisfaction

SAŽETAK

Uvod: upravljanje ljudskim resursima u savremenim farma ceutskim organizacijama predstavlja ključni faktor u osiguravanju kvaliteta zdravstvenih usluga, profesionalnog razvoja zaposlenika i ukupne organizacijske efikasnosti. Razvoj menadžerskih kompetencija ima poseban značaj jer direktno utiče na motivaciju, zadovoljstvo i radni učinak zdravstvenih radnika. Cilj: analizirati prakse upravljanja ljudskim resursima i nivo menadžerskih kompetencija u farmaceutskim organizacijama Crne Gore. Materijali i metode: provedeno je deskriptivno-kvantitativno istraživanje među 82 zaposlena u deset farmaceutskih organizacija. Podaci su prikupljeni strukturiranim anketnim upitnikom, a statistička analiza provedena je u programu IBM SPSS Statistics 26.0. Rezultati: većinu ispitanika činile su žene (72%). Najviše prosječne ocjene zabilježene su za orijentaciju na klijente (4,46) i tehnološku opremljenost (4,29), dok su najniže ocjene dobili sistemi nagrađivanja (3,65). Utvrđena je statistički značajna povezanost između motivacije i zadovoljstva zaposlenika ($r = 0,71$; $p = 0,001$), kao i između nivoa obrazovanja i percepcije menadžerskih kompetencija ($p = 0,022$). Zaključak: rezultati potvrđuju značaj profesionalnog razvoja i adekvatnih motivacijskih sistema u jačanju menadžerskih kompetencija i unapređenju zadovoljstva zaposlenika u farmaceutskim organizacijama.

Ključne riječi: upravljanje ljudskim resursima, menadžerske kompetencije, farmaceutske organizacije, motivacija, zadovoljstvo zaposlenika

INTRODUCTION

Human resource management (HRM) in the healthcare sector represents a key mechanism for ensuring the quality, safety, and efficiency of healthcare services (1). Healthcare professionals are recognized as the most important resource of any healthcare organization, and effective workforce governance, competencies development, motivation, and job satisfaction play a crucial role in workforce well-being and overall system performance (2).

Contemporary human resource management approaches increasingly emphasize a shift from traditional administrative functions toward strategic workforce management, with a strong focus on competency development, performance management, and continuous professional education as drivers of organizational effectiveness and sustainability (1).

Healthcare systems in Western Balkan countries, including Montenegro, face persistent challenges such as shortages of qualified personnel, migration of healthcare workers, limited financial resources, and rising expectations of healthcare service users (3,4). These challenges are particularly pronounced in the pharmaceutical sector, which occupies a central position within the healthcare delivery chain.

At the operational level, pharmacists' counseling practices represent an important component of patient-centered pharmaceutical care (5). Beyond medication distribution, modern pharmaceutical organizations increasingly contribute to the quality and efficiency of healthcare services through organizational human resource management mechanisms, appropriate workforce planning, and the development of professional competencies, thereby supporting improved organizational performance and service quality (6).

Human resource management in pharmaceutical organizations in Montenegro requires continuous adaptation to contemporary technological and organizational trends, including digitalization, process standardization, professionalization of management, and alignment with international quality standards. Managers in this sector are expected to balance operational efficiency, ethical business practices, employee satisfaction, and long-term service sustainability (7,8).

Furthermore, the Healthcare 4.0 concept highlights the growing importance of digital solutions, innovative management models, and the development of new managerial competencies within healthcare organizations (9). Although numerous studies have examined human resource management practices and managerial competencies in the healthcare sector, evidence specifically addressing pharmaceutical organizations in Montenegro remains limited and fragmented (10). Therefore, there is a clear need for a systematic investigation of actual management practices, levels of managerial competencies, employee motivation, and job satisfaction within this sector.

AIM

The aim of this study was to analyze human resource manage ment practices and the level of managerial competencies in pharmaceutical organizations in Montenegro, as well as to examine their association with employee motivation and job satisfaction.

MATERIALS AND METHODS

Study design

A descriptive, quantitative, cross-sectional study involving human participants was conducted among employees of pharmaceutical organizations in Montenegro in 2024. The study aimed to analyze human resource management practices, the level of managerial competencies, and their association with employee motivation and job satisfaction.

Study setting and participants

The study was conducted using a survey-based approach among employees from ten pharmaceutical organizations in Montenegro. The study included 82 employees working in various pharmaceutical organizations. Participants were invited to participate via electronic mail and voluntarily completed an anonymous online questionnaire created using Google Forms.

Inclusion and exclusion criteria

Inclusion criteria were: (1) employment in a pharmaceutical organization in Montenegro at the time of the study; (2) a minimum of six months of work experience within the organization; and (3) voluntary agreement to participate in the study by providing informed consent.

Exclusion criteria included incomplete questionnaires, duplicate responses, and respondents not directly involved in organizational or operational processes within the pharmaceutical organization.

Data collection instrument

Data were collected using a structured questionnaire developed for the purposes of this study, informed by relevant literature and theoretical concepts in the fields of healthcare management and pharmaceutical practice. The questionnaire consisted of three sections: (1) sociodemographic characteristics of participants, (2) managerial practices and competencies, and (3) employee motivation and job satisfaction.

The questionnaire items assessed employees' perceptions of human resource management elements, work organization, communication, innovativeness, and motivational factors. Responses were rated using a five-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree).

Reliability of the instrument

The internal consistency of the self-developed questionnaire was assessed using Cronbach's alpha coefficient. Reliability analysis was conducted separately for each multi-item scale: managerial competencies (10 items), employee motivation (5 items), and job satisfaction (4 items). In addition, split-half reliability with Spearman–Brown correction was calculated to further evaluate the stability of the instrument.

According to commonly accepted psychometric standards, Cronbach's alpha values ≥ 0.70 were considered acceptable, ≥ 0.80 good, and ≥ 0.90 excellent.

Statistical analysis

Data analysis was performed using descriptive statistics (frequencies, means, and standard deviations) and inferential statistical methods. Non-parametric tests were applied due to the ordinal nature of Likert-scale data. The Mann–Whitney U test was used for comparisons between two independent groups, while the Kruskal–Wallis test was applied for comparisons across more than two groups. Correlations between variables were analyzed using Spearman's rank correlation coefficient due to the ordinal nature of the data. The level of statistical significance was set at $p < 0.05$. All statistical analyses were conducted using IBM SPSS Statistics version 26.0.

Ethical considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki (11). All participants were informed about the purpose and procedures of the study prior to participation and provided informed consent. Anonymity, data confidentiality, and voluntary participation were ensured throughout the research process.

RESULTS

Reliability analysis of the questionnaire

The reliability analysis demonstrated satisfactory to excellent internal consistency of the constructed scales. The managerial competencies scale showed excellent reliability (α = 0.906), the employee motivation scale demonstrated acceptable reliability (α = 0.777), and the job satisfaction scale showed good reliability (α = 0.809). The overall 19-item instrument achieved excellent internal consistency (α = 0.921).

Following confirmation of instrument reliability, the main study findings are presented in four tables and summarize the assessment of managerial competencies, statistically significant differences among selected variables, correlations between employee motivation and job satisfaction, and the association between education level and the perception of managerial competencies. Sociodemographic characteristics of the participants are described in the text.

The sample consisted predominantly of women (72%), while men accounted for 28% of participants. The largest proportion of respondents belonged to the 35–49 age group (42.7%), followed by the 24–34 age group (41.5%). Participants aged 50 years and older represented 15.8% of the total sample.

Descriptive statistics of managerial competency dimensions are presented in Table 1. The highest mean scores were recorded for customer orientation (mean = 4.46; SD = 0.72) and technological preparedness (mean = 4.29; SD = 0.70), indicating a strong focus on service quality and the use of modern technologies. Lower mean scores were observed for employee reward systems (mean = 3.65; SD = 1.13) and continuous education (mean = 3.93; SD = 0.99), suggesting areas with potential for further improvement.

Table 1 **Descriptive statistics of managerial competency dimensions.**

Criterion	Mean	SD
Customer orientation	4.46	0.72
Innovativeness	4.21	0.77
Technological preparedness	4.29	0.70
Employee reward systems	3.65	1.13
Continuous education	3.93	0.99

Differences in the assessment of selected managerial practices according to sociodemographic characteristics were analyzed using non-parametric tests due to the ordinal nature of the Likert-scale data. The Mann–Whitney U test was used for comparisons between two independent groups (gender), while the Kruskal–Wallis test was applied for comparisons across more than two groups (education level). Statistically significant differences were identified in business activity planning and innovativeness according to education level, and in employee motivation according to gender (Table 2).

Table 2 **Differences in the assessment of managerial practices.**

Variable	Compared groups	Test	p-value
Business activity planning	Education level	Kruskal –Wallis	0.003
Innovativeness	Education level	Kruskal –Wallis	0.016
Employee motivation	Gender	Mann –Whitney U	0.033

Correlations between employee motivation, job satisfaction, and selected managerial aspects were analyzed using Spearman's rank correlation. A strong positive Spearman correlation was identified between motivation and job satisfaction (ρ = 0.71; p = 0.001). In addition, moderate positive correlations were observed between motivation and innovativeness (ρ = 0.58; p = 0.004) and between job satisfaction and employee reward systems (ρ = 0.66; p = 0.002), as presented in Table 3.

Table 3 **Correlations between motivation, job satisfaction and selected managerial aspects.**

Variables compared	ρ	p-value
Motivation – job satisfaction	0.71	0.001
Motivation – innovativeness	0.58	0.004
Job satisfaction – reward systems	0.66	0.002

The association between participants' level of education and their perception of managerial competencies was analyzed using the Kruskal–Wallis test. A statistically significant difference in the perception of managerial competencies was identified across education levels (p = 0.022). Higher mean managerial competency scores were observed among participants with higher educational attainment. The mean managerial competency scores according to education level are presented in Table 4.

Table 4 **Association between education level and perception of managerial competencies.**

Education level	Mean score
Secondary education	3.68
College / university degree	4.12
Master's / doctoral degree	4.34

DISCUSSION

The findings of this study indicate that managerial competencies, employee motivation, and reward systems represent key determinants of organizational effectiveness in pharmaceutical organizations. High mean scores for customer orientation and technological preparedness suggest that pharmaceutical organizations in Montenegro are increasingly focused on improving service quality and adopting modern technologies.These findings are consistent with Healthcare 4.0–related trends described in the human resource management literature, which emphasize the growing importance of digitalization, innovation, and technologically supported management practices in healthcare organizations (9).

The strong and statistically significant correlation between employee motivation and job satisfaction confirms the central role of motivational factors in shaping a positive work environment. This result aligns with the conceptual framework proposed by Franco LM, et al. (12), which highlights intrinsic motivation, work autonomy, and organizational and contextual factors as key determinants of job satisfaction and performance among healthcare workers.

Furthermore, statistically significant differences were identified in the perception of managerial competencies across education levels. Employees with higher educational attainment demonstrated higher expectations and a more critical evaluation of managerial practices, which is consistent with findings reported by Štimac D, et al. (8) within the Croatian healthcare system.

Lower ratings of employee reward systems and continuous professional education point to potential weaknesses in existing human resource management practices within pharmaceutical organizations. Comparable challenges related to workforce development, motivation, and staff retention have been documented by Dieleman M, et al. (2), who emphasize the role of effective governance mechanisms and coherent health workforce policies in supporting employee motivation and organizational sustainability. In addition, Kabene SM, et al. (6) note that insufficiently developed human resource management systems, limited investment in professional development, and inadequately structured reward mechanisms may adversely affect employee satisfaction and overall organizational performance in healthcare settings.

International reports further underline the importance of comprehensive workforce retention strategies, particularly in smaller and transitional healthcare systems. Both the Organization for Economic Co-operation and Development and the World Health Organization stress the need for balanced human resource policies that simultaneously address workforce motivation, retention, and continuous professional development (3,4).

The relevance of communication skills, strategic thinking, and change management as essential managerial competencies is also supported by the findings of Ndayishimiye C, et al. (10). In addition, earlier studies conducted in healthcare environments have consistently demonstrated that effective human resource management practices contribute to improved organizational functioning and higher levels of employee satisfaction (13).

Moreover, the observed association between job satisfaction and employee reward systems reinforces the importance of transparent performance evaluation processes and fair compensation mechanisms in healthcare organizations.

Finally, the results of this study are in line with previous research indicating that innovative managerial competencies are associated with improved quality of work, enhanced organizational efficiency, and greater user satisfaction among healthcare professionals (14). Overall, these findings support the primary objective of the study by demonstrating a measurable impact of managerial competencies and motivational factors on employees' perceptions of management practices and job satisfaction within pharmaceutical organizations in Montenegro.

Despite the valuable insights provided by this study, several limitations should be acknowledged. The use of a self-developed questionnaire and the cross-sectional design may limit the generalizability of the findings. However, reliability analysis confirmed high internal consistency of the instrument, supporting the credibility of the findings. Nevertheless, the results provide relevant evidence for understanding managerial practices in pharmaceutical organizations within transitional healthcare systems.

CONCLUSION

This study demonstrated that managerial competencies, employee motivation, and job satisfaction are closely interrelated factors that significantly influence employees' perceptions of management practices within pharmaceutical organizations in Montenegro. Statistically significant differences in the perception of managerial competencies were observed across education levels, along with strong associations between employee motivation, job satisfaction, and employee reward systems. The findings emphasize the importance of implementing an integrated human resource management approach that includes continuous professional development of managers, structured motivational strategies, and strengthened organizational communication. In transitional healthcare systems, reinforcing managerial competencies may enhance organizational stability, employee retention, and overall service performance. These results provide a basis for future research aimed at exploring the broader impact of managerial competencies on the quality, efficiency, and sustainability of healthcare services.

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Direct-Acting Antiviral Therapy for Patients with Hepatitis C Infection Who Inject Drugs

Terapija direktno djelujućim antivirusnim lijekovima kod intravenskih korisnika droga sa infekcijom virusom hepatitisa C

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ABSTRACT

Introduction: Hepatitis C virus (HCV) infection is highly prevalent among people who inject drugs, with an estimated prevalence of 40-50%. Treatment of individuals on opioid substitution therapy (OST) with direct-acting antiviral (DAA) therapy provides multiple benefits, both for the individual and public health. Aim: to evaluate the therapeutic response to direct-acting antiviral therapy in patients receiving opioid substitution treatment with chronic hepatitis C, after structured patient motivation and comprehensive diagnostic assessment. Materials and methods: a retrospective, descriptive study was conducted at the Institute for Addiction Diseases of Sarajevo Canton in the period between 2022 and 2024. The study included 42 OST patients diagnosed with HCV infection. Prior to therapy, patients underwent motivational interventions and comprehensive diagnostic evaluation, including serological and molecular tests (anti-HCV, HCV RNA PCR, genotype determination) and liver assessment using ultrasound and FibroScan® method. DAA therapy (glecaprevir/pibrentasvir or sofosbuvir/velpatasvir) was administered for 8 or 12 weeks. Results: the majority of participants were male (88%), with a mean age of 46.5 years. The most common HCV genotype was 1a (66.7%). Therapy was successfully completed by 34 patients (81%), while 7 patients (16.7%) discontinued treatment. One patient died due to hepatocellular carcinoma. Sustained virologic response at 12 weeks post-treatment (SVR12) was achieved in all patients who completed therapy (100%). Conclusion: DAA therapy in OST patients demonstrates high efficacy when combined with adequate motivation and continuous support during the treatment. Improving access and care for this population is essential to reduce HCV prevalence and the risk of reinfection.

Keywords: hepatitis C, opioid substitution therapy, people who inject drugs, DAA therapy, SVR

SAŽETAK

Uvod: infekcija virusom hepatitisa C (HCV) izrazito je učestala među intravenskim korisnicima droga, s procijenjenom prevalencijom od 40–50%. Liječenje osoba na opioidnoj supstitucionoj terapiji (OST) direktno djelujućom antivirusnom terapijom (DAA) ima višestruke koristi, kako za pojedinca, tako i za javno zdravlje. Cilj: procijeniti terapijski odgovor na direktno djelujuću antivirusnu terapiju kod pacijenata s hroničnim hepatitisom C koji primaju opioidnu supstitucijsku terapiju, nakon strukturirane motivacije pacijenata i sveobuhvatne dijagnostičke procjene. Materijali i metode: provedena je retrospektivna deskriptivna studija u Zavodu za bolesti ovisnosti Kantona Sarajevo u periodu od 2022. do 2024. godine. U istraživanje je uključeno 42 pacijenta na OST s dijagnosticiranom HCV infekcijom. Prije uključivanja u terapiju provedena je motivacija za liječenje te kompletna dijagnostička obrada koja je uključivala serološke i molekularne testove (anti-HCV, HCV RNA PCR, genotipizaciju) te procjenu stanja jetre ultrazvučnim pregledom i FibroScan® metodom. Primijenjena je DAA terapija (glekaprevir/pibrentasvir ili sofosbuvir/velpatasvir) u trajanju od 8 ili 12 sedmica. Rezultati: većinu ispitanika činili su muškarci (88%), s prosječnom dobi od 46,5 godina. Najzastupljeniji HCV genotip bio je 1a (66,7%). Terapiju je uredno završilo 34 pacijenta (81%), dok je 7 pacijenata (16,7%) prekinulo liječenje. Jedan pacijent je preminuo zbog hepatocelularnog karcinoma. Kod svih ispitanika koji su završili terapiju postignut je trajni virusološki odgovor (SVR12) – 100%. Zaključak: DAA terapija kod korisnika OST pokazuje visoku učinkovitost uz uslov dobre motivacije i kontinuirane podrške tokom liječenja. Unapređenje pristupa ovoj populaciji ključno je za smanjenje prevalencije HCV infekcije i rizika od reinfekcija.

Ključne riječi: hepatitis C, opioidna supstituciona terapija, intravenski korisnici droga, DAA terapija, SVR

INTRODUCTION

The use of psychoactive substances represents a major threat to individual health and to the wider social environment, with substantial medical, social, and economic consequences. The increasing number of people who inject drugs represent a significant public health problem, closely associated with a high burden of infectious diseases and increased morbidity and mortality (1).

According to the World Health Organization (WHO) definition, addiction is a mental, and sometimes physical, condition resulting from the interaction between a living organism and psychoactive substances. It is characterized by behavioral and psychological processes that include a strong compulsion to take substances periodically or continuously in order to experience their effects or to avoid discomfort related to their absence (1,2).

Injection drug use is strongly associated with blood-borne viral infections, most notably hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). According to the World Drug Report, approximately 12 million people worldwide inject drugs, of whom about 1.6 million are living with HIV infection and more than half are infected with HCV. Among this population, HCV infection accounts for a substantially higher number of deaths than HIV, with an estimated 222,000 deaths annually attributed to hepatitis C compared with approximately 60,000 deaths due to HIV infection (3).

Epidemiological studies from the United States and several European countries report anti-HCV seroprevalence rates of 70–92% among people who inject drugs. Global data from 187 countries indicate that deaths from viral hepatitis increased from 1.1 million in 2019 to 1.3 million in 2022, with hepatitis B accounting for approximately 83% and hepatitis C for 17% of these deaths. A large proportion of mortality is related to complications such as liver cirrhosis and hepatocellular carcinoma (4).

Hepatitis C virus is transmitted primarily through blood exposure, most commonly via the sharing of contaminated injecting equipment, including syringes, needles, filters, and other paraphernalia. Sexual transmission is rare, while vertical transmission from mother to child may occur during pregnancy or childbirth. In most cases, acute HCV infection progresses to a chronic form, which may lead to progressive liver fibrosis, cirrhosis, and hepatocellular carcinoma. Globally, the most prevalent HCV genotype is genotype 1 (subtypes 1a and 1b), while genotype 3 is particularly common among people who inject drugs (5,6).

Current clinical guidelines of the European Association for the Study of the Liver (EASL) and the World Health Organization recommend that all patients with chronic HCV infection be considered for antiviral therapy, regardless of disease stage. Special emphasis is placed on treating populations at high risk of transmission, including people who currently inject drugs and patients receiving opioid substitution therapy (7).

The introduction of direct-acting antiviral agents (DAA) has fundamentally changed the management of chronic hepatitis C and represents a cornerstone of the WHO strategy aimed at eliminating HCV as a public health threat by 2030. However, achieving this goal depends not only on the availability of effective therapy but also on improving testing, diagnosis, linkage to care, and long-term retention in treatment, particularly among marginalized and high-risk populations (8-10).

Sustained virologic response (SVR), defined as undetectable HCV RNA 12 weeks after completion of therapy, is considered a surrogate marker of virologic cure and is associated with long-term clinical benefit. More than 99% of patients who achieve SVR are considered cured of HCV infection (11,12).

AIM

The aim of this study was to evaluate the therapeutic response to direct-acting antiviral therapy in patients receiving opioid substitution treatment with chronic hepatitis C, after structured patient motivation and comprehensive diagnostic assessment.

MATERIALS AND METHODS

A retrospective clinical-epidemiological study was conducted at the Institute for Addiction Diseases of Sarajevo Canton, during the period from 2022 to 2024. The study included 42 patients of both sexes, aged 27 to 66 years, who were injecting drug users and were receiving opioid substitution therapy (methadone, buprenorphine, or buprenorphine/naloxone) in combination with direct-acting antiviral therapy for chronic hepatitis C.

Inclusion criteria: adult patients (≥18 years) with confirmed chronic HCV infection (positive HCV RNA), receiving opioid substitution therapy, and treated with a DAA regimen during the study period.

Exclusion criteria: incomplete medical records, absence of follow-up data for sustained virologic response, and coinfection with hepatitis B virus or human immunodeficiency virus, if present.

Before starting antiviral therapy, all patients underwent a structured motivation process and comprehensive diagnostic evaluation, including serological testing (anti-HCV), molecular testing (HCV RNA by polymerase chain reaction), HCV genotyping, and assessment of liver status by abdominal ultrasound and transient elastography (FibroScan®).

Sustained virologic response was assessed 12 weeks after completion of therapy by HCV RNA testing (SVR12).

All patients provided written informed consent for participation in the study.

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Institute for Addiction Diseases of Sarajevo Canton.

Statistical analysis was performed using descriptive statistics, including frequencies, percentages, and mean values with standard deviation. The chi-square test was used to assess differences between categorical variables, with a level of statistical significance set at $p < 0.05$. Data analysis was performed using MedCalc statistical software, version 15.0 (MedCalc Software, Antwerp, Belgium).

RESULTS

During the study period (2022-2024), there were 339 patients at the Institute on the OST program, and 114 (33.6%) had chronic hepatitis C. During that period, 42 users of the Institute on OST with chronic hepatitis C received direct-acting antiviral therapy without interferon (DAA).

A descriptive overview of the respondents by gender, age, OST, HCV genotype and DAA therapy is shown in Table 1.

Table 1 **Descriptive sample overview by gender, age, OST, genotype and DAA therapy.**

		N	%	p
Gender	Male	37	88.1	0.0001
	Female	5	11.9	
Age (year)		46.52±7.59 (27–66)		
OST	Buprenorphine	8	19.0	0.0001
	Buprenorphine & Naloxone	5	11.9	
	Methadone	29	69.0	
HCV genotype	1a	28	66.7	0.004
	1b	4	9.5	
	3	10	23.8	
DAA therapy (weeks)	1 (8)	32	76.2	0.0001
	1 (12)	6	14.3	
	2 (12)	4	9.5	
Total		42	100.0	

Legend: N – Number of respondents; % – Percentage; OST – Opioid Substitution Therapy; DAA – Direct-acting antiviral therapy; 1 – Glecaprevir+Pibrentasvir 100/40 mg; 2 – Sofosbuvir/Velpatasvir 400/100 mg.

Therapy was completed in 34 (34/42; 80.9%) and interrupted in seven (7/42; 16.7%) cases. Discontinuation of therapy was done by subjects on buprenorphine and methadone. Application of HCV therapy according to age, sex, OST, genotype and DAA therapy is shown in Table 2.

Table 2 **Application of HCV therapy by gender, age, OST, HCV genotype and DAA.**

		HCV THERAPY N (%)			p
		Completed	Interupted	Died	
Gender	Male	30 (71.4)	6 (14.3)	1 (2.4)	0.917
	Female	4 (9.5)	1 (2.4)	0 (0.0)	
Age (mean±SD)		46.56±7.75	44.29±4.82	61.00± 0.00	0.119
OST	Buprenorphine	4 (9.5)	4 (9.5)	0 (0.0)	0.072
	Buprenorphine & Naloxone	5 (11.9)	0 (0.0)	0 (0.0)	
	Methadone	25 (59.5)	3 (7.2)	1 (2.4)	
HCV genotype	1a	23 (54.7)	4 (9.5)	1 (2.4)	0.931
	1b	3 (7.1)	1 (2.4)	0 (0.0)	
	3	8 (19.1)	2 (4.8)	0 (0.0)	
DAA (weeks)	1 (8)	28 (66.6)	0 (0.0)	1 (2.4)	0.002
	1 (12)	5 (11.9)	4 (9.5)	0 (0.0)	
	2 (12)	1 (2.4)	3 (7.2)	0 (0.0	
Total		34 (80.9)	7 (16.7)	1 (2.4)	

Legend: N – Number of respondents; % – Percentage; OST – Opioid Substitution Therapy; DAA – Direct-acting antiviral therapy; 1 – Glecaprevir+Pibrentasvir 100/40 mg; 2 – Sofosbuvir/Velpatasvir 400/100 mg.

SVR was achieved in all subjects who completed therapy (100%).
The achieved SVR by OST, HCV genotype, type of HCV therapy, and statistical significance are shown in Table 3.

Table 3 **SVR achieved in 34 subjects, according to OST, HCV genotype and DAA therapy.**

	SVR ACHIEVED	N	%	p
OST	Buprenorphine	4	11.8	0.004
	Buprenorphine & Naloxone	5	14.7	
	Methadone	25	73.5	
HCV genotype	1a	23	67.6	0.010
	1b	3	8.8	
	3	8	23.6	
DAA therapy (weeks)	1 (8)	28	82.4	0.0001
	1 (12)	5	14.7	
	2 (12)	1	2.9	
Total		34	100.0	

Legend: OST – Opioid Substitution Therapy; DAA – Direct-acting antiviral therapy; 1 – Glecaprevir+Pibrentasvir 100/40 mg; 2 – Sofosbuvir/Velpatasvir 400/100 mg.

DISCUSSION

According to the latest EASL and WHO guidelines, all patients with chronic hepatitis C who are untreated or unsuccessfully treated, who wish to receive therapy and have no contraindications, should be considered for DAA treatment (7).

In Croatia, DAA therapy was introduced into protocols in 2019 (10). In Bosnia and Herzegovina, DAA therapy is funded by the Solidarity Fund since 2019 and became available at the end of 2020 for all patients with chronic HCV infection. The use of medications for addiction therapy, at any dose, is not considered a contraindication to HCV treatment (13).

At the Sarajevo Canton Institute for Addiction Diseases, patients received DAA therapy for chronic hepatitis C during 2022–2024. Of 339 patients enrolled in the OST program, 114 (33.6%) had chronic HCV infection.

A 2022 study in Croatia reported high HCV prevalence among people who inject drugs: Rijeka (37.2%), Zagreb (37.2%), and Split (59.5%). Compared to 2015, the prevalence increased. Among those with anti-HCV antibodies, 41.1% were previously HCV positive; one-third (35.3%) received treatment, and only 13.4% were cured (14).

In this retrospective study, 42 patients in the OST program with chronic HCV were analyzed. Subjects were predominantly male (88%), on methadone (69%) or buprenorphine, with HCV genotype 1a (66.7%), and most received an 8-week DAA regimen (76.2%) ($p < 0.05$). The male-to-female ratio was 7.4:1, and mean age was 46.52 ± 7.59 years (range 27-66).

Adherence to DAA therapy among drug users can be challenging, and discontinuation is common despite the short duration and minimal side effects of therapy. Proper patient motivation and support are essential to achieve treatment success. Expanding access to harm-reduction services, diagnostic testing, and timely treatment is crucial to prevent new HCV infections and related comorbidities.

In Croatia, the incidence of treatment and cure among people who inject drugs remains low: less than half of previously diagnosed patients received therapy, and only one-third were healed. Regular testing for HCV and HIV is recommended at least annually; however, in the study, four out of five participants in all three cities (Rijeka, Zagreb, Split) had not been tested within the previous 12 months. Rapid testing and counseling by trained non-medical staff could improve coverage, in line with WHO recommendations (14).

At the Sarajevo Institute, 34/42 patients (81%) completed DAA therapy, while seven (16.7%) discontinued treatment and one (2.4%), aged 61, died due to hepatocellular carcinoma. Therapy discontinuation occurred in both sexes, OST types, and HCV genotypes ($p > 0.05$), with longer therapy duration (12 weeks) associated with higher discontinuation ($p < 0.05$).

Similar findings were reported by Macías J, et al., where 17% of patients were lost to follow-up and 3.5% experienced reinfection. They concluded that PWID achieve high SVR12 rates with DAA therapy, regardless of OST status, although slightly lower than patients without a history of drug use (12).

Habchi J, et al. in the US observed that among 275 patients starting DAA therapy, 57.5% had genotype 1a, with 8.7% dropouts. Three years post-therapy, approximately 1% experienced HCV reinfection (15).

In the Sarajevo cohort, all 34 patients completing therapy achieved SVR12 (100%, $p < 0.05$). McDonald et al. in Scotland reported good outcomes in treating PWID with chronic HCV, although evidence regarding extrahepatic outcomes and mortality remains limited (16).

In Germany, among 10,298 patients with genotypes 1 and 3 receiving DAA therapy, SVR was achieved in 95%, with fewer adverse events than prior treatments. Patients with cirrhosis, HIV co-infection, and on OST showed slightly lower but still high SVR rates (17).

In Bosnia and Herzegovina, future studies will determine the long-term SVR and reinfection rates, particularly among OST patients. Okić R, et al. emphasize the importance of supporting patients to maximize OST program benefits as a gateway to HCV therapy. Active engagement of healthcare professionals and societies is essential to promote HCV testing and prevent reinfection (18).

WHO and the European Union Drugs Agency (EUDA) recommend biobehavioral surveillance among PWID every 3-5 years in high-concentration areas to monitor HCV infection and guide public health interventions (14,19).

CONCLUSION

This study highlights the challenges in managing patients who have difficulty remaining in the OST program and completing their chronic hepatitis C treatment. Despite the advantages of DAA therapy short treatment duration and minimal side effects which serve as strong motivation for patients on opioid substitution therapy to initiate HCV treatment, treatment interruptions remain common. The results demonstrate that treatment outcomes in PWID patients on OST with chronic hepatitis C are excellent when patients adhere to recommendations and treatment guidelines. A multidisciplinary approach at all levels of healthcare is essential to prevent HCV infections and reinfections, reduce stigmatization, and ensure appropriate support and care for this vulnerable population.

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Antimicrobial Resistance Profiles of Clinical Isolates at the Clinical Center University of Sarajevo: A 2023-2024 Comparative Analysis

Profil rezistencije na antimikrobne lijekove kliničkih izolata u Kliničkom centru Univerziteta u Sarajevu: komparativna analiza 2023-2024 godina

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ABSTRACT

Introduction: the emergence of antimicrobial resistance (AMR) represents a growing global health concern, requiring systematic surveillance and rational antibiotic use. Aim: to analyze the antimicrobial susceptibility patterns of clinical isolates in 2024 at the Clinical Center University of Sarajevo (CCUS) and to compare them with data from 2023. Materials and methods: the 2024 surveillance included Staphylococcus aureus, Enterococcus faecium, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis, Acinetobacter baumannii, and Pseudomonas aeruginosa. Antibiotic susceptibility testing was performed using a standardized disk diffusion method, VITEK 2 Compact system (BioMérieux) and broth microdilution, following the standards of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Detection of resistance mechanisms included ESBL, carbapenemases, MRSA, VRE, and glycopeptide resistance. The data were compared with those previously published in 2023. Results: showed an increase in cephalosporin resistance among E. coli and K. pneumoniae, indicating the spread of ESBL-producing strains. Carbapenem resistance was also observed among Enterobacteriaceae, representing a significant therapeutic challenge. Extensively drug-resistant (XDR) A. baumannii and P. aeruginosa were identified, with colistin remaining the only effective agent. Among Gram-positive organisms, E. faecium displayed increased resistance to vancomycin and teicoplanin, while S. aureus exhibited a slight increase in oxacillin-resistant isolates. These local trends align with global and European AMR surveillance data, highlighting the importance of continuous monitoring, rational antibiotic use, and effective infection prevention strategies. Conclusion: local surveillance of antimicrobial resistance enables timely recognition of newly emerging resistant strains, provides a basis for the rational selection of empirical therapy, and supports antimicrobial stewardship and infection control measures in healthcare settings.

Keywords: antimicrobial resistance, ESBL, carbapenem-resistance, MRSA, VRE, EUCAST, colistin

SAŽETAK

Uvod: pojava antimikrobne rezistencije (AMR) predstavlja sve veći globalni zdravstveni problem, koji zahtijeva sistematski nadzor i racionalnu upotrebu antibiotika. Cilj: analizirati obrasce osjetljivosti kliničkih izolata na antimikrobne lijekove u 2024. godini u Kliničkom centru Univerziteta u Sarajevu (KCUS) i uporediti ih sa podacima iz 2023. godine. Materijali i metode: u 2024. godini nadzor je obuhvatio Staphylococcus aureus, Enterococcus faecium, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis, Acinetobacter baumannii i Pseudomonas aeruginosa. Testiranje osjetljivosti na antibiotike provedeno je primjenom standardizirane metode disk-difuzije, sistema VITEK 2 Compact (bioMérieux) i metode mikrodilucije u bujonu, u skladu sa standardima Evropskog komiteta za testiranje antimikrobne osjetljivosti (EUCAST). Detekcija mehanizama rezistencije obuhvatala je ESBL, karbapenemaze, MRSA, VRE i rezistenciju na glikopeptide. Dobijeni podaci upoređeni su s prethodno objavljenim rezultatima iz 2023. godine. Rezultati su pokazali porast rezistencije na cefalosporine kod E. coli i K. pneumoniae, što ukazuje na širenje sojeva koji produciraju ESBL. Rezistencija na karbapeneme također je zabilježena među enterobakterijama, što predstavlja značajan terapijski izazov. Ekstenzivna rezistencija je uočena kod A. baumannii i P. aeruginosa, pri čemu je kolistin ostao jedini efikasan antimikrobni lijek. Među gram-pozitivnim bakterijama, E. faecium je pokazao povećanu rezistenciju na vankomicin i teikoplanin, dok je kod S. aureus zabilježen blagi porast izolata rezistentnih na oksacilin. Ovi lokalni trendovi u skladu su s globalnim i evropskim podacima nadzora nad AMR-om, naglašavajući značaj kontinuiranog praćenja, racionalne upotrebe antibiotika i efikasnih strategija prevencije infekcija. Zaključak: lokalni nadzor antimikrobne rezistencije omogućava pravovremeno prepoznavanje rezistentnih sojeva, pruža osnovu za racionalan izbor empirijske terapije, te podržava mjere prevencije i kontrole infekcija u zdravstvenim ustanovama.

Ključne riječi: antimikrobna rezistencija, ESBL, rezistencija na karbapeneme, MRSA, VRE, EUCAST, kolistin

INTRODUCTION

Antimicrobial resistance (AMR) is a major global health threat, with considerable variability in resistance patterns reported across EU/EEA countries (1,2). This variability is influenced by bacterial species, antibiotic class, local antibiotic usage, and geographic region (3,4).

Although general trends in bacterial resistance are documented, local resistance profiles can differ significantly, highlighting the need for hospitals and healthcare institutions to monitor their own isolates against commonly used antibiotics (5,6). Resistance evolves over time, so clinicians must remain informed about current susceptibility profiles to optimize empirical therapy, guide infection control, and prevent further spread of resistant organisms (7,8).

WHO recommends systematic detection and reporting of bacterial resistance, alongside monitoring antibiotic usage in hospital and outpatient settings, as a global responsibility shared by all countries (9,10). Identification and tracking of multidrug-resistant organisms within and beyond healthcare settings is essential for outbreak mitigation and infection prevention strategies (11,12).

Surveillance AMR at the Clinical Center University of Sarajevo (CCUS) began in 2013. The microbiology laboratory adheres to EUCAST standards (13) and participates in external quality control programs to ensure methodological reliability (14,15).

AIM

The aim of the study was to analyze antimicrobial susceptibility patterns of clinical isolates in 2024 and compare them with the 2023 data (16).

MATERIALS AND METHODS

Bacterial isolates

During 2024, the following bacterial species were monitored at CCUS: Staphylococcus aureus, Enterococcus faecium, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis, Acinetobacter baumannii, and Pseudomonas aeruginosa
Basic principles of surveillance methodology:

1. AST was performed according to EUCAST guidelines
2. All isolates were tested using a standardized antibiotic panel.
3. Duplicate strains (same species from the same patient within 30 days) were excluded

Special isolates included: MRSA, VRSA, VRE, ESBL-producing E. coli and K. pneumoniae, carbapenem-resistant Enterobacterales, and colistin-resistant Acinetobacter and Pseudomonas.

Antimicrobial susceptibility testing

AST was performed using Kirby-Bauer disk diffusion on Mueller-Hinton agar according to EUCAST standards (13). Tested antibiotics included penicillin, ampicillin, amoxicillin, amoxicillin/clavulanic acid, cefoxitin, erythromycin, azithromycin, clarithromycin, cefazolin, cefuroxime, ceftazidime, cefepime, piperacillin/tazobactam, gentamicin, amikacin, chloramphenicol, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, vancomycin, imipenem, meropenem, fusidic acid, tetracycline, linezolid, colistin, tobramycin, teicoplanin, and tigecycline.

Bacteria were classified as Sensitive (S), Susceptible with Increased Exposure (I), or Resistant (R) following EUCAST breakpoints (13). MICs were determined using VITEK 2 Compact (BioMérieux) and broth microdilution for colistin (MIC-Strip Colistin, Merlin Diagnostika). Quality control used ATCC reference strains

Detection of resistance mechanisms

Resistance mechanisms were detected using standard phenotypic methods in accordance with EUCAST guidelines (17). ESBL production was identified by the double-disc synergy test (DDST), combination disc testing (CDT), ESBL E-test, or the VITEK 2 Compact system. Carbapenemase production was assessed using inhibitor-based combined disk tests for class A, class B, and OXA-48-like enzymes, with confirmatory testing when indicated. Methicillin/oxacillin resistance in Staphylococcus aureus was determined by cefoxitin disk diffusion or PBP2a latex agglutination. Glycopeptide resistance was evaluated by broth microdilution for S. aureus and by disk diffusion or VITEK 2 Compact for enterococci.

RESULTS

A total of 4473 bacterial isolates were identified from various clinical specimens submitted to the Clinical Microbiology Laboratory of the Clinical Center University of Sarajevo between January and December 2024.

The proportion of isolates in the total number of detected was as follows: Enterococcus faecalis 803 (17.95%), Staphylococcus aureus 656 (14.66%), Escherichia coli 628 (14.03%), Klebsiella pneumoniae 725 (16.2%), Pseudomonas aeruginosa 277 (6.2%), Enterobacter cloacae 388 (8.67%), Acinetobacter baumannii complex (ACB) 500 (11.17%), Proteus mirabilis 346 (7.73%), and Enterococcus faecium 150 (3.35%). The pattern of antimicrobial susceptibility/resistance for all isolates in 2023 and 2024 is presented in Tables 1-9.

Antimicrobial resistance patterns of Escherichia coli

Resistance of E. coli to ampicillin remained consistently high (83.0% in 2023 vs. 80.85% in 2024). Similarly, high resistance was observed for amoxicillin/clavulanic acid, although a slight decrease was noted (55.06% vs. 51.89%). In contrast, resistance to piperacillin/tazobactam increased substantially from 17.19% to 26.54%.

Among cephalosporins, resistance to cefazolin decreased slightly but remained high (49.7% vs. 44.97%). Resistance to cefuroxime increased from 30.0% in 2023 to 39.02% in 2024. A marked increase was observed for higher-generation cephalosporins: ceftazidime resistance nearly doubled (20.8% vs. 40.33%), while cefepime resistance increased from 19.64% to 34.18%. The significant rise in ceftazidime resistance is consistent with an increased prevalence of extended-spectrum β-lactamase (ESBL)-producing E. coli, in line with EUCAST indicator criteria (17).

Fluoroquinolone resistance also increased notably, with ciprofloxacin resistance rising from 34.69% to 42.43% and levofloxacin from 37.2% to 49.15%.

No resistance to imipenem was detected in either year (0%), confirming preserved susceptibility to carbapenems. Among aminoglycosides, amikacin retained high activity with very low resistance (4.35% vs. 3.08%), whereas gentamicin resistance increased moderately (21.05% vs. 26.46%). Resistance to trimethoprim/sulfamethoxazole further increased from 41.21% to 47.35% (Table 1).

Overall, E. coli exhibited increasing resistance to multiple antimicrobial classes, most prominently to higher-generation cephalosporins and fluoroquinolones. A significant increase in ceftazidime resistance strongly suggests an expanding burden of ESBL-producing strains. Carbapenems remain fully active, while amikacin continues to show high effectiveness.

Table 1 **Antimicrobial resistance patterns of Escherichia coli in 2023 and 2024.**

Antibiotic	2023 (%)	2024 (%)	Trend / Notes
Ampicillin	83.00	80.85	High, slight decrease
Amoxicillin/clavulanic acid	55.06	51.89	Slight decrease
Piperacillin/tazobactam	17.19	26.54	Increase
Cefazolin	49.70	44.97	Slight decrease
Cefuroxime	30.00	39.02	Increase
Ceftazidime	20.80	40.33	A marked increase, ESBL
Cefepime	19.64	34.18	Increase, ESBL
Ciprofloxacin	34.69	42.43	Increase
Levofloxacin	37.20	49.15	Increase
Imipenem	0.00	0.00	Fully susceptible
Amikacin	4.35	3.08	Remains active
Gentamicin	21.05	26.46	Moderate increase
Trimethoprim/sulfamethoxazole	41.21	47.35	Moderate increase

Antimicrobial resistance patterns of Klebsiella pneumoniae

Resistance to amoxicillin/clavulanic acid decreased slightly from 76.96% in 2023 to 68.06% in 2024, while piperacillin/tazobactam resistance decreased moderately from 66.32% to 58.55%.

Among cephalosporins, resistance decreased across all generations: cefazolin 72.84% to 67.30%, cefuroxime 68.14% to 57.54%, ceftazidime 66.67% to 58.70%, and cefepime 64.75% to 57.28%. The high resistance to third- and fourth-generation cephalosporins indicates a substantial prevalence of ESBL-producing K. pneumoniae. Aminoglycoside resistance increased for amikacin (11.53% to 18.23%) and fluctuated for gentamicin (61.85% to 47.51%). Fluoroquinolone resistance decreased for ciprofloxacin (from 62.76% to 53.94%) and levofloxacin (from 56.50% to 49.73%). Carbapenem resistance declined: imipenem 36.54% to 29.04% and meropenem 34.69% to 27.57%. Resistance to trimethoprim/sulfamethoxazole increased slightly (50.13% to 53.24%), while colistin remained low (8.48% vs. 8.17%) (Table 2).

Overall, K. pneumoniae exhibited high ESBL-associated cephalosporin resistance, with a general decrease in other cephalosporins and fluoroquinolones. Beta-lactam and carbapenem resistance remained high, emphasizing the need for continuous ESBL surveillance and antimicrobial stewardship.

Table 2 **Antimicrobial resistance patterns of Klebsiella pneumoniae in 2023 and 2024.**

Antibiotic	2023 (%)	2024 (%)	Trend / Notes
Amoxicillin/clavulanic acid	76.96	68.06	Slight decrease
Piperacillin/tazobactam	66.32	58.55	Moderate decrease
Cefazolin	72.84	67.30	Slight decrease
Cefuroxime	68.14	57.54	Decrease
Ceftazidime	66.67	58.70	High, ESBL prevalent
Cefepime	64.75	57.28	ESBL prevalent
Amikacin	11.53	18.23	Increase
Gentamicin	61.85	47.51	Decrease
Ciprofloxacin	62.76	53.94	Decrease
Levofloxacin	56.50	49.73	Decrease
Imipenem	36.54	29.04	Decrease
Meropenem	34.69	27.57	Decrease
Trimethoprim/sulfamethoxazole	50.13	53.24	Slight increase
Colistin	8.48	8.17	Remains low

Antimicrobial resistance patterns of Enterobacter cloacae

Resistance to ampicillin and amoxicillin/clavulanic acid remained at 100% in both years. Piperacillin/tazobactam resistance decreased slightly (62.83% to 53.14%).

Among cephalosporins, cefazolin resistance remained at 100%, while ceftazidime resistance increased from 49.09% to 61.71%, and cefepime resistance increased from 45.26% to 52.72%. The increase in third- and fourth-generation cephalosporin resistance suggests a rising prevalence of ESBL-producing E. cloacae. Aminoglycoside resistance increased for amikacin (11.11% to 23.47%) and decreased for gentamicin (56.28% to 51.29%). Fluoroquinolone resistance increased for ciprofloxacin (38.10% to 45.00%) and levofloxacin (16.59% to 21.24%). Carbapenem resistance showed divergent trends: imipenem decreased (15.53% to 11.91%), while meropenem remained low (12.32% to 11.73%). Trimethoprim/sulfamethoxazole resistance increased modestly (42.76% to 46.55%) (Table 3).

Overall, E. cloacae displayed increasing ESBL-associated resistance, particularly in third- and fourth-generation cephalosporins, while other classes showed variable trends. Continuous surveillance and targeted stewardship are essential.

Table 3 **Antimicrobial resistance patterns of Enterobacter cloacae in 2023 and 2024.**

Antibiotic	2023 (%)	2024 (%)	Trend / Notes
Ampicillin / Amoxicillin/clavulanic acid	100	100	Fully resistant
Piperacillin/tazobactam	62.83	53.14	Slight decrease
Cefazolin	100	100	Fully resistant
Ceftazidime	49.09	61.71	Increase, ESBL
Cefepime	45.26	52.72	Increase, ESBL
Amikacin	11.11	23.47	Increase
Gentamicin	56.28	51.29	Slight decrease
Ciprofloxacin	38.10	45.00	Increase
Levofloxacin	16.59	21.24	Increase
Imipenem	15.53	11.91	Decrease
Meropenem	12.32	11.73	Low, stable
Trimethoprim/sulfamethoxazole	42.76	46.55	Slight increase

Antimicrobial resistance patterns of Proteus mirabilis

Resistance to ampicillin remained high but decreased from 85.92% to 63.08%, and amoxicillin/clavulanic acid resistance decreased slightly (39.22% to 34.19%). Piperacillin/tazobactam resistance increased (7.59% to 11.17%).

Among cephalosporins, cefazolin decreased from 50.60% to 43.93%, cefuroxime from 32.33% to 21.97%, ceftazidime from 21.98% to 20.67%, and cefepime increased from 14.35% to 15.14%. Persistent resistance to third-generation cephalosporins indicates the presence of ESBLs in a subset of P. mirabilis. Aminoglycoside resistance decreased for amikacin (8.33% to 1.65%) and gentamicin (23.53% to 18.89%). Fluoroquinolone resistance showed mixed trends: ciprofloxacin decreased from 34.94% to 30.99%, and levofloxacin remained stable at 31.43% to 30.25%. Carbapenem resistance remained extremely low, with only one resistant isolate in 2024. Trimethoprim/sulfamethoxazole resistance increased from 45.65% to 53.25% (Table 4).

Overall, P. mirabilis showed moderate ESBL-associated resistance with decreasing aminoglycoside and cephalosporin resistance, while ampicillin and trimethoprim/sulfamethoxazole resistance remained moderate to high.

Table 4 **Antimicrobial resistance patterns of Proteus mirabilis in 2023 and 2024.**

Antibiotic	2023 (%)	2024 (%)	Trend / Notes
Ampicillin	85.92	63.08	Decrease
Amoxicillin/clavulanic acid	39.22	34.19	Slight decrease
Piperacillin/tazobactam	7.59	11.17	Slight increase
Cefazolin	50.60	43.93	Decrease
Cefuroxime	32.33	21.97	Decrease
Ceftazidime	21.98	20.67	Stable, some ESBL
Cefepime	14.35	15.14	Stable
Amikacin	8.33	1.65	Decrease
Gentamicin	23.53	18.89	Decrease
Ciprofloxacin	34.94	30.99	Decrease
Levofloxacin	31.43	30.25	Stable
Imipenem / Meropenem	0.00	0.08	Extremely low
Trimethoprim/sulfamethoxazole	45.65	53.25	Increase

Antimicrobial resistance patterns of Pseudomonas aeruginosa

Resistance to amikacin increased slightly (14.78% to 18.45%) and piperacillin/tazobactam (29.10% to 31.07%). Ceftazidime resistance increased significantly from 37.15% to 56.34%, and cefepime from 26.94% to 31.05%. Fluoroquinolone resistance rose for ciprofloxacin (39.26% to 49.33%) and levofloxacin (40.06% to 50.75%). Carbapenem resistance increased: imipenem from 30.09% to 46.43%, meropenem from 30.41% to 43.97%. Colistin resistance remained absent (0%) (Table 5).

Overall, P. aeruginosa demonstrated a general increase in resistance, particularly to cephalosporins, fluoroquinolones, and carbapenems, while colistin retained full activity.

Table 5 **Antimicrobial resistance patterns of Pseudomonas aeruginosa in 2023 and 2024.**

Antibiotic	2023 (%)	2024 (%)	Trend / Notes
Amikacin	14.78	18.45	Slight increase
Piperacillin/tazobactam	29.10	31.07	Slight increase
Ceftazidime	37.15	56.34	Significantly increase
Cefepime	26.94	31.05	Increase
Ciprofloxacin	39.26	49.33	Increase
Levofloxacin	40.06	50.75	Increase
Imipenem	30.09	46.43	Increase
Meropenem	30.41	43.97	Increase
Colistin	0.00	0.00	Fully active

Antimicrobial resistance patterns of Acinetobacter baumannii

A. baumannii exhibited extremely high resistance across most antibiotic classes in both years. Amikacin resistance increased slightly (98.57% to 99.80%), while ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, and tobramycin all exceeded 90% resistance. Colistin resistance emerged in one isolate in 2024 (0.2%). Trimethoprim/sulfamethoxazole resistance remained high (Table 6).

Overall, A. baumannii showed extensive multidrug resistance, with only colistin retaining near-universal activity, highlighting the need for vigilant surveillance and strict infection control.

Table 6. **Antimicrobial resistance patterns of Acinetobacter baumannii in 2023 and 2024.**

Antibiotic	2023 (%)	2024 (%)	Trend / Notes
Amikacin	98.57	99.80	Very high
Ciprofloxacin	>90	>90	Very high
Gentamicin	>90	>90	Very high
Imipenem	>90	>90	Very high
Levofloxacin	>90	>90	Very high
Meropenem	>90	>90	Very high
Tobramycin	>90	>90	Very high
Colistin	0.00	0.20	Emergent resistance

Antimicrobial resistance patterns of Staphylococcus aureus

Oxacillin resistance (MRSA) increased modestly (9.78% to 13.41%). Erythromycin resistance rose from 11.73% to 16.09%, and clarithromycin from 11.14% to 19.01%. Gentamicin remained stable (7.10% to 7.79%), and tetracycline increased slightly (13.06% to 16.05%). Ciprofloxacin resistance increased (2.60% to 6.82%), while trimethoprim/sulfamethoxazole and fusidic acid remained low. Vancomycin retained full activity (0%) (Table 7).

Overall, S. aureus showed a moderate increase in MRSA prevalence and macrolide/tetracycline resistance, while vancomycin and fusidic acid remained fully effective.

Table 7 **Antimicrobial resistance patterns of Staphylococcus aureus in 2023 and 2024.**

Antibiotic	2023 (%)	2024 (%)	Trend / Notes
Oxacillin	9.78	13.41	Slight increase, MRSA
Erythromycin	11.73	16.09	Increase
Clarithromycin	11.14	19.01	Increase
Gentamicin	7.10	7.79	Stable
Tetracycline	13.06	16.05	Slight increase
Ciprofloxacin	2.60	6.82	Increase
Trimethoprim/sulfamethoxazole	0.69	0.78	Low
Fusidic acid	0.43	0.78	Negligible
Vancomycin	0.00	0.00	Fully active

Antimicrobial resistance patterns of Enterococcus faecalis

E. faecalis remained fully susceptible to beta-lactams (ampicillin, amoxicillin, amoxicillin/clavulanic acid) and vancomycin (0% resistance). Ciprofloxacin resistance decreased slightly (33.6% to 32.48%), and nitrofurantoin resistance decreased (8.3% to 4.26%) (Table 8).

Overall, E. faecalis maintained high susceptibility to beta-lactams and vancomycin, with moderate fluoroquinolone resistance and low

Table 8 **Antimicrobial resistance patterns of Enterococcus faecalis in 2023 and 2024.**

Antibiotic	2023 (%)	2024 (%)	Trend / Notes
Ampicillin / Amoxicillin / Amox -Clav	0.00	0.00	Fully susceptible
Vancomycin	0.00	0.00	Fully susceptible
Ciprofloxacin	33.60	32.48	Slight decrease
Nitrofurantoin	8.30	4.26	Decrease

Antimicrobial resistance patterns of Enterococcus faecium

E. faecium exhibited universal beta-lactam resistance. Resistance increased substantially for vancomycin (44.78% to 64.00%) and teicoplanin (38.94% to 51.16%). Nitrofurantoin resistance increased (81.44% to 91.11%). Tigecycline resistance remained low (2.84% □ 4.85%), and linezolid retained full activity (0%) (Table 9).

Overall, E. faecium exhibited extensive drug resistance, with only linezolid and tigecycline retaining reliable activity. The rise in vancomycin and teicoplanin resistance underscores the need for continuous antimicrobial surveillance and stewardship.

Table 9 **Antimicrobial resistance patterns of Enterococcus faecium in 2023 and 2024.**

Antibiotic	2023 (%)	2024 (%)	Trend / Notes
Ampicillin / Amoxicillin / Amox -Clav	100	100	Fully resistant
Vancomycin	44.78	64.00	Substantial increase
Teicoplanin	38.94	51.16	Substantial Increase
Nitrofurantoin	81.44	91.11	Increase
Tigecycline	2.84	4.85	Low
Linezolid	0.00	0.00	Fully active

DISCUSSION

The emergence of antimicrobial resistance (AMR) on a global scale is increasingly becoming a critical issue. Consequently, implementing a systematic resistance monitoring system is essential, providing data to guide antibiotic usage, mitigate current resistance, and slow the emergence of new resistance (1,2). This information also serves as a reference for empirical antibiotic treatments, particularly in severely ill patients (3).

At the Clinical Center University of Sarajevo (CCUS), systematic monitoring began in 2013, with methodologies developed through inter-laboratory standardization and high-quality preparation of bacterial susceptibility tests.

Comparative analysis of 2023 (16) and 2024 isolates revealed concerning increases in resistance among several clinically relevant pathogens. Among Gram-negative bacteria, Escherichia coli, Klebsiella pneumoniae, and Enterobacter cloacae exhibited rising resistance to third- and fourth-generation cephalosporins, consistent with the spread of ESBL-producing strains. Notably, E. coli showed a marked increase in ceftazidime and cefepime resistance, while K. pneumoniae maintained high resistance to beta-lactams, with modest decreases in cephalosporins but persistent ESBL activity. E. cloacae and Proteus mirabilis also displayed increased cephalosporin resistance, signaling emerging ESBL prevalence. Fluoroquinolone resistance rose in E. coli, Pseudomonas aeruginosa, and E. cloacae, whereas carbapenem resistance remained negligible in Enterobacteriaceae but increased in P. aeruginosa. Acinetobacter baumannii remained highly resistant across nearly all classes, with colistin largely retaining activity, although a colistin-resistant isolate emerged in 2024.

Among Gram-positive organisms, Enterococcus faecium exhibited substantial increases in vancomycin and teicoplanin resistance, while Staphylococcus aureus showed a modest rise in oxacillin resistance, indicating ongoing local circulation of MRSA and VRE strains. Nitrofurantoin remained largely effective against E. faecalis, and linezolid retained full activity against E. faecium.

These patterns align with global AMR trends reported by WHO through the Global Antimicrobial Resistance and Use Surveillance System (GLASS). The WHO GLASS 2024 report indicated that a substantial proportion of laboratory-confirmed bacterial infections worldwide exhibited resistance to first-line antibiotics, with increases observed across multiple pathogen–antibiotic combinations (10,11). Gram-negative pathogens such as E. coli and K. pneumoniae show high resistance to third-generation cephalosporins and fluoroquinolones, with notable increases in carbapenem resistance in many regions (10,12).

Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) further emphasize the severity of AMR in Europe. The EARS-Net 2024 Annual Epidemiological Report identified rising rates of third-generation cephalosporin-resistant E. coli and carbapenem-resistant K. pneumoniae bloodstream infections, despite ongoing interventions aimed at EU 2030 reduction targets (2,7,14). While MRSA incidence in invasive infections has declined in some European countries, resistance patterns vary considerably by pathogen and antibiotic class, with vancomycin-resistant E. faecium and multidrug-resistant non-fermenters remaining significant concerns (14).

Our local data align with these broader surveillance patterns in several ways. The observed increase in fluoroquinolone resistance among E. coli and P. aeruginosa mirrors global and European surveillance reports, often linked to extensive antibiotic use in community and hospital settings (1,6,8). High levels of resistance in A. baumannii, with only colistin retaining effective activity and rare emergence of colistin resistance in 2024, reflect global concerns regarding this pathogen's ability to accumulate resistance determinants and the shrinking efficacy of last-line antibiotics (4,16,18).

Among Gram-positive organisms, the rise in vancomycin and teicoplanin resistance in E. faecium underscores a particularly troubling trend. EARS-Net data indicate that vancomycin-resistant enterococci are increasingly documented in nosocomial infections in several European regions (14). Similarly, our data showing a modest rise in oxacillin resistance in S. aureus contrasts with decreasing MRSA bloodstream infection incidence in Europe, suggesting possible local epidemiological dynamics that differ from invasive surveillance data (2,7,14).

WHO's global overview highlights substantial regional heterogeneity: resistance rates are highest in the Southeast Asian and Eastern Mediterranean Regions, where up to one in three infections may be resistant to key antibiotics, and lower but still significant in the Americas and Europe (10,11). This underscores that AMR is a global issue with regional variability, influenced by antibiotic use policies, surveillance capacity, and healthcare infrastructure (1,4).

Collectively, these findings emphasize the urgent need for strengthened antimicrobial stewardship, enhanced laboratory and surveillance infrastructure, and tailored infection prevention strategies (14, 19). The global escalation of resistance across both commonly used and last-line antibiotics signals that, without coordinated action at local, national, and international levels, treatment options will continue to narrow, with significant implications for patient outcomes and healthcare systems (8,20).

CONCLUSION

Surveillance at the Clinical Center University of Sarajevo in 2024 revealed a high prevalence of multidrug-resistant Gram-negative and Gram-positive pathogens, highlighting the ongoing challenge of antimicrobial resistance in local healthcare settings. Notably, an increase in cephalosporin resistance among Escherichia coli and Klebsiella pneumoniae confirms the spread of ESBL-producing strains, while carbapenem resistance in Enterobacteriaceae poses a serious therapeutic concern. Resistance in Acinetobacter baumannii and Pseudomonas aeruginosa, with colistin remaining the only reliably effective agent, underscores the vulnerability of last-line antibiotics. Among Gram-positive organisms, Enterococcus faecium displayed increased vancomycin and teicoplanin resistance, whereas Staphylococcus aureus exhibited a slight rise in oxacillin resistance, emphasizing the need for continued monitoring of MRSA and VRE. These findings underscore the importance of local AMR surveillance to guide empiric therapy, inform infection control measures, and support antimicrobial stewardship. Continuous antimicrobial resistance surveillance, adherence to EUCAST-guided stewardship, and rigorous infection prevention measures are essential to mitigate the spread of multidrug-resistant organisms.

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Effect of Hospital-Based Physical Rehabilitation on Beck Depression Inventory Scores in Patients with Lumbar Pain Syndrome

Uticaj stacionarne fizikalne rehabilitacije na rezultate Beckove skale depresije kod pacijenata sa lumbalnim bolnim sindromom

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ABSTRACT

Lumbar pain syndrome is a leading cause of disability worldwide and is frequently associated with depressive symptoms that adversely affect rehabilitation outcomes. Therefore, addressing psychological factors is essential for comprehensive patient management. Aim: to evaluate the effect of inpatient physical rehabilitation on depressive symptoms in patients with lumbar pain syndrome using the Beck Depression Inventory (BDI) and to examine changes in depression in relation to functional abilities and daily activities. Materials and methods: this prospective clinical study included 60 patients of both sexes hospitalized at the Clinic of Physical Medicine and Rehabilitation, Clinical Center University of Sarajevo. Depressive symptoms were assessed using the Beck Depression Inventory at the time of admission and discharge. Functional status and participation were evaluated according to the International Classification of Functioning, Disability, and Health (ICF). Statistical analysis included descriptive statistics, t-test, chi-square test, and ANOVA, with significance set at $p < 0.05$. Results: the mean BDI score significantly decreased from 14.3 ± 0.7 at admission to 12.5 ± 0.6 at discharge ($t = 5.085$, $p = 0.001$). A significant redistribution of patients across depression categories was observed, with the elimination of moderate depression at discharge ($\chi^2 = 11.291$, $p = 0.001$). Improvements in depressive symptoms were significantly associated with enhanced physical function, reduced pain, improved mobility, better sleep, and increased exercise tolerance ($p < 0.001$). Furthermore, improvements in activities and participation, including gait, work ability, self-care, and household activities, were significantly correlated with lower BDI scores at the time of discharge ($p < 0.05$). Conclusion: inpatient physical rehabilitation leads to statistically significantly reduced depressive symptoms in patients with lumbar pain syndrome. Improvements in functional abilities and daily activities are closely associated with better emotional status. These findings emphasize the importance of an integrated multidisciplinary approach that addresses both the physical and psychological aspects of rehabilitation.

Keywords: low back pain, depressive disorder, Beck Depression Inventory, rehabilitation, quality of life, functional status

SAŽETAK

Lumbalni bolni sindrom predstavlja jedan od vodećih uzroka invaliditeta u svijetu i često je povezan s depresivnim simptomima koji nepovoljno utiču na ishode rehabilitacije. Stoga je u sveobuhvatnom zbrinjavanju pacijenata neophodno uzeti u obzir i psihološke faktore. Cilj: ispitati efekat stacionarne fizikalne rehabilitacije na depresivne simptome kod pacijenata sa lumbalnim bolnim sindromom primjenom Beckove skale za depresiju (BDI), te analizirati promjene depresivnosti u odnosu na funkcionalne sposobnosti i svakodnevne aktivnosti. Materijali i metode: ova prospektivna klinička studija obuhvatila je 60 pacijenata oba spola hospitaliziranih na Klinici za fizikalnu medicinu i rehabilitaciju Kliničkog centra Univerziteta u Sarajevu. Depresivni simptomi procjenjivani su primjenom Beckove skale za depresiju pri prijemu i otpustu. Funkcionalni status i učešće u aktivnostima evaluirani su prema Međunarodnoj klasifikaciji funkcionisanja, invaliditeta i zdravlja (ICF). Statistička analiza obuhvatila je deskriptivnu statistiku, t-test, hi-kvadrat test i ANOVA test, uz nivo statističke značajnosti $p < 0,05$. Rezultati: prosječna vrijednost BDI skora značajno je smanjena sa 14,3 ± 0,7 pri prijemu na 12,5 ± 0,6 pri otpustu ($t = 5,085$; $p = 0,001$). Utvrđena je značajna redistribucija pacijenata unutar kategorija depresije, uz potpuno odsustvo umjerene depresije pri otpustu ($\chi^2 = 11,291$; $p = 0,001$). Poboljšanje depresivnih simptoma bilo je značajno povezano s unapređenjem fizičkih funkcija, smanjenjem bola, poboljšanjem pokretljivosti, kvaliteta sna i tolerancije napora ($p < 0,001$). Također, unapređenje aktivnosti i participacije, uključujući hod, radnu sposobnost, samostalnu njegu i obavljanje kućnih poslova, bilo je značajno korelirano s nižim BDI skorovima pri otpustu ($p < 0,05$). Zaključak: stacionarna fizikalna rehabilitacija dovodi do statistički značajnog smanjenja depresivnih simptoma kod pacijenata sa lumbalnim bolnim sindromom. Poboljšanje funkcionalnih sposobnosti i svakodnevnih aktivnosti usko je povezano s boljim emocionalnim statusom pacijenata. Ovi nalazi naglašavaju značaj integrisanog, multidisciplinarnog pristupa koji obuhvata i fizičke i psihološke aspekte rehabilitacije.

Ključne riječi: lumbalni bolni sindrom, depresija, Beckova skala za depresiju, rehabilitacija, funkcionalna sposobnost, kvalitet života.

INTRODUCTION

Lumbar pain syndrome is one of the most common social health problems in everyday clinical practice. Globally, it is one of the leading causes of disability and absence from work. Data show that almost 80% of people will experience an episode of lower back pain at least once in their lifetime. In addition to the fact that it is primarily a health problem, lumbar pain syndrome also has social and psychological repercussions for the patient, due to the fact that patients, due to physical disability, have a consequent reduced working capacity, or reduced social contact, which ultimately contributes to a reduced quality of life (1,2).

Due to the strong social implications for the patient, lumbar pain syndrome brings a new dimension to the treatment and rehabilitation of patients. As the duration of lumbar pain increases, the influence of social, economic, and psychological factors on the course of the disease increases compared to physical factors, and their neglect significantly reduces the effects of treatment (3,4). Assessing the patient's psychological state and directing the activities of all team members towards adequate psychological support significantly improves the effects of other forms of conservative treatment (5). Patient education is important: introducing the patient to the nature, course, and expected positive outcome of the disease, providing ergonomic advice, and encouraging the patient to develop their own behavioral patterns that will help them heal faster (6).

Due to the complexity of the overall condition of such patients, lumbar pain syndrome is increasingly viewed through a psychosocial model, where the connection between physical symptoms and social and psychological factors is indicated (4). Such patients often develop depressive symptoms, which can affect the overall rehabilitation process, adherence to therapy, and final outcome of treatment. The prevalence of major depression in patients with chronic low back pain is approximately three to four times greater than that in the general population (7,8). Patients with depression are more likely to experience poorer health outcomes, contributing to higher rates of disability. This results in significant economic burdens due to reduced productivity, higher medical costs, and poorer social outcomes associated with reduced functional capacity (9,10).

The Beck Depression Inventory (BDI) is a 21-item self-report scale designed to assess depressive symptoms and severity in a clinically diagnosed group. In clinical practice, an instrument for assessing the intensity of depressive symptoms is often used (11).

The main goal of rehabilitation is to improve the functional status of patients while reducing pain and enabling them to perform daily activities as fully as possible (12). The use of this scale in patients with lumbar pain syndrome allows for an objective assessment of the patient's emotional state and monitoring during the rehabilitation process (11). Adequate rehabilitation procedures in patients will not only lead to the withdrawal of somatic symptoms but also to a reduction or complete loss of depressive symptoms through gradual progress in the functional independence of patients. The lack of accurate knowledge of the role of depression in the course of low back pain prevents clinicians from providing appropriate information and advice for patients with both conditions (11-13).

Bearing in mind the previously mentioned psychological component in the treatment of lumbar pain syndrome, we aimed to examine the effect of inpatient rehabilitation on the values of the Beck depression scale in patients with lumbar pain syndrome and to assess changes in depressive symptoms at the beginning and end of rehabilitation.

AIM

The aim of the study was to evaluate the effect of inpatient physical rehabilitation on depressive symptoms in patients with lumbar pain syndrome using the Beck Depression Inventory (BDI) and to examine changes in depression in relation to functional abilities and daily activities

MATERIALS AND METHODS

The research was designed as a clinical, prospective study that was conducted at the Clinic of Physical Medicine and Rehabilitation, Clinical Center University of Sarajevo, in the period of one year. A total of 60 patients of both sexes were included in the study. All patients signed an informed consent form to participate in the study before being included in the study and were fully familiar with the research protocol. The study was conducted with the approval of the Ethics Committee of the University Clinical Center Sarajevo and in accordance with the Helsinki Declaration on Patients' Rights (14). The authors of this study guarantee the confidentiality of patient data.

The inclusion criteria for the study were as follows: patients over 18 years of age, confirmed diagnosis of lumbar pain syndrome by clinical examination and medical documentation, patients hospitalized at the Clinic for Physical Medicine and Rehabilitation, University Clinical Center Sarajevo and included in inpatient physical treatment, stable general condition that allows participation in the rehabilitation program and testing, and patients with preserved cognitive ability that allows understanding and completing the questionnaire. The exclusion criteria were as follows: minor patients; patients who refuse to participate in the study; presence of a severe psychiatric disorder (severe depression, bipolar disorder, psychosis) diagnosed before admission; active treatment with antidepressants or other psychotropic medications initiated immediately before or during hospitalization; presence of malignant diseases, systemic inflammatory diseases, or other serious chronic conditions that can significantly affect the psychological state. After providing written informed consent, all participants underwent a standardized physical examination and comprehensive clinical assessment. Depressive symptoms were evaluated using the Beck Depression Inventory (BDI) at two time points: upon admission to the inpatient rehabilitation program and at discharge following completion of the rehabilitation course. The BDI is a 21-item self-report instrument designed to assess the presence and severity of depressive symptoms in clinically evaluated populations. Each item is scored according to a standardized grading system, and the total score is calculated as the sum of individual item scores. Depression severity was categorized as follows: 1-10 points, no clinically significant depression; 11-16, mild mood disturbance; 17-20, borderline clinical depression; 21-30, moderate depression; 31-40, severe depression; and >40, extreme depression (11).

The International Classification of Functioning, Disability, and Health (ICF) was used as a predictor of program evaluation and treatment outcomes in rehabilitation (15). Using this scale, the components of "Body Function and Structure" and "Activities and Participation" were compared with the assessment areas and specific items that appear in the Beck Depression Inventory.

Randomization was performed using a randomization list prepared by an independent statistician employing the Random Sorting Using Maximum Allowable % Deviation algorithm. This method ensures a well-balanced longitudinal allocation by measuring the deviation of the actual sample size for each group from the expected size after each assignment. If the deviation exceeded the pre-specified maximum allowable percentage, the list was discarded, and the randomization process was repeated until the criteria for individual allocation were satisfied.

Statistical analysis

Statistical data processing was performed using IBM SPSS Statistics v. 20.0 for Windows. Descriptive statistics were applied according to the distributional characteristics of the data. Continuous variables were summarized as mean ± standard deviation or median with interquartile range, as appropriate.

Categorical variables were presented as frequencies and percentages. Between-group differences in categorical variables were assessed using the chi-square (X^2) test. For comparisons of continuous variables across independent groups, analysis of variance (ANOVA) was performed when assumptions of normality and homogeneity of variance were satisfied. Statistical significance was defined as a two-sided p-value < 0.05.

RESULTS

A statistically significant reduction in depressive symptom severity was observed following completion of the inpatient rehabilitation program. The mean BDI score decreased from 14.25 ± 5.76 at admission to 12.50 ± 4.74 at discharge. The mean paired difference was 1.75 points (SD = 2.66), and the paired-samples t-test demonstrated statistical significance (t = 5.09, df = 59, p = 0.001). These findings indicate a significant improvement in depressive symptoms over the course of rehabilitation (Table 1).

Table 1 **Average value of Back's depression scale in subjects with lumbar pain syndrome (n=60).**

Time Point	N	Mean ± SD
Admission	60	14.25 ± 5.76
Discharge	60	12.50 ± 4.74

Paired-samples t-test (t = 5.09, df = 59, p = 0.001)

Table 2 **Patient profile according to Back's depression scale in subjects with lumbar pain syndrome (n=60).**

BDI Category	Admission n (%)	Discharge n (%)
Normal	16 (26.7%)	19 (31.7%)
Mild mood disorder	18 (30.0%)	28 (46.7%)
Borderline clinical depression	19 (31.7%)	13 (21.7%)
Moderate depression	7 (11.7%)	0 (0.0%)
Total	60 (100%)	60 (100%)

x2=30.437; p=0.001

The distribution of depressive symptom severity categories significantly changed between admission and discharge ($\square^2 = 30.44$, p = 0.001). At baseline, the majority of patients were classified within the mild (30.0%) or borderline clinical depression (31.7%) categories, while 11.7% exhibited moderate depressive symptoms. Following completion of the inpatient rehabilitation program, a shift toward lower severity categories was observed. The proportion of patients with mild mood disturbance increased from 30.0% to 46.7%, and the proportion classified as normal increased from 26.7% to 31.7%. Notably, no patients met criteria for moderate depression at discharge.

Concurrently, the proportion of patients with borderline clinical depression decreased from 31.7% to 21.7%, indicating a clinically relevant downward shift in depressive severity. These findings suggest a significant redistribution of depressive symptom burden toward lower severity categories following rehabilitation, supporting the association between structured physical rehabilitation and improvement in psychological outcomes (Table 2).

Table 3 **Relationship between physical functions and Back's depression scale at admission and discharge in subjects with lumbar pain syndrome (n=60).**

Functional Domain	Category	N	BDI Admission (Mean±SD)	BDI Discharge (Mean±SD)	Mean Change	p-value[1]
Pain functions	Yes	60	14.25 ± 5.76	12.50 ± 4.74	−1.75	<0.001
	No	23	16.00 ± 5.43	12.91 ± 4.36	−3.09	<0.001
Emotional function	Anxiety	42	12.02 ± 5.39	11.04 ± 4.57	−0.98	<0.001
	No anxiety	18	19.44 ± 2.09	15.88 ± 3.21	−3.56	0.014
Muscle strength	Weakness	60	14.25 ± 5.76	12.50 ± 4.74	−1.75	<0.001
Joint mobility	Limited	28	15.39 ± 5.43	13.00 ± 4.21	−2.39	<0.001
	Preserved	32	13.25 ± 5.95	12.06 ± 5.19	−1.19	<0.001
Exercise tolerance	Fatigue	37	13.16 ± 5.77	12.24 ± 5.00	−0.92	<0.001
Sleep function	Disturbed	25	11.36 ± 5.13	11.08 ± 5.02	−0.28	<0.001
	Normal	35	16.31 ± 5.34	13.51 ± 4.32	−2.80	<0.001
Muscle endurance	Intolerance	40	14.45 ± 5.85	12.67 ± 4.69	−1.78	<0.001
	No intolerance	20	13.85 ± 5.71	12.15 ± 4.95	−1.70	<0.001
Muscle tone	Increased	46	13.73 ± 5.49	12.28 ± 4.66	−1.45	<0.001
	Normal	14	15.92 ± 6.53	13.21 ± 5.10	−2.71	0.002
Joint stability	Yes	60	14.25 ± 5.76	12.50 ± 4.74	−1.75	<0.001
Energy functions	Motivated	60	14.25 ± 5.76	12.50 ± 4.74	−1.75	<0.001

[1]Paired-samples t-test

The analysis of BDI scores across functional domains demonstrated a consistent reduction in depressive symptom severity following inpatient rehabilitation (Table 3). Across all examined domains, mean BDI scores were lower at discharge compared with admission, and the observed within-group differences were statistically significant. In the domain of pain functions, patients reporting pain-related limitations showed a reduction in BDI scores from 14.25 ± 5.76 to 12.50 ± 4.74 (mean change -1.75; $p < 0.001$). A larger absolute change was observed among patients without reported pain limitations (-3.09; $p < 0.001$), although baseline BDI values were higher in this subgroup.

Within the emotional function domain, patients categorized with anxiety had lower baseline BDI scores compared with those without anxiety; however, both subgroups demonstrated statistically significant improvement at discharge. Notably, patients without anxiety exhibited a greater mean reduction (-3.56; $p = 0.014$), which is consistent with their higher initial depressive burden. Regarding joint mobility, muscle endurance, and muscle tone, reductions in BDI scores ranged between -1.19 and -2.71 points, with statistically significant within-group differences across categories. Similar patterns were observed in exercise tolerance and sleep function domains. The magnitude of change was generally larger among subgroups with higher baseline depressive scores, suggesting regression toward lower severity categories following rehabilitation. Importantly, the direction of change was uniform across domains. No functional category demonstrated worsening of depressive symptoms at discharge. This consistency indicates that the reduction in BDI scores was not confined to a single physical impairment but occurred broadly across functional strata. Given the repeated within-subject design, these findings reflect temporal improvement rather than between-group comparisons.

However, the observational nature of the study precludes causal inference. The data support an association between functional rehabilitation and reduced depressive symptom severity, with the magnitude of change appearing greater in patients presenting with higher baseline psychological burden.

Across all examined functional domains, mean BDI scores decreased between admission and discharge, with statistically significant within-subject differences observed in every category (all $p < 0.001$) (Table 4). The magnitude of reduction was consistent, ranging from -1.68 to -1.87 points. At admission, mean BDI values were highly comparable across domains (approximately 14.20–14.28), indicating a uniform baseline level of depressive symptom severity throughout the cohort. At discharge, mean values decreased to a narrow range of 12.41–12.52, suggesting a homogeneous pattern of improvement. The mean change in BDI scores was modest but stable across domains: -1.75 for maintaining body position, -1.87 for lifting objects, -1.68 for maintaining posture, -1.77 for gait, and -1.73 for work ability. Similar reductions were observed in domestic activities, dressing, stress management, family relationships, toileting, and employment status.

Importantly, the near-identical magnitude of change across domains indicates that the observed improvement in depressive symptoms was not domain-specific. Rather, it reflects a global reduction in depressive symptom severity over the course of rehabilitation.

Given the paired design, these results represent a temporal within-cohort improvement. The consistency of findings across functional categories strengthens the internal coherence of the data. However, as no control group was included, the observed changes should be interpreted as associations with rehabilitation rather than definitive causal effects.

Table 4 **Analysis of activity and participation in relation to the Back Depression Scale at admission and discharge in patients with lumbar pain syndrome (n=60).**

Functional Domain	N	BDI Admission (Mean±SD)	BDI Discharge (Mean±SD)	Mean Change	p-value[1]
Maintaining body position	60	14.25 ± 5.78	12.50 ± 4.76	−1.75	<0.001
Lifting object	60	14.28 ± 5.71	12.41 ± 4.71	−1.87	<0.001
Maintaining posture	60	14.20 ± 5.64	12.52 ± 4.78	−1.68	<0.001
Gait	60	14.27 ± 5.67	12.50 ± 4.70	−1.77	<0.001
Ability to work	60	14.20 ± 5.69	12.47 ± 4.74	−1.73	<0.001
Housework	60	14.24 ± 5.70	12.45 ± 4.75	−1.79	<0.001
Dressing	60	14.26 ± 5.66	12.48 ± 4.73	−1.78	<0.001
Stress management	60	14.25 ± 5.78	12.42 ± 4.77	−1.83	<0.001
Family relationship	60	14.26 ± 5.72	12.49 ± 4.74	−1.77	<0.001
Using toilet	60	14.24 ± 5.74	12.46 ± 4.76	−1.78	<0.001
Employment status	60	14.25 ± 5.73	12.48 ± 4.75	−1.77	<0.001

[1] Paired-samples t-test

DISCUSSION

Mental and physical health is connected to a large extent through negative stereotypes about the loss of health and physical and psychological functions with aging. Negative stereotypes can increase psychological susceptibility to illness if people internalize them and therefore do not seek help, and if such stereotypes are internalized by caregivers (16).

Functional abilities are required to perform daily activities (17,18). Along with physical health, functional abilities are the best predictors of mental health (19). Weaker functional abilities are a strong predictor of further weakening of these abilities. With greater difficulties in physical functional abilities, depressive symptoms increase, and life satisfaction decreases (20).

It is well established that functional disability increases with age (21). Chronic health conditions contribute to the reduction of functional abilities, and functional incapacity leads to depression. Declining physical activity is associated with poor mental health. The ability to move and greater physical activity are generally shown to be significantly related to life satisfaction and subjective well-being (19,20). In most cases, older adults perceive mobility as a condition they cannot live without because it is a leading force for life expectations (21). Good health is associated with activity, and activities imply that a person has goals. The association between physical activity, even moderate physical activity, and better cognitive functioning has been proven in many studies (22). Adequate rehabilitation changes circulation, muscle tone, and a whole range of other aspects of physical functioning. Consequently, the risk of depression can be prevented (23).

In the present study, lumbar pain syndrome was significantly associated with the presence of depressive symptoms. Furthermore, participation in a structured inpatient rehabilitation program was associated with a significant reduction in depressive symptom severity. The majority of patients exhibited mild depressive symptoms at baseline. A statistically significant difference in Beck Depression Inventory (BDI) scores was observed between admission and discharge ($p = 0.001$), indicating improvement following rehabilitation. These findings are consistent with previous evidence. Srivastava S, et al. reported that patients with major depressive disorder demonstrated poorer sleep quality, reduced quality of life, and greater functional disability. Moreover, individuals with severe functional disability due to low back pain exhibited more pronounced depressive symptoms, poorer sleep quality, and diminished quality of life, with depression severity positively correlated with the degree of functional impairment (24).

Cai L, et al. found that lumbar disk herniation induced depressive behavior that persisted for 6 weeks after surgery. These behavioral changes were associated with decreased 5-HT concentrations and increased TNF-α mRNA levels. However, fluoxetine treatment improved depressive behavior and moderately alleviated pain by increasing 5-HT concentrations and inhibiting TNF-α mRNA expression (25).

An additional structural analysis of depression showed that the number of patients with moderate depression decreased from 7 to 0 after rehabilitation, while the number of patients with borderline clinical depression decreased from 19 to 13. Simultaneously, an increase in the number of participants with normal findings and mild mood disorders was recorded. A statistically significant difference ($p=0.001$) indicated that rehabilitation affected not only the average value of the scale but also the redistribution of patients within depression categories. An individually created rehabilitation protocol for each patient leads to a decrease in overall pain and greater functional ability (26), and we have shown that the alleviation of these symptoms consequently has a direct positive impact on the emotional state of the patient. In our study, no patient had a moderate degree of depression upon discharge, suggesting that a proper rehabilitation approach may also have a preventive role in the progression of depressive symptoms in people with lumbar pain syndrome.

Additional analyses of physical function demonstrated a strong association between depressive symptom severity and the degree of functional limitation. Patients with more pronounced functional impairment at admission exhibited significantly higher BDI scores compared with those with milder functional deficits. Following completion of the rehabilitation program, statistically significant improvements were observed across all assessed domains of functional ability ($p < 0.001$). These findings underscore the multidimensional nature of chronic lumbar pain, suggesting that its impact extends beyond physical disability and substantially affects emotional well-being. The observed relationship between functional impairment and depressive symptoms aligns with previous research. Tsuji T, et al. reported that patients with chronic low back pain and comorbid depression experienced significantly greater pain intensity compared with non-depressed patients ($p < 0.001$), and that depression was associated with more pronounced reductions in health-related quality of life (27). Sagheer MA, et al. investigated the prevalence of anxiety and depression among patients with chronic low back pain in a tertiary care setting and reported a high risk of psychological comorbidity, particularly among female patients (28). In contrast, our findings did not demonstrate statistically significant sex-based differences in depressive symptom severity.

This discrepancy may reflect differences in study design, population characteristics, cultural context, or sample size, and highlights the importance of considering demographic and contextual factors when interpreting the relationship between chronic pain and psychological outcomes. In the present study, patients reporting sleep disturbances exhibited more pronounced depressive symptoms, supporting previous findings that chronic pain, sleep disorders, and depression are closely interrelated (29-31). Improvement in pain intensity following structured rehabilitation may have contributed to better sleep quality, which in turn was associated with a reduction in depressive symptom severity. Although causality cannot be inferred due to the observational design, the findings suggest a clinically relevant interaction between pain modulation, sleep regulation, and psychological well-being.

Further analysis examining the relationship between psychological status and domains of activity and participation revealed that patients who experienced greater difficulties in daily activities at admission had significantly higher BDI scores. After completion of the rehabilitation program, a statistically significant improvement was observed in both functional performance and depressive symptoms ($p < 0.05$). These findings are consistent with those of Haghgoo HA, et al., who reported a strong association between daily activity performance, depression severity, and quality of life, suggesting that promoting engagement in daily activities may enhance overall well-being (32).

Collectively, the results indicate that increasing functional independence and facilitating reintegration into daily activities are associated with meaningful psychological benefits in patients with chronic lumbar pain.

The absence of severe depressive symptoms in patients in our study may be due to the timely inclusion of patients in the rehabilitation program, which may have a protective effect. Our research was conducted at the tertiary level of health care, where all patients were provided with a multidisciplinary approach that included not only physical therapy but also continuous medical supervision and support, which we believe contributed to greater motivation and cooperation of the patient during rehabilitation. In the clinical context, this was reflected as an improvement in the patients' emotional state. Previous research has also emphasized the role of patient motivation in the final rehabilitation outcome (33,34).
Our study had certain limitations. The follow-up period was limited exclusively to hospitalization, without long-term follow-up of the effect. In addition, our study did not include a control group; therefore, we cannot conclude that the improvement was solely the result of an adequate rehabilitation program.

CONCLUSION

Inpatient physical rehabilitation was associated with a statistically significant reduction in depressive symptom severity among patients with low back pain syndrome. Given the observational design of the study, causal inferences cannot be established; however, the findings suggest a clinically meaningful relationship between structured rehabilitation and improvements in psychological outcomes. These results reinforce the relevance of an integrated, multidisciplinary approach that concurrently addresses both the somatic and psychological components of chronic low back pain. Structured rehabilitation programs may therefore represent an important component of comprehensive management strategies aimed at enhancing functional recovery while supporting emotional well-being in routine clinical practice.

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Impact of Arteriovenous Fistula on Carpal Tunnel Syndrome and Peripheral Neuropathy in Hemodialysis Patients: A Prospective Study at the Clinical Center University of Sarajevo

Učinak arteriovenske fistule na znakove sindroma karpalnog kanala i perifernu neuropatiju kod pacijenata na hemodijalizi: prospektivna studija u Kliničkom centru Univerziteta u Sarajevu

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ABSTRACT

Introduction: peripheral neuropathy is a common complication in patients with advanced chronic kidney disease (CKD), affecting up to 60–100% of those on dialysis. Hemodialysis patients also frequently develop carpal tunnel syndrome (CTS), due to systemic factors like β_2-microglobulin amyloidosis and local influences such as arteriovenous (AV) fistulas, which may cause nerve damage through venous hypertension or ischemia. Aim: to evaluate the impact of arteriovenous fistulas on the incidence of carpal tunnel syndrome and on the occurrence and severity of peripheral neuropathy in hemodialysis patients. Materials and methods: we conducted a three-month prospective study involving 30 hemodialysis patients who met strict inclusion criteria. All underwent clinical evaluations and nerve conduction studies to assess CTS and polyneuropathy, with analysis of their correlation to AV fistula laterality. Results: CTS was confirmed in 66.7% of patients, and in 33.3% the signs were on the same side as the fistula. Polyneuropathy was found in 80%, with 80% showing greater involvement on the fistula side. No significant association was found between fistula side and CTS (p=0.991) or neuropathy presence (p=0.719). However, neuropathy severity significantly correlated with the fistula side (p=0.023). Conclusion: while AV fistulas did not influence the presence of CTS or neuropathy, they were associated with more severe neuropathy in the affected limb. Close neurological monitoring is advised, and larger studies are needed to further investigate these relationships.

Keywords: arteriovenous fistula, carpal tunnel syndrome, peripheral neuropathy, hemodialysis, chronic kidney disease

SAŽETAK

Uvod: periferna neuropatija je česta komplikacija kod pacijenata sa uznapredovalom hroničnom bubrežnom bolešću (HBB), prisutna kod 60–100% pacijenata na dijalizi. Hemodijalizirani bolesnici često razvijaju i karpalni tunelski sindrom (KTS), koji je češći u ovoj populaciji nego u općoj. Uzrok tome su sistemski faktori poput □□-mikroglobulinske amiloidoze, ali i lokalni, uključujući arterio-venske (AV) fistule koje mogu izazvati neuropatije šake zbog venske hipertenzije i ishemije. Cilj: procijeniti utjecaj arteriovenskih fistula na incidenciju sindroma karpalnog tunela te na pojavu i težinu periferne neuropatije kod pacijenata na hemodijalizi. Materijali i metode: u tromjesečnoj prospektivnoj studiji analizirano je 30 pacijenata na hemodijalizi koji su ispunjavali stroge kriterije uključenja. Provedeni su klinički pregledi i elektromioneurografija s ciljem utvrđivanja prisutnosti KTS-a i polineuropatije, uz korelaciju s lateralizacijom AV fistule. Rezultati: KTS je potvrđen kod 66,7% pacijenata, a kod 33,3% znakovi su bili na istoj strani kao fistula. Polineuropatija je otkrivena kod 80% ispitanika, a kod njih 80% bila je izraženija u ruci s fistulom. Nije utvrđena statistički značajna povezanost između strane fistule i prisutnosti KTS-a (p=0,991) ni polineuropatije (p=0,719). Ipak, ozbiljnost neuropatije značajno je korelirala sa stranom fistule (p=0,023). Zaključak: AV fistule nisu značajno utjecale na pojavu KTS-a ili neuropatije, ali su bile povezane s većom težinom neuropatije u toj ruci. Potrebno je pažljivo neurološko praćenje ovih pacijenata, a daljnja istraživanja s većim uzorkom mogla bi dodatno rasvijetliti ove odnose.

Ključne riječi: arteriovenska fistula, karpalni tunelski sindrom, periferna neuropatija, hemodijaliza, hronična bubrežna bolest.

INTRODUCTION

Peripheral neuropathy (PN) is a frequent complication of advanced CKD and end-stage renal disease, attributed to the accumulation of uremic toxins. By the time patients require dialysis, a large majority develop a distal symmetric polyneuropathy. Symptoms range from mild distal paresthesias to severe motor-sensory deficits, often progressing despite dialysis. In hemodialysis (HD) populations, additional neuropathies can coexist with uremic PN, compounding morbidity. One of the most prevalent is carpal tunnel syndrome (CTS), an entrapment of the median nerve at the wrist. CTS occurs in HD patients at higher rates than in the general population (reported in up to ~9% of HD patients) and some reports suggest it is more frequent in the arm bearing an AV fistula. The etiology of CTS in this setting is multifactorial. Long-term HD leads to dialysis-related amyloidosis (DRA): β microglobulin accumulates and deposits as amyloid in osteoarticular structures, with the carpal tunnel being a common site (1). As a result, CTS is a well-recognized manifestation of DRA in patients on dialysis for many years. Additionally, the hemodynamic and structural changes from an arteriovenous fistula - the preferred vascular access for HD - may contribute to nerve dysfunction. Elevated venous pressure and blood flow steal phenomena in the fistula arm can cause chronic ischemia of the median nerve or surrounding tissues (2). Repetitive needle trauma and local edema or hematomas around the fistula may further increase compartment pressure in the carpal tunnel. Indeed, prior studies have documented CTS in approximately 10% to over 40% of dialysis patients with AV fistulas, with higher prevalence among those with longer dialysis vintage. Despite these observations, it remains unclear to what extent an AV fistula per se predisposes patients to CTS or exacerbates underlying peripheral neuropathy, as opposed to systemic factors (3).

AIM

The aim of this study was to evaluate the impact of arterio venous fistulas on the incidence of carpal tunnel syndrome and on the occurrence and severity of peripheral neuropathy in hemodialysis patients. We hypothesized that if the AV fistula contributes significantly to focal or regional neuropathic processes, patients would show higher rates of CTS and more severe neuropathic changes in the fistula-arm compared to the non-fistula arm.

MATERIALS AND METHODS

This single-center observational study enrolled 30 patients undergoing maintenance hemodialysis at the Clinical Center University of Sarajevo. Patients were recruited over a three-month period (October-December 2025) and were selected based on inclusion and exclusion criteria designed to isolate the effects of the AV fistula on neuropathic outcomes. Inclusion criteria were: age ≥18, end-stage renal disease on HD via a mature AV fistula for at least 6 months, and willingness to undergo neurological assessment. Exclusion criteria included: presence of diabetes mellitus or other known causes of peripheral neuropathy (e.g. alcoholism, B12 deficiency), prior carpal tunnel release surgery, active cervical radiculopathy or plexopathy, and any acute neurological illness. Patient demographic and clinical data were recorded, including age, sex, primary kidney disease, dialysis vintage (years on dialysis), and AV fistula details (location and age of fistula). The research was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and all relevant national ethical standards.

A neurologic examination was performed on each patient, with emphasis on detecting peripheral neuropathy signs and focal median nerve compression signs. For peripheral neuropathy, we assessed symptoms such as distal limb numbness, tingling, burning pain, and weakness. A standardized neuropathy symptom score and examination score (including reflexes and sensory testing) were used to categorize neuropathy severity as absent, mild, moderate, or severe. Carpal tunnel syndrome signs were evaluated using Tinel's test and Phalen's maneuver at both wrists. Nocturnal hand paresthesia and hand grip weakness were noted. If a patient reported or exhibited CTS symptoms, these were recorded separately for each hand.

Nerve conduction studies (NCS) were conducted using a Dantec™ keypoint EMG/NCS machine, according to standard protocols. Median nerve conduction was tested across the wrist to confirm CTS: prolonged distal latency or slowed conduction velocity in the median nerve (with normal ulnar nerve parameters in the same hand) was taken as electrophysiological evidence of CTS. For peripheral neuropathy evaluation, both motor and sensory NCS were performed in at least four nerves (e.g., median, ulnar, peroneal, sural nerves). Abnormalities in amplitude, latency, or conduction velocity consistent with a length-dependent polyneuropathy (typically symmetric) were documented. We defined neuropathy severity in electrophysiological terms as follows: mild - minimal reduction in distal sensory amplitudes or borderline slowing of velocities; moderate - clear demyelination or axonal loss in distal nerves (usually legs) without proximal involvement; severe - marked abnormalities in both distal and proximal nerve segments, often with absent potentials in distal nerves. These electrophysiologic grades were cross-correlated with the clinical severity categories.

The primary outcomes were the presence of CTS and the presence of peripheral neuropathy in each patient. CTS was considered present if clinical signs and symptoms were confirmed by NCS criteria in either hand. Peripheral neuropathy was considered present if NCS showed a polyneuropathy pattern in the legs (with or without arm involvement). A critical aspect of analysis was the laterality of findings relative to the AV fistula. For each patient, we determined whether CTS (if present) was in the fistula arm or the contralateral arm. "CTS on fistula side" was defined as a positive clinical and NCS diagnosis of CTS in the wrist of the arm with the AV fistula. Patients with bilateral CTS were counted as having CTS on the fistula side as well. Similarly, we noted whether neuropathy signs (weakness, reflex loss) or abnormal NCS findings were present in the upper limb containing the fistula. "Neuropathy overlap with fistula" was defined as the presence of any peripheral neuropathy manifestation in the fistula arm (for example, significantly reduced sensory amplitude in the fistula-arm ulnar nerve, or hand muscle weakness on that side in the context of polyneuropathy).

Categorical data (such as presence/absence of CTS) are presented as counts and percentages. Continuous variables (such as age) are presented as mean ± standard deviation. We used the chi-square test (or Fisher's exact test when appropriate) to evaluate associations between fistula laterality (left vs right arm) and the occurrence of CTS, and between fistula laterality and the occurrence of peripheral neuropathy. We also analyzed the relationship between neuropathy severity category (none, mild, moderate, severe) and the occurrence of CTS on the fistula side. A chi-square test for trend was applied to assess whether higher neuropathy severity was associated with higher probability of CTS in the fistula arm. A p-value < 0.05 was considered statistically significant. Data were analyzed using SPSS v25.0 (IBM Corp).

RESULTS

The study included 30 hemodialysis patients (18 males and 12 females), with a mean age 55.2 ± 11.4 years (range 34-77 years). The etiologies of end-stage renal disease in the cohort were primarily hypertensive and glomerular diseases, with a minority of patients having polycystic kidney disease or other causes. The median dialysis vintage was 5.5 years (IQR 3–8 years). All patients had an upper-extremity AV fistula (26 radiocephalic at the wrist, 4 brachiocephalic at the elbow); 20 patients (66.7%) had the fistula in the left arm and 10 (33.3%) in the right arm. No patient had a failed fistula or was using a dialysis catheter at the time of evaluation. By design, none of the patients had diabetes or other major neuropathic comorbidities outside of uremia.

Peripheral neuropathy was common in this cohort. Based on clinical and NCS criteria, 24 out of 30 patients (80%) were found to have a uremic peripheral neuropathy (of at least mild degree). The remaining 6 patients (20%) had no evidence of peripheral neuropathy. Among the neuropathy-positive patients, we classified 5 patients as having mild neuropathy, 2 as moderate, and 17 as severe. The distribution of neuropathy severity generally correlated with dialysis vintage - for example, all patients with ≥10 years on dialysis fell into the severe neuropathy category.

Signs of carpal tunnel syndrome were detected in a substantial subset. Overall, 20 patients (66.7%) had clinical symptoms and NCS findings consistent with CTS in at least one hand. The majority of CTS cases were bilateral or occurred in the non-dominant hand; however, the relationship to fistula laterality was of special interest. Out of these, 10 patients (33.3% of the total sample) had CTS localized to the same side as their AV fistula (i.e., CTS in the fistula arm).

An additional 10 patients had CTS affecting the contralateral (non-fistula) hand (in a few cases CTS was bilateral, but for analysis those were counted as including the fistula side). Thus, the cohort was evenly split between those with CTS on the fistula side and those with CTS only on the opposite side.

We analyzed whether having an AV fistula in a given arm predisposed patients to ipsilateral CTS or neuropathy. The chi-square test for association between fistula laterality (left vs right) and occurrence of CTS did not show any significant relationship (X^2 = 0.0004, p = 0.991). In other words, patients with left-arm fistulas were just as likely to develop CTS in the right hand as in the left (fistula) hand, and vice versa. Similarly, there was no significant association between fistula laterality and the presence of peripheral neuropathy (p = 0.719) – virtually all long-term HD patients tended to develop neuropathy, regardless of which arm contained the access.

However, when considering neuropathy severity and its overlap with the fistula limb, a notable pattern emerged. Patients with more severe polyneuropathy tended to show a greater concordance between the neuropathy and the fistula arm. Specifically, among patients with mild neuropathy, only 1 out of 5 (20%) exhibited any neuropathic signs (including CTS) in the fistula arm. None of the patients with moderate neuropathy (0/2) showed involvement of the fistula arm. In contrast, in the severe neuropathy group, 9 of 17 patients (52.94%) had their neuropathy (including CTS or marked weakness/numbness) predominating in the fistula arm. These differences corresponded to a statistically significant association between neuropathy severity and CTS overlap with the fistula side (X^2 for trend = 5.17, p = 0.023). Notably, while the presence of CTS or neuropathy alone did not differ by fistula side (both yielded non-significant p-values), the laterality of severe neuropathy/CTS did show a significant skew toward the fistula side in the sickest nerves.

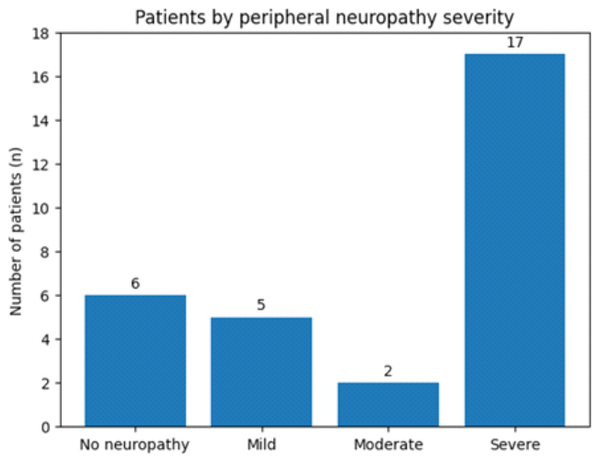

Figure 1 **Overlap of carpal tunnel syndrome (CTS) signs with the AV fistula side, stratified by peripheral neuropathy severity. Patients without neuropathy (n = 6) or with moderate neuropathy (n = 2) showed no cases of CTS occurring in the fistula arm, whereas 20% of those with mild neuropathy (n = 5) and 52.94% of those with severe neuropathy (n = 17) had CTS signs localized to the fistula arm. This trend of increasing CTS overlap in the fistula limb with greater neuropathy severity was statistically significant (p = 0.023).**

Table 1 **Association of AV Fistula with Neurological Outcomes (CTS = carpal tunnel syndrome; PN = peripheral neuropathy).**

Outcome Measure	Observation in Patients	Chi-square p - value
CTS on fistula side	10/30 patients (33.3%)	p = 0.991 (NS)
Peripheral neuropathy on fistula side	24/30 patients (80%)	p = 0.719 (NS)
CTS-fistula overlap by neuropathy severity	None: 0%; Mild: 20%; Moderate: 0%; Severe: 52.94%	p = 0.023 (S)

The key findings and statistical associations were further summarized in Table 1. The first row indicates the proportion of all patients who had CTS occurring in the same limb as the fistula (33.3%); the second row indicates the proportion with any neuropathic findings in the fistula limb (80%). Neither of these proportions differs from random expectation based on fistula laterality (non-significant p-values). The third row illustrates the increasing overlap of CTS in the fistula limb with greater neuropathy severity (from 0% in none/moderate neuropathy up to ~53% in severe neuropathy), a difference that was statistically significant ($p = 0.023$). These results suggest that while fistula location alone does not determine whether a patient gets CTS or neuropathy, it may influence where the neuropathy is worst when the overall neuropathic burden is high.

DISCUSSION

The present study found a high frequency of neurologic abnormalities among chronic hemodialysis patients with arteriovenous fistulas (AVFs). In particular, features of peripheral neuropathy were common, in keeping with the literature on uremic neuropathy. For example, Camargo CR, et al. (2019) reviewed uremic neuropathy and noted that distal symmetric sensorimotor polyneuropathy occurs in 60-100% of patients on dialysis. They emphasized the importance of early diagnosis and intervention to prevent progression. Similarly, we observed that many patients exhibited sensory deficits or nerve conduction slowing in a glove-stocking pattern, consistent with uremic neuropathy. This agreement suggests that chronic kidney disease and dialysis itself remain the dominant determinants of diffuse neuropathy in our cohort, as reported by Camargo CR, et al. (1).

Carpal tunnel syndrome (CTS) and other focal neuropathies were also frequently encountered in our AVF patients. Our findings agree with prior reports that CTS is a common complication of AVF creation. MacRae JM (2016) (2) reported that up to 9% of hemodialysis patients develop CTS, a markedly higher prevalence than in the general population. He noted that factors such as diabetes, uremia, β2-microglobulin deposition and especially the presence of an AV access can promote median nerve compression. We also took care to distinguish CTS and peripheral neuropathy from ischemic neuropathies. MacRae JM (2016) emphasized the importance of differentiating median nerve entrapment from ischemic steal or ischemic monomelic neuropathy (IMN) after AVF creation. In line with this guidance, we evaluated each patient's symptoms and vascular status; for example, patients with hand pain and weakness but warm hand temperature and preserved pulses were carefully assessed for IMN. Grant Y, et al. (2021) (3) similarly found that CTS occurs in roughly 10.4-42.6% of hemodialysis patients with an AVF, and that longer dialysis duration was associated with CTS development.

Kopeć J, et al. (2011) (4) provided a benchmark for CTS incidence and risk factors in dialysis populations. They performed nerve conduction studies in 386 patients and found CTS in 10.4% of cases. They identified dialysis vintage as a highly significant risk factor (mean dialysis 16.05 vs. 4.51 years for patients with vs. without CTS; $p<0.0001$). Notably, Kopeć J, et al. found no significant relationship between CTS and the anatomical side of the AVF or patient sex. Our findings were similar: we saw a strong association between dialysis age and CTS, whereas CTS occurrence did not differ significantly between dominant versus non-dominant arm fistulas.

In our cohort we observed no cases of acute IMN. This is consistent with the literature that IMN is rare. Sheetal S, et al. (2017) (5) reported IMN as a very uncommon but "devastating complication" of AVF creation, characterized by immediate, diffuse hand paralysis and sensory loss despite a warm well-perfused hand. The absence of IMN in our series is congruent with its low incidence; however, our awareness of this syndrome is important because of its potential for permanent disability.

Large database studies (6) have confirmed that CTS is substantially more common in dialysis-dependent populations compared to the general population. Larson et al. demonstrated a significantly increasing incidence of CTS and carpal tunnel release in dialysis patients over time. Our findings complement these epidemiological observations by providing electrophysiological data and by exploring the spatial relationship between CTS and the AV fistula limb.

Hatano M, et al. (2022) demonstrated that dialysis-related amyloidosis and β2-microglobulin accumulation represent the major systemic drivers of CTS development in long-term hemodialysis patients (7). Our findings support this systemic mechanism but suggest that local factors, such as the presence of an AV fistula, may influence the regional expression of neuropathic damage when the overall neuropathic burden becomes severe.

CONCLUSION

Our study demonstrates that while arteriovenous fistulas are not conclusively associated with a higher incidence of carpal tunnel syndrome or peripheral neuropathy per se, they do have a notable impact on neuropathy severity in the affected limb. Patients with AV fistulas should be closely observed for neurologic symptoms, as early detection of CTS or asymmetric neuropathy allows timely interventions that can significantly improve patient outcomes. The creation of a dialysis access, although life-saving, introduces unique physiologic changes in the limb; awareness of these changes is crucial for comprehensive dialysis patient care. Our findings highlight the importance of a multidisciplinary approach, involving nephrologists, neurologists, and vascular surgeons, to monitor and manage neuropathic complications in hemodialysis patients. We recommend further studies with larger sample sizes and longitudinal follow-up to better elucidate the causal pathways and to verify these preliminary findings. Ensuring optimal neurological health in dialysis patients will ultimately contribute to better functional status and quality of life in this vulnerable population.

Study limitations

This study has several limitations. The sample size (n=30) was relatively small, limiting the statistical power especially for subgroup analyses. Our population was from a single center and excluded diabetics, which, while done to isolate the effect of fistulas, also means our findings may not generalize to the broader HD population (where diabetes is a common cause of neuropathy). We also relied on cross-sectional observations; a longitudinal study could better assess causality (e.g., do neuropathy and CTS worsen after fistula creation in the same patients?). The grading of neuropathy severity, though based on objective criteria, has some semi-quantitative elements and might have classification bias. We did not directly measure steal syndrome severity (e.g., via ultrasound or digital pressures) in our patients, which could have strengthened the link between ischemia and neuropathy. Despite these limitations, the study provides valuable preliminary data on a topic that has been sparsely studied – namely, the localized impact of dialysis access on nerve function. Further research is warranted to explore the mechanisms behind our observations. A larger multi-center study could confirm whether neuropathy severity truly correlates with fistula-sided deficits. It would also be interesting to investigate if certain fistula characteristics (e.g., high flow vs low flow, proximal vs distal creation, left vs right side) influence neurological outcomes. Advanced imaging of the median nerve (using ultrasound or MRI) in HD patients could reveal subclinical nerve compression or ischemia associated with fistulas. Ultimately, understanding these relationships could guide modifications in vascular access practices - for instance, in patients with pre-existing neuropathy or prior CTS, clinicians might choose one arm over the other for fistula creation or opt for grafts to mitigate risk.

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Clinical and Anatomical Features of Venous Thrombosis: The Role of Provoking Factors and Biomarkers

Kliničke i anatomske karakteristike venske tromboze: uloga provocirajućih faktora i biomarkera

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ABSTRACT

Clinical presentation of venous thrombosis and risk factor profiles vary substantially across anatomical locations, and malignancy remains one of the most relevant provoking factors. Aim: to analyze clinical and anatomical characteristics of hospitalized patients with venous thrombosis, with emphasis on provoking factors and biomarker profiles across different thrombosis sites. Materials and methods: this retrospective study included 380 patients hospitalized with venous thrombosis at the Clinical Center University of Sarajevo (2020-2024). Clinical, laboratory, and imaging data were collected from electronic medical records. Thrombosis was categorized as proximal or distal lower extremity deep venous thrombosis (DVT), upper extremity DVT, central, or visceral venous thrombosis. Results: proximal lower extremity DVT predominated (88.9%), with iliac involvement being the most frequent site. Malignancy was the leading provoking factor (23.9%). D-dimer levels were significantly higher in patients with previous VTE, malignancy, and proximal thrombosis (all $p < 0.05$), while fibrinogen showed no significant variation. Age correlated positively with D-dimer and negatively with fibrinogen. Provoked thrombosis and malignancy were more frequent among women, whereas pulmonary embolism was more frequent in men. Enoxaparin was more frequently prescribed on discharge in patients with iliac thrombosis and malignancy. Conclusion: Proximal thrombosis, particularly iliac, was most common and linked to higher D-dimer and older age, while malignancy remained the main provoking factor, underscoring the need for improved risk stratification.

Keywords: deep venous thrombosis; venous thromboembolism; D-dimer; fibrinogen; malignancy

SAŽETAK

Klinička prezentacija venske tromboze i profili rizikofaktora značajno variraju u zavisnosti od anatomske lokalizacije, a malignitet ostaje jedan od najvažnijih provocirajućih faktora. Cilj: analizirati kliničke i anatomske karakteristike hospitalizovanih pacijenata s venskom trombozom, s posebnim naglaskom na provocirajuće faktore i profile biomarkera u različitim lokalizacijama tromboze. Materijal i metode: u ovu retrospektivnu studiju uključeno je 380 pacijenata hospitalizovanih zbog venske tromboze na Kliničkom centru Univerziteta u Sarajevu (2020–2024). Klinički, laboratorijski i radiološki podaci prikupljeni su iz elektronskih medicinskih zapisa. Tromboze su kategorizirane kao proksimalna ili distalna duboka venska tromboza (DVT) donjih ekstremiteta, DVT gornjih ekstremiteta, centralna ili visceralna venska tromboza. Rezultati: proksimalna DVT donjih ekstremiteta bila je najzastupljenija (88.9%), s najčešćim zahvatanjem ilijačnog segmenta. Malignitet je bio vodeći provocirajući faktor (23.9%). Vrijednosti D-dimera bile su značajno više kod pacijenata s prethodnim VTE, malignitetom i proksimalnom trombozom (sve $p < 0.05$), dok fibrinogen nije pokazao značajne razlike. Dob je pozitivno korelirala s D-dimerom, a negativno s fibrinogenom. Provocirane tromboze i malignitet bili su češći kod žena, dok je plućna embolija bila češća kod muškaraca. Enoksaparin je češće propisivan pri otpustu pacijentima s ilijačnom trombozom i malignitetom. Zaključak: proksimalna tromboza, posebno ilijačna, bila je najčešća i povezana s višim vrijednostima D-dimera i starijom dobi, dok je malignitet bio glavni provocirajući faktor, naglašavajući potrebu za boljom procjenom rizika.

Ključne riječi: duboka venska tromboza; venski tromboembolizam; D-dimer; fibrinogen; malignitet

INTRODUCTION

Venous thrombosis represents a significant clinical entity encompassing thrombus formation within the deep venous system of the extremities as well as in central and visceral veins. Deep venous thrombosis (DVT) of the lower extremities remains the most common manifestation. Venous thrombosis can occur as a provoked event, associated with factors such as surgery, trauma, immobilization, or malignancy, or as an unprovoked event when no clear precipitating factor is identified (1-3). Malignancy is one of the strongest contributors to thrombosis and frequently influences both the clinical course and treatment decisions (4). The anatomical distribution of DVT plays an important role in prognosis. Proximal lower extremity DVT (PLEDVT) carries a higher risk of embolization and complications compared to distal lower extremity DVT (DLEDVT) (1,5,6). Upper extremity, visceral, and central venous thrombosis, although less common, are increasingly recognized in hospitalized patients (7,8). D-dimer and fibrinogen are routine diagnostic biomarkers, yet their relationship with clinical features and thrombosis location is not fully understood (9-11).

AIM

The primary aim of this study was to evaluate the anatomical distribution of venous thrombosis and its association with biomarker levels (D-dimer and fibrinogen) in hospitalized patients. Secondary objectives included the analysis of provoking factors, demographic and clinical characteristics, and their relationship with thrombosis location.

MATERIALS AND METHODS

A total of 380 patients hospitalized with venous thrombosis at the Clinic for Heart, Blood Vessel and Rheumatic Diseases, Clinical Center University of Sarajevo (KCUS), between 2020 and 2024 were included in this retrospective study. DVT was confirmed by compression ultrasonography, while central and visceral venous thromboses were diagnosed using contrast-enhanced computed tomography (CT). Clinical, laboratory, and imaging data were obtained from patients' medical records. The analysis included demographic data, clinical characteristics (thrombosis location and laterality, pulmonary embolism (PE), previous venous thromboembolism (VTE), malignancy, and other provoking factors), laboratory parameters (D-dimer and fibrinogen), anticoagulant therapy at discharge, and in-hospital mortality.

D-dimer levels were measured using an immunoturbidimetric assay (STA®-Liatest® D-Di PLUS, Diagnostica Stago) on the STA-R MAX 3 analyzer (Diagnostica Stago, Asnières-sur-Seine, France). Fibrinogen levels were determined by the Clauss clotting method using the STA®-Liquid Fib reagent (Diagnostica Stago) on the same analyzer. Laboratory reference ranges were <0.55 mg/L for D-dimer and 2-4 g/L for fibrinogen. Measurements were performed at the time of admission or during the early phase of hospitalization. Because of the retrospective study design, information on prior anticoagulant therapy was not consistently available for all patients.

Thrombosis locations were categorized as follows: PLEDVT (popliteal, superficial femoral, common femoral, external and common iliac veins), DLEDVT (anterior/posterior tibial, peroneal, gastrocnemius, and soleal veins), upper extremity DVT (UEDVT) (internal jugular, subclavian, axillary, brachial veins), central venous thrombosis (inferior vena cava and renal vein), and visceral venous thrombosis (portal, splenic, superior and inferior mesenteric veins). PLEDVT was further classified into iliac, femoral, and popliteal segments.

Statistical Analysis

Statistical analysis was performed using SPSS (version 25; IBM Corp.). Normality of continuous variables was assessed using the Kolmogorov–Smirnov test; all showed a non-parametric distribution and were presented as median (interquartile range, IQR), while categorical variables were summarized as counts and percentages. Group comparisons were conducted using the Mann–Whitney U test for binary categorical variables and the Kruskal–Wallis test for variables with ≥3 categories. Associations between categorical variables were evaluated using the Chi-square test or Fisher's exact test when assumptions were not met. Effect sizes were calculated to assess the magnitude of observed differences. For Mann–Whitney U tests, effect size was expressed as r ($Z/\sqrt{N}$), while for Kruskal–Wallis tests, eta squared (η^2) was calculated. Effect sizes were interpreted as small ($r = 0.10$; $\eta^2 = 0.01$), moderate ($r = 0.30$; $\eta^2 = 0.06$), and large ($r = 0.50$; $\eta^2 = 0.14$). Correlations between continuous variables were assessed using Spearman's rank correlation coefficient. Variables with subgroup sizes <10% of the total sample were excluded from comparative analysis and described only. Statistical significance was set at $p < 0.05$.

RESULTS

The median age of the study population was 64 years (IQR 45–73), ranging from 17 to 93 years, with a similar gender distribution. Most cases involved PLEDVT (338 patients, 88.9%), with the iliac vein (176 patients, 52.2%) being the most frequently affected site. PE was confirmed in 50 patients (13.2%), and a previous history of VTE was documented in 63 patients (16.6%). Overall, 162 patients (42.6%) had provoked thrombosis. The most common provoking factor was malignancy, present in 91 patients (23.9%). The in-hospital mortality rate was 2.9% (11 patients). Regarding treatment at discharge, rivaroxaban was prescribed in 174 patients (47.2%), followed by enoxaparin in 113 patients (30.6%). Descriptive statistics of patient data are summarized in Table 1.

Table 1 **Descriptive statistics of patients hospitalized with venous thrombosis (2020 and 2024).**

Variable	Category	n (%) or Median (IQR)
Age	—	64 (45–73)
Gender	Female	195 (51.3)
	Male	185 (48.7)
Side of thrombosis	Right	152 (40.8)
	Left	211 (56.6)
	Bilateral	8 (2.1)
Location of thrombosis	PLEDVT	338 (88.9)
	Iliac vein	*176 (52.2)*
	Femoral vein	*136 (40.4)*
	Popliteal vein	*25 (7.4)*
	DLEDVT	10 (2.6)
	UEDVT	24 (6.3)
	IVC thrombosis	5 (1.3)
	Visceral vein thrombosis	3 (0.8)
Previous VTE	No	317 (83.4)
	Yes	63 (16.6)
PE	No	324 (85.3)
	Yes	50 (13.2)
	Suspected	6 (1.6)
Malignancy	No	279 (73.4)
	Yes	93 (24.4)
	Suspected	8 (2.1)
Recent surgery	No	366 (96.3)
	Yes	14 (3.7)
Immobilization or trauma	No	345 (90.8)
	Yes	35 (9.2)
Pregnancy and postpartum period	No	361 (95.0)
	Pregnancy	13 (3.4)
	Postpartum period	6 (1.6)
Thrombophilia	No	370 (97.4)
	Yes	10 (2.6)
SARS-CoV-2 infection	No	375 (98.7)
	Yes	5 (1.3)
Drug abuse	No	377 (99.2)
	Yes	3 (0.8)
Hormone therapy	No	379 (99.7)
	Yes	1 (0.3)
Provoked thrombosis	No	218 (57.4)
	Yes	162 (42.6)
D-dimer (mg/L)	—	7.1 (3.47–15.0)
Fibrinogen (g/L)	—	4.4 (3.4–5.4)
In-hospital mortality	No	369 (97.1)
	Yes	11 (2.9)
Treatment on discharge	Rivaroxaban	174 (47.2)
	Enoxaparin	113 (30.6)
	Apixaban	71 (19.2)
	Acenocoumarol	10 (2.7)
	UFH	1 (0.3)

Data are presented as median (interquartile range) for continuous variables and number (percentage) for categorical variables. Abbreviations: DVT – deep venous thrombosis; PLEDVT- proximal lower extremity DVT; DLEDVT- distal lower extremity DVT; UEDVT- upper extremity DVT; VTE – venous thromboembolism; PE – pulmonary embolism; UFH – unfractionated heparin; IVC- inferior vena cava; n- number of participants; IQR – interquartile range.

A moderate positive correlation was found between age and D-dimer ($r_s = 0.357$, $p < 0.001$), while age showed a weak negative correlation with fibrinogen ($r_s = –0.155$, $p = 0.004$). D-dimer and fibrinogen were also negatively correlated ($r_s = –0.208$, $p < 0.001$). Age differed significantly across thrombosis locations, with the oldest patients in the PLEDVT group (median age 65 years, $p = 0.010$), and within this group in the iliac segment (median age 67 years, $p < 0.001$). The youngest patients in the cohort were those with UEDVT (median age 45 years, $p = 0.010$). Patients with malignancy were older than those without malignancy (median age 67 vs. 60 years, $p < 0.001$), with no additional age-related differences.

Patients with previous VTE had higher D-dimer values ($p = 0.029$, $r = 0.12$). D-dimer levels also differed significantly by thrombosis location, with the highest values in patients with PLEDVT ($p < 0.001$, $\eta^2 = 0.04$), and within this group, among patients with iliac vein thrombosis ($p < 0.001$, $\eta^2 = 0.07$). Higher D-dimer values were also observed in patients with malignancy ($p = 0.006$, $r = 0.16$) and in those discharged on enoxaparin compared with those receiving oral anticoagulants ($p = 0.024$, $\eta^2 = 0.02$). These findings are summarized in Table 2. In contrast, fibrinogen levels did not differ significantly across clinical or anatomical subgroups, and effect sizes were negligible.

Table 2 **Comparison of D-dimer and fibrinogen levels according to clinical and anatomical characteristics.**

Variable	Category	D-dimer (mg/L), Median (IQR)	*p*-value	Effect size	Fibrinogen (g/L), Median (IQR)	*p*-value	Effect size
Gender	Female	8.76 (3.73-17.39)	0.093	0.10	4.35 (3.40-5.50)	0.476	0.04
	Male	6.86 (3.54-13.39)			4.25 (3.30-5.25)		
Side of thrombosis	Right	6.86 (3.7-15.27)	0.611	0	4.40 (3.40-5.40	0.237	0.003
	Left	7.49 (3.65-16.47)			4.10 (3.30-5.375)		
	Bilateral	3.92 (3.87-4.00)			4.40 (4.10-4.50)		
Location of thrombosis	PLEDVT	7.40 (3.65-15.75)	<0.001*	0.04	4.30 (3.40-5.40)	0.199	0.004
	DLEDVT	6.02 (2.23-15.27)			5.50 (4.05-5.72)		
	UEDVT	1.79 (0.88-6.76)			5.40 (3.65-6.00)		
PLEDVT	Iliac	9.67 (3.99-18.50)	<0.001*	0.07	4.40 (3.40-5.45)	0.551	0
	Femoral	6.81 (3.60-12.65)			3.90 (3.30-5.30)		
	Popliteal	3.65 (1.55-6.80)			4.90 (3.95-5.27)		
PE	No	7.53 (3.60-15.25)	0.487	0.04	4.30 (3.40-5.40)	0.885	0.008
	Yes	7.06 (3.86-12.00)			4.40 (3.50-5.50)		
Previous VTE	No	7.03 (3.57-14.52)	0.029*	0.12	4.25 (3.40-5.30)	0.452	0.04
	Yes	9.12 (4.10-21.00)			4.40 (3.30-5.55)		
Malignancy	No	6.86 (3.50-12.61)	0.006*	0.16	4.40 (3.40-5.40)	0.294	0.06
	Yes	10.82 (3.78-23.27)			3.85 (2.72-5.40)		
Provoked thrombosis	No	6.98 (3.71-12.84)	0.119	0.09	4.20 (3.40-5.20)	0.606	0.03
	Yes	9.20 (3.51-18.87)			4.35 (3.30-5.50)		
Treatment on discharge	Enoxaparin	10.62 (3.82-22.69)	0.024*	0.02	4.60 (3.30-5.70)	0.328	0.001
	Apixaban	6.83 (2.66-13.07)			3.90 (3.30-4.90)		
	Rivaroxaban	6.77 (3.69-12.80)			4.30 (3.40-5.20)		

Mann-Whitney U or Kruskal-Wallis tests were used as appropriate. Effect size was calculated as r for Mann–Whitney U tests and η^2 for Kruskal–Wallis tests. Continuous variables are presented as median (interquartile range). Abbreviations: IQR- interquartile range; DVT- deep venous thrombosis; PLEDVT- proximal lower extremity DVT; DLEDVT- distal lower extremity DVT; UEDVT- upper extremity DVT; PE- pulmonary embolism; VTE- venous thromboembolism. *Statistical significance was defined as $p < 0.05$.

Significant associations with gender were observed for PE ($p = 0.003$), malignancy ($p = 0.014$), and provoked thrombosis ($p < 0.001$). PE was more frequent in men, while malignancy and provoked thrombosis were more frequent in women. No other gender-related associations were found, and neither PE nor previous VTE showed significant relationships with other categorical variables. A significant association was found between PLEDVT and provoked thrombosis ($p = 0.007$), with the highest proportion of provoked events in iliac vein thrombosis. Iliac thrombosis, as well as malignancy and provoked thrombosis, were more frequently treated with enoxaparin at discharge (all $p < 0.05$).

DISCUSSION

This study provides a comprehensive analysis of clinical characteristics, anatomical distribution, provoking factors, and biomarker profiles in patients hospitalized with venous thrombosis at a tertiary care center over a five-year period. Proximal lower extremity thrombosis, especially iliac involvement, was the most common pattern, with a higher prevalence among older patients. Another large retrospective analysis from our center, involving 1,154 patients over a ten-year period, reported a predominance of lower extremity thrombosis and a high proportion of iliac segment involvement (12). Consistent with published evidence, UEDVT occurred predominantly in younger patients, a pattern previously described in primary (effort-related) and catheter-associated thrombosis (7,13).

D-dimer levels were higher in patients with previous VTE, malignancy, and proximal, particularly iliac, thrombosis, consistent with a greater thrombotic burden, whereas fibrinogen showed no meaningful differences across subgroups. Although several of these associations reached statistical significance, effect sizes were generally small to moderate, indicating modest differences in biomarker levels between clinical subgroups (12,14). The positive correlation between age and D-dimer and the negative correlation with fibrinogen further support age-related variation in biomarker expression (15). Our findings regarding anatomical distribution and biomarker variations correspond closely with earlier research conducted at the same institution (11). A previous study of 1,142 patients demonstrated that both D-dimer and fibrinogen values were highest in iliofemoral DVT, and similarly, we observed elevated D-dimer levels in proximal thrombosis. Unlike that study, fibrinogen did not differ significantly in our cohort, possibly due to differences in patient characteristics or the inclusion of central and visceral thromboses. Both studies reinforce that, despite consistent trends, D-dimer and fibrinogen cannot be used in isolation to determine thrombosis localization (11).

Provoking factors represented an important aspect of this analysis. In our cohort, provoked thrombosis was identified in 42.6% of patients, almost identical to the 45.7% reported in a previous ten-year study from the same institution (16). Malignancy was the leading provoking factor in both cohorts, reinforcing its significance (17,18). Our findings regarding gender-specific patterns are consistent with previous research conducted at our institution, where provoked DVT and malignancy-associated thrombosis were also significantly more common in women. Although broader literature does not uniformly confirm sex-based differences in malignancy-associated VTE, local data suggest a reproducible pattern in which women more frequently present with provoked events, driven largely by pregnancy-, hormone-, and cancer-related factors (19-22). Conversely, although population-based studies generally report a similar incidence of first-time PE in men and women, men have been shown to carry a higher risk of recurrent VTE and more severe thromboembolic presentations (23,24). This may partly explain why PE was more frequent among men in our cohort.

The higher use of enoxaparin at discharge among patients with iliac thrombosis, malignancy, and provoked events likely reflects current clinical preference for low-molecular-weight heparin in patients with higher thrombotic burden or malignancy-associated thrombosis. However, contemporary guidance supports direct oral anticoagulants as effective treatment options for many patients with cancer-associated VTE, with LMWH preferred in those at increased bleeding risk, unresected gastrointestinal or genitourinary cancer, or with significant drug interactions (25-27).

This study has several strengths, including a well-defined hospitalized cohort, detailed anatomical classification of thrombosis, and simultaneous evaluation of provoking factors and biomarker profiles. However, certain limitations must be acknowledged. As a retrospective, single-center study, findings may not fully represent the broader population. Some subgroups were too small (<10% of the sample) to allow meaningful comparative testing, limiting the ability to draw conclusions for less common thrombosis locations such as visceral or central venous thrombosis. Additional limitations include the absence of standardized timing for biomarker measurement, since laboratory testing was performed as part of routine clinical care, and the lack of data on pre-hospital anticoagulant use or thrombus burden on imaging. Furthermore, several clinically relevant comorbidities were not fully captured, and long-term outcomes were not assessed.

CONCLUSION

This study demonstrates that proximal lower extremity thrombosis, especially iliac involvement, is linked with higher D-dimer levels and older age, while malignancy remains the most frequent provoking factor with clear clinical impact. D-dimer varied across anatomical and clinical subgroups, whereas fibrinogen showed no meaningful differences. These findings contribute to a better understanding of thrombosis patterns in hospitalized patients and highlight the need for improved risk stratification and targeted preventive strategies.

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Frequency of Elevated Titer of Antithyroid Antibodies in Pregnant Women with Newly Diagnosed and Previously Diagnosed Hypothyroidism

Učestalost povišenog titra antitireoidnih antitijela kod trudnica s novodijagnosticiranom i ranije dijagnosticiranom hipotireozom

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ABSTRACT

Hypothyroidism in pregnancy represents an important clinical issue due to the associated risks for both the mother and the fetus. Although Hashimoto's thyroiditis is the most common cause, hypothyroidism in pregnant women without elevated thyroid antibody levels is frequently encountered in clinical practice. Aim: to investigate the frequency of elevated and reference titers of anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase (anti-TPO) antibodies in pregnant women with newly diagnosed and previously diagnosed hypothyroidism, and to analyze their association with maternal age, prior presence of hypothyroidism, and the number of previous pregnancies. Materials and methods: a retrospective analysis was performed of endocrinological findings from 94 pregnant women with hypothyroidism who attended the Endocrinology Outpatient Clinic of the Clinical Center University of Sarajevo from the Sarajevo Canton during the first trimester of 2025. Recorded data included TSH, FT4, FT3, anti-Tg, and anti-TPO levels, as well as maternal age, previous diagnosis of hypothyroidism, and number of prior pregnancies. Statistical analysis was conducted using SPSS version 26. The chi-square test was used for comparison of categorical variables, and a p-value <0.05 was considered statistically significant. Results: the mean age of the pregnant women was 31.8 ± 5.4 years. Newly diagnosed hypothyroidism was present in 48 (51.1%) participants, while 46 (48.9%) had previously diagnosed hypothyroidism. Only six women had overt hypothyroidism, whereas the majority had a subclinical form. The mean anti-Tg and anti-TPO antibody levels were 312.7 ± 645.3 IU/mL and 186.4 ± 242.1 IU/mL, respectively. Elevated anti-TPO levels were significantly more frequent in women with previously diagnosed hypothyroidism (65.2% vs. 27.1%; p<0.001), while anti-Tg levels showed no significant differences. Maternal age and the number of previous pregnancies were not statistically associated with the presence of elevated antibody levels. Conclusion: the majority of pregnant women with hypothyroidism in our study presented with a subclinical form of the disease. A considerable proportion of newly diagnosed pregnant women had normal anti-Tg and anti-TPO levels, suggesting that hypothyroidism may develop during pregnancy in the absence of autoimmune markers. These findings support the need for careful monitoring of thyroid function in all pregnant women, including those without classical risk factors or detectable thyroid antibodies. Further studies are needed to identify non-autoimmune mechanisms of hypothyroidism in pregnancy

Keywords: hypothyroidism, pregnancy, anti-Tg, anti-TPO, subclinical hypothyroidism

SAŽETAK

Hipotireoza u trudnoći predstavlja važan klinički problem zbog rizika za majku i fetus. Iako je Hashimotov tireoiditis najčešći uzrok, u praksi se često javlja hipotireoza kod trudnica bez povišenih vrijednosti antitijela na štitnu žlijezdu. Cilj: ispitati učestalost povišenog i referentnog titra anti-Tg i anti-TPO antitijela kod trudnica s novodijagnosticiranom i ranije dijagnosticiranom hipotireozom te analizirati povezanost s dobi trudnica, prethodnom prisutnošću hipotireoze i brojem ranijih trudnoća. Materijali i metode: Retrospektivno su analizirani endokrinološki nalazi 94 trudnice s hipotireozom koje su se javile u Endokrinološko savjetovalište KCUS-a iz Kantona Sarajevo u prvom tromjesečju 2025. godine. Zabilježene su vrijednosti TSH, FT4, FT3, anti-Tg i anti-TPO, te podaci o dobi, prethodnoj hipotireozi i broju ranijih trudnoća. Statistička analiza provedena je u SPSS v.26; za usporedbu kategorijskih varijabli korišten je Hi-kvadrat test, p<0,05 smatrano je statistički značajnim. Rezultati: prosječna starost trudnica bila je 31,8 ± 5,4 godine. Novoustanovljena hipotireoza prisutna je kod 48 (51,1%), a ranije dijagnosticirana kod 46 (48,9%) ispitanica. Samo 6 trudnica imalo je ispoljeni hipotireoidizam, dok je većina imala subklinički oblik. Prosječne vrijednosti anti-Tg i anti-TPO antitijela iznosile su 312,7 ± 645,3IU/mL i 186,4 ± 242,1IU/mL. Povišene vrijednosti anti-TPO bile su značajno češće kod trudnica sa ranije dijagnosticiranom hipotireozom (65,2% vs. 27,1%; p<0,001), dok vrijednosti anti-Tg nisu pokazale značajne razlike. Dob trudnica i broj prethodnih trudnoća nisu bili statistički povezani s prisustvom povišenih antitijela. Zaključak: većina trudnica s hipotireozom u našoj studiji imala je subklinički oblik bolesti. Značajan broj novodijagnosticiranih trudnica imao je normalne vrijednosti anti-Tg i anti-TPO, što sugerira da hipotireoza može nastati u trudnoći bez prisustva autoimunih markera. Nalazi podržavaju potrebu za pažljivim praćenjem funkcije štitnjače kod svih trudnica, uključujući one bez klasičnih rizik faktora ili prisutnih antitijela. Dalja istraživanja su potrebna za identifikaciju neautoimunih mehanizama hipotireoze u trudnoći.

Ključne riječi: hipotireoza, trudnoća, anti-Tg, anti-TPO, subklinički hipotireoidizam

INTRODUCTION

Hypothyroidism is a disorder of thyroid gland function characterized by reduced production of thyroid hormones, most commonly resulting from an autoimmune process accompanied by elevated titers of antithyroid antibodies, with Hashimoto's thyroiditis being the predominant cause in the general population (1). Hypothyroidism during pregnancy requires special attention due to potential complications for both the mother and the fetus. Clinical manifestations may be nonspecific and overlap with common pregnancy-related symptoms, which may also be absent, such as fatigue, weight gain, and constipation. Routine screening of thyroid function in all pregnant women remains a subject of debate. Measurement of thyroid-stimulating hormone (TSH) is recommended in pregnant women with the presence of goiter, type 1 diabetes mellitus, prior thyroid surgery, positive thyroid antibodies, a history of spontaneous miscarriages or preterm delivery, previous pregnancies, treatment with amiodarone or lithium, a family history of thyroid disease, advanced maternal age, or residence in iodine-deficient areas. The diagnosis of hypothyroidism in pregnancy is established according to standard criteria, while therapeutic thresholds for subclinical hypothyroidism are specific. Treatment with levothyroxine is initiated when TSH exceeds the upper limit of the reference range or is >4.0 IU/mL, regardless of symptoms or the presence of antithyroid antibodies, or when TSH levels are between 2.6 and 4.0 mU/L in the presence of thyroid antibodies, with the aim of maintaining TSH levels below 2.5 mIU/mL (2). In clinical practice, an increasing number of pregnant women with newly diagnosed hypothyroidism have been observed without elevated antibody titers or other known risk factors; in some cases, this condition is transient and resolves after pregnancy.

AIM

The aim of the study was to analyze the frequency of elevated antithyroid antibody titers in pregnant women with hypothyroidism in relation to reference values and to examine the association between antibody titers and maternal age, previously diagnosed hypothyroidism, and the number of previous pregnancies.

MATERIALS AND METHODS

This retrospective study included pregnant women with hypothyroidism from the Sarajevo Canton who attended the Endocrinology Outpatient Clinic of the Clinical Center University of Sarajevo during the first trimester of 2025. Data on TSH, FT4, FT3, anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibody titers, maternal age, previously diagnosed hypothyroidism, and the number of previous pregnancies were extracted from the Hospital Information System (HIS).

Statistical data analysis was performed using the statistical software SPSS version 26. Reference ranges for antibodies and thyroid hormones were as follows: anti-Tg 0–115 IU/mL, anti-TPO 0–34 IU/mL, TSH 0.27–4.2 IU/mL, FT4 12–22 pmol/L, and FT3 3.1–6.8 pmol/L.

Descriptive statistics were presented as absolute numbers and percentages for categorical variables. Continuous variables with an approximately normal distribution were expressed as mean ± standard deviation, whereas variables with a non-normal distribution were presented as median and interquartile range (IQR). Assessment of normality was based on data distribution, variability, and the deviation of the mean from the median.

Associations between categorical variables were analyzed using the chi-square test, with a p-value <0.05 considered statistically significant.

RESULTS

The study included 94 pregnant women with hypothyroidism. The mean age was 31.8 ± 5.4 years (range 20–43). Regarding parity, 45 women (47.9%) were in their first pregnancy, while 49 (52.1%) had one or more previous pregnancies. A history of hypothyroidism was documented in 46 women (48.9%), whereas 48 women (51.1%) were newly diagnosed. The mean TSH level was 6.14 ± 10.84 mIU/mL, FT4 14.18 ± 13.85 pmol/L, (due to the pronounced variations in TSH and FT4 test results in pregnant women, a high standard deviation indicates an asymmetric distribution) and FT3 4.86 ± 1.62 pmol/L. The mean concentration of anti-Tg antibodies was 312.7 ± 645.3 IU/mL, and anti-TPO antibodies 186.4 ± 242.1 IU/mL. Due to the high variability in antibody values, the median and interquartile range (IQR) provide a more accurate representation of the distribution than the mean and standard deviation. For anti-Tg antibodies (n = 94), the median was 20 IU/mL, IQR 14–253 IU/mL, with a minimum of 0.6 and a maximum of 4000 IU/mL. For anti-TPO antibodies (n = 94), the median was 30 IU/mL, IQR 14–202 IU/mL, with a minimum of 3 and a maximum of 600 IU/mL. Analysis of anti-Tg antibody values by age showed that among women ≤30 years (n = 42), 35 (83.3%) had normal levels and 7 (16.7%) had elevated levels. Among women >30 years (n = 52), 35 (67.3%) had normal levels and 17 (32.7%) had elevated levels. The difference was not statistically significant (x^2 = 3.1384; p = 0.07) (Table 1).

Table 1 **Number of pregnant women with normal and elevated anti-Tg levels according to age.**

Age (years)	Number of pregnant women with normal levels *n (%)*	Number of pregnant women with elevated levels *n (%)*	χ^2	p-value
≤ 30	35 (83.33)	7 (16.66)		
>30	35 (67.30)	17 (32.69)	3.1384	0.07

Similarly, anti-TPO antibody levels did not show a significant difference according to age: ≤30 years, 64.3% normal; >30 years, 46.2% normal (x^2 = 3.0777; p = 0.07) (Table 2).

Table 2 **Number of pregnant women with normal and elevated anti-TPO levels according to age.**

Age (years)	Number of pregnant women with normal levels *n (%)*	Number of pregnant women with elevated levels *n (%)*	χ^2	p-value
≤ 30	27 (64.28)	15 (35.71)		
>30	24 (46.15)	28 (53.84)	3.0777	0.07

Analysis according to the timing of hypothyroidism diagnosis showed that among newly diagnosed pregnant women (n = 48), 38 (79.2%) had normal and 10 (20.8%) had elevated anti-Tg levels, whereas among previously diagnosed women (n = 46), 32 (69.6%) had normal and 14 (30.4%) had elevated anti-Tg levels. The difference was not statistically significant ($x^2 = 1.1389$; p = 0.28) (Table 3).

Table 3 **Number of pregnant women with normal and elevated anti-Tg levels according to the timing of hypothyroidism diagnosis.**

Patient history regarding the timing of hypothyroidism diagnosis	Number of pregnant women with normal levels *n (%)*	Number of pregnant women with elevated levels *n (%)*	χ^2	p-value
Patients with newly diagnosed hypothyroidism	38 (79.16)	10 (20.83)		
Patients with previously diagnosed hypothyroidism	32 (69.56)	14 (30.43)	1.1389	0.28

On the other hand, elevated anti-TPO levels were significantly more frequent in pregnant women with previously diagnosed hypothyroidism compared to newly diagnosed women (65.2% vs. 27.1%; p < 0.001) (Table 4).

Table 4 **Number of pregnant women with normal and elevated anti-TPO levels according to patient history regarding the timing of hypothyroidism diagnosis.**

Patient history regarding the timing of hypothyroidism diagnosis	Number of pregnant women with normal levels *n (%)*	Number of pregnant women with elevated levels *n (%)*	χ^2	p-value
Patients with newly diagnosed hypothyroidism	35 (72.91)	13 (27.08)		
Patients with previously diagnosed hypothyroidism	16 (34.78)	30 (65.21)	13.763	<0.001

In the analysis according to parity, anti-Tg levels did not show significant differences: first pregnancy, 73.3% normal vs. ≥1 previous pregnancies, 75.5% normal ($x^2 = 0.0585$; p = 0.8) (Table 5).

Table 5 **Number of pregnant women with normal and elevated anti-Tg values in relation to the number of previous pregnancies.**

Number of previous pregnancies	Number of pregnant women with normal levels *n (%)*	Number of pregnant women with elevated levels *n (%)*	χ^2	p-value
0	33 (73.33)	12 (26.66)		
≥1	37 (75.51)	12 (24.48)	0.0585	0.8

A similar analysis of anti-TPO showed that elevated values were not significantly associated with the number of previous pregnancies, first pregnancy: 37.77%, ≥1 pregnancies: 53.06%, $x^2 = 2.2077$, p = 0.13 (Table 6).

Table 6 **Number of pregnant women with normal and elevated anti-TPO values in relation to the number of previous pregnancies.**

Number of previous pregnancies	Number of pregnant women with normal levels *n (%)*	Number of pregnant women with elevated levels *n (%)*	χ^2	p-value
0	28 (62.22)	17 (37.77)		
≥1	23 (46.93)	26 (53.06)	2.2077	0.13

DISCUSSION

In our study, most cases of hypothyroidism among pregnant women were subclinical (93.6%), while overt hypothyroidism was rare (6.4%). These results are consistent with previous studies, where the prevalence of subclinical hypothyroidism in pregnancy ranged from 2–3%, and overt hypothyroidism from 0.3–0.5. (3). In our sample of 94 pregnant women, 48 (51.1%) had newly diagnosed hypothyroidism, while 46 (48.9%) had previously diagnosed hypothyroidism. Only 6 women had overt hypothyroidism, evenly distributed between the groups. Most newly diagnosed women had negative anti-Tg and anti-TPO antibody titers, suggesting that hypothyroidism during pregnancy can occur even in the absence of an autoimmune process. Data from other studies show similar trends. In a study of 1,200 pregnant women with a mean age of 27.5 ± 5.1 years, hypothyroidism was found in 12.4% of participants, of which 9.3% had subclinical and 3.1% had overt hypothyroidism. Subclinical hypothyroidism was defined as TSH 2.5–10 mIU/L with normal FT4, and overt hypothyroidism as TSH ≥ 10 mIU/L or reduced FT4. Negative outcomes associated with untreated hypothyroidism during pregnancy include miscarriage, preterm delivery, intrauterine growth restriction, low birth weight, postpartum hemorrhage, and neonatal respiratory distress syndrome (4). According to the 2024 American Thyroid Association (ATA) guidelines, women with TSH >10 mIU/L should be treated, whereas treatment for TSH between 2.5–10 mIU/L depends on the presence of anti-TPO or anti-Tg antibodies (5). In our study, there was no significant association between antibody levels and maternal age, which aligns with the findings of Potlukova et al., who did not observe an increase in the prevalence of autoimmune thyroid disease with maternal age (6). The study by Konishi S, et al. showed that women with anti-thyroid peroxidase (anti-TPO) ≥16 IU/mL had lower conception rates compared to those with anti-TPO <16 IU/mL (7). In the study by Kumari S, et al., out of 281 pregnant women with thyroid disorders, 277 were hypothyroid; of these, 180 were anti-TPO positive and 97 were anti-TPO negative. Thyroid dysfunction was newly diagnosed during pregnancy in 166 cases (59.1%). Most participants were aged 26-31 years (48.4%), with a mean age of 27.95 years, consistent with the typical reproductive age range for pregnant women. These findings highlight the need for universal thyroid screening in early pregnancy to identify undiagnosed thyroid dysfunction and ensure timely intervention (8). Furthermore, the number of previous pregnancies was not significantly associated with elevated anti-TPO or anti-Tg antibody levels, although some studies suggest that multiple pregnancies may increase the risk of developing new subclinical or overt hypothyroidism (9). One possible explanation for the higher number of pregnant women with negative antibody titers in our study is that pregnancy is a state of relative immunosuppression, during which pro-inflammatory responses are suppressed to allow fetal tolerance (10). This may temporarily reduce autoimmune activity, explaining the absence of elevated antibody levels in some newly diagnosed cases. Our findings underscore the need for careful monitoring of thyroid function during pregnancy, including in women without classic risk factors or detectable antibodies, as hypothyroidism can occur de novo and potentially affect pregnancy outcomes. Further research is needed to better understand the factors contributing to hypothyroidism in pregnancy.

CONCLUSION

The study showed that most pregnant women with hypo thyroidism in our sample had the subclinical form of the disease, while overt hypothyroidism was rare. A significant number of women with newly diagnosed hypothyroidism had normal anti-Tg and anti-TPO antibody levels, indicating that hypothyroidism during pregnancy can develop even in the absence of classical autoimmune markers. Anti-TPO antibody levels were significantly higher in women with previously diagnosed hypothyroidism, whereas anti-Tg antibody levels did not show significant differences based on the timing of diagnosis. Maternal age and the number of previous pregnancies were not statistically associated with the presence of elevated antibodies. These findings suggest that thyroid function screening and monitoring during pregnancy should also include women without classic risk factors or detectable antibodies, as hypothyroidism can occur de novo and potentially negatively impact pregnancy outcomes. Further research is needed to assess the role of iodine status, the immunosuppressive effects of pregnancy, and other non-autoimmune mechanisms in the development of hypothyroidism in pregnant women.

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Diagnostic Value of Tumor Markers CEA and CA 19-9 and Their Ratio in Colorectal Cancer

Dijagnostička vrijednost tumorskih markera CEA, CA 19-9 i odnosa CA19-9/CEA kod kolorektalnog karcinoma

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ABSTRACT

Colorectal cancer (CRC) is a significant problem of the modern health system, given its incidence and mortality, which is why early diagnosis of this disease is important. The aim of this study was to examine the diagnostic value of tumor markers CEA, CA 19-9 and their ratio (CA19-9/CEA) in colorectal cancer in relation to the healthy population. Materials and methods: 100 participants were retrospectively analyzed, divided into two groups: 50 healthy participants and 50 participants with histopathologically confirmed colorectal cancer. Tumor markers were determined by the chemiluminescent microparticle immunoassay method, and then the data were statistically processed with a statistical significance threshold of $p<0,05$. Results: CEA, CA 19-9 and CA 19-9/CEA ratios were significantly higher in the CRC group compared to the control group. A moderate positive Spearman correlation was found between CEA and CA 19-9 ($\rho=0,456$; $p<0,001$), while the CA 19-9/CEA ratio was dominantly associated with CA 19-9 ($\rho=0,799$; $p<0,001$). ROC analysis showed the highest diagnostic value for CEA (AUC=0,844), followed by CA 19-9 (AUC=0,816), and the lowest diagnostic value was shown by the CA 19-9/CEA ratio (AUC=0,657). Conclusion: the analyzed tumor markers, CEA and CA 19-9 demonstrate significant diagnostic value in CRC, especially in combination, and can be used in practice to monitor the therapeutic response, assess the prognosis of the disease, but also as additional, early diagnostic markers for CRC.

Keywords: colorectal cancer, diagnostic value, tumor markers, CEA, CA 19-9, CA 19-9/CEA ratio, AUC, ROC analysis

SAŽETAK

Kolorektalni karcinom (CRC) predstavlja značajan problem savremenog zdravstvenog sistema, obzirom na učestalost i mortalitet, zbog čega je rana dijagnostika ovog oboljenja. Cilj ovog rada je ispitati dijagnostičku vrijednost tumorskih markera CEA, CA 19-9 i njihovog odnosa (CA19-9/CEA) kod kolorektalnog karcinoma u odnosu na zdravu populaciju. Materijal i metode: retrospektivno je analizirano 100 ispitanika pri čemu su podijeljeni u dvije grupe: 50 zdravi ispitanici i 50 ispitanici sa histopoatološki potvrđenim kolorektalnim karcinomom. Tumorski markeri su određeni metodom hemiluminiscentnog mikročestičnog imunoeseja, a potom su podaci obrađeni statistički uz prag statističke značajnosti $p<0,05$. Rezultati: vrijednosti CEA, CA 19-9 i odnosa CA 19-9/CEA bile su značajno veće u grupi ispitanika sa CRC u odnosu na kontrolnu grupu. Umjerena pozitivna Spearman-ova korelacija utvrđena je između CEA i CA 19-9 ($\rho=0,456$; $p<0,001$), dok je odnos CA 19-9/CEA dominantno bio povezan sa CA 19-9 ($\rho=0,799$; $p<0,001$). ROC analiza pokazala je najveću dijagnostičku vrijednost za CEA (AUC=0,844), potom za CA 19-9 (AUC=0,816), a najmanju dijagnostičku vrijednost pokazao je odnos CA 19-9/CEA (AUC=0,657). Zaključak: analizirani tumorski markeri, CEA i CA 19-9 pokazuju imaju značajnu dijagnostičku vrijednost kod CRC, posebno u kombinaciji, te se u praksi mogu koristiti kod praćenja terapijskog odgovora, procjeni prognoze bolesti, ali i kao dodatni, rani dijagnostički markeri za CRC.

Ključne riječi: kolorektalni karcinom, dijagnostička vrijednost, tumorski markeri, CEA, CA 19-9, Odnos CA 19-9/CEA, AUC, ROC analiza

INTRODUCTION

Colorectal cancer (CRC) is a multifactorial disease. It is a malignant neoplasm originating from the epithelial cells of the colorectal mucosa, and its development involves the progressive transformation of benign precancerous lesions into invasive cancer, conditioned by the complex interaction of carcinogenic factors that cause structural changes in DNA (1).

In today's public health system, colorectal cancer is one of the leading problems of the modern era, given its high incidence and mortality. According to Global Cancer Statistics data from 2022, colorectal cancer ranks third in incidence and second in mortality among all malignant diseases globally. According to available data, more than 1,9 million new cases of colorectal cancer were registered in 2022. with almost 904,000 deaths. The incidence of colorectal cancer shows very pronounced geographical differences, with the highest incidence in developed countries in Europe, North America, Australia and New Zealand, and the lowest in countries in Africa and Asia. Particularly worrying data relate to the increase in incidence in countries in transition, as well as the increase in the incidence among the population under 50 years of age in highly developed countries (2).

Diagnosis of colorectal cancer, in accordance with the recommendations of the World Health Organization (WHO), is based on a combination of clinical examination, endoscopic, radiological, histopathological and molecular methods. Endoscopic methods, colonoscopy or sigmoidoscopy, are key diagnostic tests because they allow direct examination of the colonic mucosa and tissue sampling for histopathological confirmation of malignancy. Imaging methods are used to assess local tumor spread and determine distant metastases, and molecular diagnostic methods are primarily used to identify specific gene mutations that are important for choosing the most optimal therapeutic approach (3).

Early diagnosis of colorectal cancer is of key importance for the prognosis of the disease, as it enables the detection of cancer in its early stages or in the phase of precancerous lesions, when the therapeutic outcome is significantly more favorable. Timely diagnosis of colorectal cancer significantly reduces its incidence and mortality with a significantly higher five-year survival rate, which justifies the importance of organized screening programs in the population at increased risk (4). Therefore, more and more attention is being paid today to the identification of early biomarkers of colorectal cancer. One of the important groups of early biomarkers are tumor markers. Tumor markers are a heterogeneous group of substances produced by cancer cells, but they can also be expressed to a certain extent by healthy tissues. Patients with malignancies often show elevated levels of markers that correlate with tumor size. Correct interpretation of these values in clinical practice enables faster diagnosis and timely therapeutic intervention, thereby improving the final outcome of treatment. In clinical practice, the concentration of tumor markers is usually determined in order to monitor the response to the applied therapy, but determining their concentration can also be a significant aid in the diagnosis of malignancy itself, early detection of disease relapse, as well as assessing the prognosis of the disease (5).

In colorectal cancer, the most important tumor markers monitored are carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9). CEA is the most studied tumor marker in colorectal cancer. It is a glycoprotein expressed in epithelial cells of the gastrointestinal tract and plays a role in biological processes such as cell adhesion and apoptosis. It is characterized by low sensitivity in the early stages of the disease, which is why it cannot be used as a screening marker, but preoperative determination of its concentration is a very significant predictor of overall survival in CRC, and high postoperative CEA levels directly indicate a worse outcome (5,6). Carbohydrate antigen 19-9 (CA 19-9) is more often used for the diagnosis of pancreatic cancer, it is characterized by lower sensitivity compared to CEA in colorectal cancer. However, there are studies that indicate its prognostic significance in colorectal cancer as an independent marker (7).

Given the characteristics of these two markers, the main goal of this study is to examine their diagnostic and prognostic value in patients with confirmed colorectal cancer compared to healthy controls, and to examine the CA 19-9/CEA ratio as a potential biomarker.

AIM

The aim of this study was to examine the diagnostic value of tumor markers CEA, CA 19-9 and their ratio (CA19-9/CEA) in colorectal cancer in relation to the healthy population.

MATERIALS AND METHODS

Patients and study protocol

The study was designed as a unicentric retrospective study in which a total of 100 samples of participants were analyzed, divided into two groups: 50 healthy participants who make up the control group and 50 participants with a histopathologically confirmed diagnosis of colorectal cancer. The complete study was conducted at the Clinical Center of the University of Sarajevo (KCUS) in the organizational unit Clinical Biochemistry and Laboratory Medicine. Data were collected through retrospective analysis from the database of the KCUS information system, covering the period July 2023-July 2024. The research was conducted with the approval of the Ethics Committee of the Clinical Center of the University of Sarajevo No. 06-04-9-4508, in compliance with the ethical principles of the Declaration of Helsinki.

The main inclusion criteria for the study are participants older than 18 years of age with complete demographic and laboratory data available, including data for tumor markers that are the subject of analysis in this study. When it comes to the group of participants with confirmed CRC, only those patients with a histopathological diagnosis of colorectal cancer were included in the study, without other malignancies, severe hepatobiliary and renal diseases, and acute and chronic inflammatory conditions that could affect the value of tumor markers, and before the start of specific oncological therapy.

The control group consisted of 15 men and 35 women, while the CRC group consisted of 27 men and 23 women. In the control group, the majority of respondents were women (70%), while the proportion of men was 30%. The largest number of respondents in this group, 66% were ≥ 65 years old and the rest of 44% were respondents aged 18 to 65 years. In the group of respondents with CRC, men were more represented (54%) compared to women (46%), whereby 60% of the respondents of this group were aged 65 years and older.

Venous blood samples from all participants were collected by standard venipuncture from the cubital vein into anticoagulant-free tubes, so-called serum tubes (BD Vacutainer®SST II Advance; Becton Dickinson, USA). After sampling, the blood was allowed to coagulate at room temperature for 20-30 min, after which centrifugation was performed on a HERMLE Type Z 446 K laboratory centrifuge (HERMLE Labortechnik GmbH, Germany) at a speed of 4000 rpm for 5 min. In this way, a serum sample was obtained, which was separated from the clot and stored at a temperature of 2 °C to 8 °C for up to 24 hours, or at a temperature of -20 °C if the analysis could not be performed within 24 hours.

Measurement of tumor markers

Tumor marker analysis was performed on an Abbott Alinity i automated analyzer (Abbott Diagnostics, Abbott Park, IL, USA) using the chemiluminescent microparticle immunoassay (CMIA) method on serum samples from participants. The analytical procedure included prior system calibration and verification of results using internal quality control, ensuring the reliability and accuracy of the results obtained. The results were expressed in appropriate units of measurement (ng/mL for CEA and U/mL for CA 19-9) in accordance with the reference intervals of the KCUS laboratory (reference interval for CEA: 0-5 ng/mL; reference interval for CA 19-9: 0-37 U/mL).

The basic principle of the CMIA method is based on a two-layer sandwich immunoassay that takes place in several steps. In the first step, incubation occurs where antigens from the sample (CEA or CA 19-9) react specifically with paramagnetic microparticles that are coated with primary monoclonal antibodies specific for the target antigen. In this phase, an antigen-antibody immune complex is formed. After incubation, washing is performed to remove unbound material. In the second step, a conjugate is added that represents a specific secondary monoclonal antibody labeled with acridinium. The washing process is repeated, after which pre-trigger and trigger solutions are added to initiate the chemiluminescent reaction. The intensity of the emitted light is measured in relative light units (RLU) and is directly proportional to the concentration of the target antigen in the sample. Quantification of the concentration of the tested analytes is performed automatically based on a calibration curve, previously generated using a calibrator, in accordance with the manufacturer's recommendations (8,9).

Statistical analysis

The results of the analyses were collected and statistically processed using IBM SPSS Statistics, version 26 (IBM Corp., NY, USA) and Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA) software. The normality of the distribution of the analyzed variables was assessed by the Shapiro-Wilk test, and in further work, since the data did not follow a normal distribution, the non-parametric Mann-Whitney U test was used. Correlations between the examined tumor markers were analyzed using Spearaman's correlation coefficient. To assess the diagnostic value of the tumor markers CEA, CA 19-9 and their ratio (CA 19-9/CEA), ROC (Receiver Operating Characteristic) analysis was used, where AUC (Area Under the Curve) values were shown for each analyzed analyte with the optimal cut off value calculated based on the Youden index. The value $p<0,05$ was considered statistically significant.

DECLARATIONS

Ethics approval

The present study was approved by the Ethics Committee of The Clinical Center University of Sarajevo. All procedures were performed in accordance with the ethical standards of the Institutional Review Board and The Declaration of Helsinki.

RESULTS

A total of 100 samples were analyzed, divided into two groups: 5 0 healthy controls and 50 participants with a histopathologically confirmed diagnosis of colorectal cancer. The gender structure of the colorectal cancer group shows a slightly higher representation of men (54%) compared to women (46%), while in the control group, women dominate (70%) compared to men (30%). According to the age structure, in the group of participants with CRC, the majority of participants were aged ≥65 years (60%), while 40% of participants were younger than 65 years. The control group of participants had a similar age structure, with the majority of participants aged 65 years and older (66%), while 34% were younger than 65 years. The demographic characteristics of the participants are shown in table 1.

Table 1 **Gender and age structure of study respondents.**

Diagnosis	**Sex**	Count	Percent %	**Age**	Count	Percent %
Colorectal cancer	Male	27	54	18-65	10	20
				≥ 65	17	34
	Female	23	46	18-65	10	20
				≥ 65	13	26
Healthy controls	Male	15	30	18-65	5	10
				≥ 65	10	20
	Female	35	70	18-65	12	24
				≥ 65	23	46

Descriptive analysis of serum values of tumor markers CEA, CA 19-9 and their ratio (CA 19-9/CEA) was performed stratified according to the diagnosis and sex of the participants. Results are presented as median with 95% confidence interval (95% CI). The obtained results are presented in table 2.

Table 2 **Median values with 95% CI for analyzed tumor markers.**

		CEA		CA 19-9		Ratio CA19-9/CEA	
Diagnosis	Sex	Median	95% CI	Median	95% CI	Median	95% CI
Colorectal cancer	Male	14,10	9,61-36,76	35,50	10,8-132,80	2,02	1,34-3,32
	Female	45,70	16,21-172,18	36,10	14,7-246,20	1,31	0,31-2,71
Healthy control	Male	6,37	5,66-7,27	4,00	2,1-8,30	0,60	0,36-1,47
	Female	7,13	6,45-7,84	3,60	3,03-7,90	0,58	0,41-1,11

The values of the analyzed tumor markers divided into two groups were analyzed using the non-parametric Mann-Whitney U test in order to determine the existence of a statistically significant difference between them. It was found that there are statistically significant differences for all tumor markers between the analyzed groups. The CEA value is statistically significantly higher in the group of participants with CRC compared to the control group (U=389; Z=-5,936; p<0,001), with a higher mean rank in participants with CRC (67,72) compared to the control group (33,28). Also, the values for CA 19-9 are statistically significantly higher in the group of participants with CRC (U=459,5; Z=-5,463; p<0,001) compared to the control group, with higher mean ranks in the group of participants with CRC (66,31) compared to healthy controls (34,69). Additionally of the CA 19-9/CEA ratio showed similar results, with statistically higher values in the CRC group (U=857,5; Z=-2,706; P=0,007) compared to the control group. The data obtained are presented in table 3. Graphically, a comparison of the mean ranks for the analyzed tumor markers is shown in Figure 1.

Table 3 **Mann-Whitney test results.**

Tumor markers	Diagnosis	N	Mean Rank
CEA	Colorectal cancer	50	67,72
	Healthy control	50	33,28
CA 199	Colorectal cancer	50	66,31
	Healthy control	50	34,69
Ratio CA199/CEA	Colorectal cancer	50	58,35
	Healthy control	50	42,65
Test Statistics			
	CEA	CA 199	Ratio CA199/CEA
Mann-Whitney U	389,000	459,500	857,500
Wilcoxon W	1664,000	1734,500	2132,500
Z	-5,936	-5,463	-2,706
Asymp. Sig. (2-tailed)	<0,001	<0,001	0,007

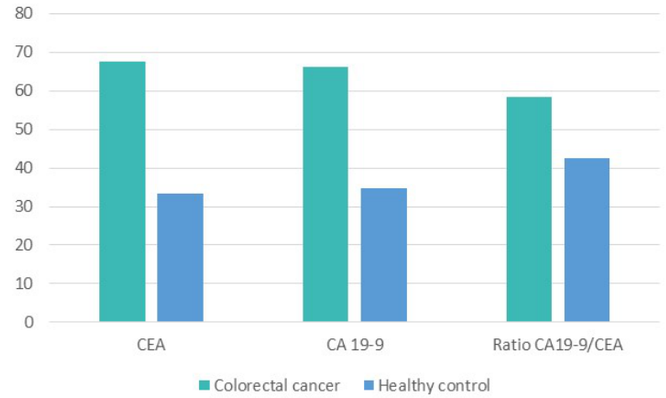

Figure 1 **Comparison of Mean Ranks of Tumor Markers (CEA, CA 19-9 and CA 19-9/CEA) in Colorectal Cancer Patients and Healthy Control.**

In further statistical analysis, correlations between tumor markers were analyzed, and Spearman's correlation coefficient was determined. The results show a moderate positive association between CEA and CA 19-9 (rho=0,459; p< 0,01). No significant correlation was observed between CEA and the CA 19-9/CEA ratio (rho=-0,007; p= 0,944). On the other hand, a strong positive correlation was found between CA 19-9 and the CA 19-9/CEA ratio (rho= 0,799, p< 0,01). The data are presented in table 4.

Table 4 **Spearman's correlation coefficient of the analyzed tumor markers.**

Spearman's rho		CEA	CA 19-9	Ratio CA19-9/CEA
CEA	Correlation Coefficient	1,000	,459**	-0,007
	Sig. (2-tailed)		<0,001	0,944
CA 19-9	Correlation Coefficient	,459**	1,000	,799**
	Sig. (2-tailed)	<0,001		<0,001
Ratio CA19-9/CEA	Correlation Coefficient	-0,007	,799**	1,000
	Sig. (2-tailed)	0,944	<0,001	

In order to assess the diagnostic value in the further statistical processing of the data, a ROC (Receiver Operating Characteristic) analysis was performed, and the obtained ROC curves for the tumor markers CEA, CA 19-9 and their ratio (CA 19-9/CEA) are shown in Figure 2. The largest area under the curve was shown by CEA (AUC= 0,844; 95% CI: 0,761–0,928; p< 0,001), CA 19-9 also showed good diagnostic ability (AUC= 0,816; 95% CI: 0,726–0,907; p< 0,001). The CA 19-9/CEA ratio had the lowest AUC value (AUC= 0,657; 95% CI: 0,541–0,773; p= 0,007). Optimal cut-off values for each tumor marker were determined using the Youden index. CEA showed the highest diagnostic performance, with a cut-off value of 9,57 (sensitivity of 70% and specificity of 98%). CA 19-9 demonstrated a cut-off value of 10,1 (sensitivity of 76% and specificity of 86%). The CA 19-9/CEA ratio showed lower diagnostic accuracy, with a cut-off value of 1,75 (sensitivity of 54% and specificity of 88%). The corresponding values of the area under the curve (AUC) with 95% confidence interval and the calculated cut-off values with sensitivity and specificity for each analyzed marker are given in Table 5.

Figure 2 **ROC curves for the analyzed tumor markers.**

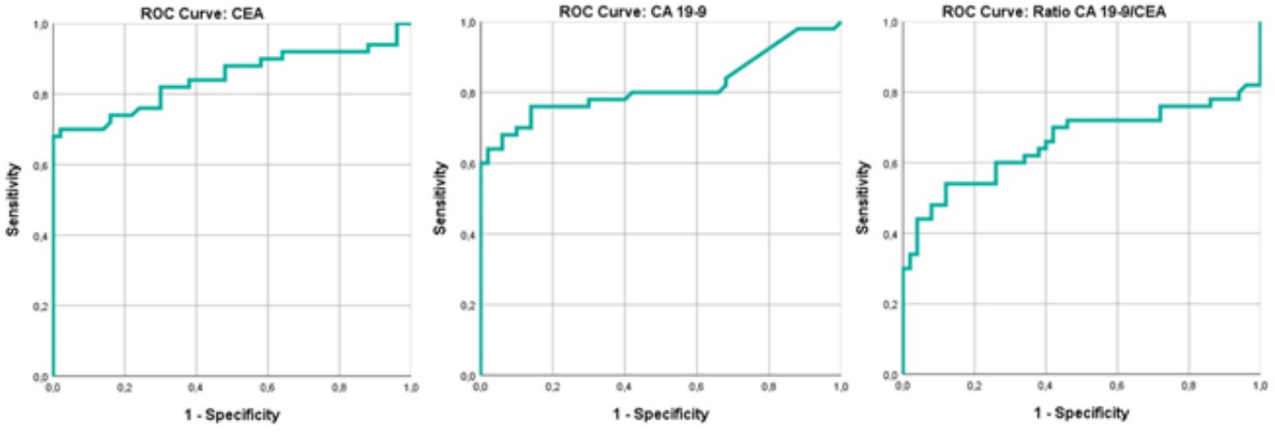

Table 5 **AUC and cut-off values for analyzed tumor markers.**

Tumor markers	AUC	p	95% CI	Cut-off	Sensitivity (%)	Specificity (%)
CEA	0,844	<0,001	0,761-0,928	9,57	70	98
CA 19-9	0,816	<0,001	0,726-0,907	10,1	76	86
Ratio CA19-9/CEA	0,657	0,007	0,541-0,773	1,75	54	88

DISCUSSION

From the presented results of the conducted study, statistically significantly higher values of the analyzed tumor markers CEA, CA 19-9 and the CA 19-9/CEA ratio can be observed in the group of participants with colorectal cancer compared to the participants representing the control group. The differences are also evident in the case of stratification of groups by gender. Significantly higher mean ranks of the analyzed tumor markers in the group of participants with CRC compared to the control group indicate systematically higher values of tumor markers in the group of participants with CRC. The differences in mean ranks were least pronounced in the CA 19-9/CEA ratio. Increased values of CEA and CA 19-9 are also mentioned by the author Lakemeyer L, et al. in their research in which they examine their diagnostic value in CRC, according to the stages of the disease (10). A statistically significantly higher concentration of CA 19-9 in colorectal cancer was also reported by the author Wannhoff A. et al. in a retrospective study in which the values of the tumor marker CA 19-9 were used in combination with other markers to create a screening algorithm (11). Significantly higher values of these markers in colorectal cancer compared to healthy participants have been confirmed by other studies examining the association of preoperative values of these markers with five-year survival rates (12,13).

The results presented below refer to the examination of correlations between CEA, CA 19-9 and the ratio of these two markers (CA19-9/CEA). The moderate positive Spearman correlation between CEA and CA 19-9 (ρ=0,456; p<0,001) indicates that in patients with high CEA values, the value of the marker CA 19-9 is often increased. The strong correlation between CA 19-9 and the ratio CA19-9/CEA (ρ=0,799; p<0,001) and the inadequate correlation between CEA and the ratio CA 19-9/CEA, indicate the dominant dependence of this index primarily on the concentration of the marker CA 19-9 in relation to CEA. The identified positive correlation between CEA and CA 19-9 is in line with studies in which the combined use of these and other markers increases the sensitivity and prognostic value in CRC (10). A similar positive correlation value between CEA and CA 19-9 (ρ=0,328; p=0,001) when it comes to preoperative values of these markers is also reported by Halilovic E, et al. (14). The significance of the CA19-9/CEA ratio is explained in the study by the author Aida T, et al., where this index is cited as a very significant independent prognostic marker in colorectal cancer (15).

The diagnostic value of the studied tumor markers was examined by ROC analysis. Based on the presented results of ROC curves and AUC values, it can be said that the tumor marker CEA (AUC=0,844), followed by CA 19-9 (AUC=0,816), and the CA19-9/CEA ratio (AUC=0,657) have the highest diagnostic value. In a study examining the diagnostic value of CEA and CA 19-9, by Zhang SY. et al., the AUC value for CEA is 0,789 for CEA and 0,690 for CA 19-9, and for the combination of these two markers, the AUC is 0,900. The combination of these two markers ensures greater sensitivity and better prognostic value (16). Similar results were published in the study by the author Bagaria B, et al., where the diagnostic value of individual tumor markers CEA, CA 19-9 and the ratio CEA/CA 19-9 in esophageal and gastric cancer and colorectal cancer was examined. Colorectal cancer data show a value of AUC=0,856 for CEA, AUC=0,580 for CA 19-9 and a sensitivity for the CEA/CA 19-9 ratio of 72% (17). ROC analysis, AUC and cat-off values for CEA and CA 19-9 were also published in a study by Bak M. et al. who examined their diagnostic value for peritoneal metastases in CRC. In this study, the value AUC=0,912 for CEA and AUC=0,741 for CA 19-9 was published (18). A study by Kamada T. et al. reported a significantly higher AUC value for the combined use of tumor markers for prognostic purposes in colorectal cancer compared to their individual use (13).

CONCLUSION

A study conducted in which the tumor markers CEA, CA 19-9 and their ratio (CA 19-9/CEA) were analyzed shows significantly higher values of these markers in patients with CRC compared to the healthy population, with CEA having the highest diagnostic significance. The established positive correlations between the markers confirm the additional value of combined monitoring, while the CA 19-9/CEA ratio alone shows the lowest diagnostic significance. These results indicate the potential clinical application of these markers not only in monitoring therapy and disease recurrence, but also as a valuable additional tool in the early diagnosis of CRC.

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Prevalence of Major Modifiable Cardiovascular Risk Factors in Patients with Acute Myocardial Infarction as Their First Ischemic Event: A Retrospective Study

Prevalenca glavnih promjenjivih kardiovaskularnih faktora rizika kod pacijenata s akutnim infarktom miokarda kao prvim ishemijskim događajem: retrospektivna studija

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ABSTRACT

Cardiovascular diseases remain the leading cause of mortality worldwide. Primary prevention of ischemic cardiovascular disease is based on the assessment of overall cardiovascular risk and the modification of major risk factors in accordance with clinical guidelines. Aim: to assess the prevalence of major modifiable cardiovascular risk factors in patients who experienced myocardial infarction as their first ischemic cardiovascular event and to analyze their distribution in relation to selected baseline characteristics in order to gain insight into the current state and potential for implementing preventive measures. Materials and methods: a retrospective study included 166 consecutive patients with acute myocardial infarction (STEMI or NSTEMI) admitted to the Clinic of Emergency Medicine, Clinical Center of the University of Sarajevo, over a nine-month period. Baseline characteristics (age, sex, diabetes mellitus) and major modifiable cardiovascular risk factors were analyzed, including systolic and diastolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, smoking status, and the use of antihypertensive therapy, statins, and aspirin. Results: In a sample with a mean age of 60.02 years, comprising 69.3% men, the majority of participants had elevated blood pressure, were active smokers, and were not receiving preventive therapy. A substantial proportion also exhibited unfavorable levels of total cholesterol and HDL cholesterol, while most participants (66.9%) had elevated LDL cholesterol levels. Conclusion: patients experiencing a first myocardial infarction show a high prevalence of major modifiable cardiovascular risk factors and low use of preventive therapy, emphasizing the urgent need for improved primary prevention and early risk factor management.

Keywords: modifiable cardiovascular risk factors, prevalence, primary prevention

SAŽETAK

Kardiovaskularne bolesti i dalje predstavljaju vodeći uzrok smrtnosti u svijetu. Primarna prevencija ishemijske kardiovaskularne bolesti zasniva se na procjeni ukupnog kardiovaskularnog rizika i modifikaciji glavnih faktora rizika u skladu s kliničkim smjernicama. Cilj: procijeniti prevalenciju glavnih promjenjivih kardiovaskularnih faktora rizika kod pacijenata koji su doživjeli infarkt miokarda kao prvi ishemijski kardiovaskularni događaj te analizirati njihovu raspodjelu u odnosu na odabrane početne karakteristike zbog uvida u trenutno stanje i potencijal za provođenje preventivnih mjera. Materijali i metode: retrospektivna studija obuhvatila je 166 uzastopnih pacijenata s akutnim infarktom miokarda (STEMI ili NSTEMI) primljenih na Kliniku urgentne medicine Kliničkog centra Univerziteta u Sarajevu tokom devetomjesečnog perioda. Analizirane su osnovne karakteristike ispitanika (dob, spol, dijabetes melitus) te glavni promjenjivi kardiovaskularni faktori rizika: sistolni i dijastolni krvni pritisak, ukupni holesterol, LDL-holesterol, HDL-holesterol, pušenje, te upotreba antihipertenzivne terapije, statina i aspirina. Rezultati: u uzorku prosječne dobi 60.02 godine, sa 69.3% muškaraca, većina ispitanika imala je povišene vrijednosti krvnog pritiska, bila aktivni pušači i nije koristila preventivnu terapiju, dok je značajan dio imao nepovoljne vrijednosti ukupnog holesterola i HDL holesterola te je većina (66.9%) imala povišene vrijednosti LDL holesterola. Zaključak: pacijenti koji su doživjeli prvi infarkt miokarda pokazuju visoku prevalenciju glavnih promjenjivih kardiovaskularnih faktora rizika i nisku upotrebu preventivne terapije, što naglašava hitnu potrebu za unapređenjem primarne prevencije i ranog upravljanja faktorima rizika.

Ključne riječi: promjenjivi kardiovaskularni faktori rizika, prevalenca, primarna prevencija

INTRODUCTION

Cardiovascular diseases remain the leading cause of mortality worldwide (1), underscoring the critical importance of effective preventive strategies. Consequently, increasing efforts have been directed toward the development and implementation of comprehensive prevention programs aimed at reducing the incidence and burden of ischemic cardiovascular events.

Primary prevention of ischemic cardiovascular disease is based on the estimation of an individual's overall risk for experiencing a first ischemic event. This risk assessment incorporates both modifiable and non-modifiable risk factors, and the calculated risk serves as the basis for targeted preventive interventions focused on the modification of reversible risk factors in accordance with established clinical guidelines. Numerous risk assessment models have been developed at national and regional levels, and these tools continue to evolve in parallel with advances in epidemiological and clinical research (2).

The American College of Cardiology/American Heart Association (ACC/AHA) risk assessment model represents one such approach to primary prevention and differs from other systems by incorporating a broad range of clinical risk determinants. Overall cardiovascular risk estimation includes systolic and diastolic blood pressure, total cholesterol levels, high-density and low-density lipoprotein cholesterol, smoking status, and the presence of diabetes mellitus. In addition, the use of preventive pharmacotherapy such as antihypertensive treatment, statin therapy, and aspirin is taken into account, further refining risk stratification. Demographic characteristics, including age and sex, are also integrated into the risk assessment framework (3).

The incidence of ischemic cardiovascular diseases is a useful indicator of the implementation and overall effectiveness of preventive strategies within a given population (4). However, interpretation may be influenced by population-specific demographic and clinical characteristics, including age distribution, sex structure, and the prevalence of diabetes mellitus. For this reason, the assessment of the prevalence of major cardiovascular risk factors among patients experiencing a first ischemic event provides a more accurate reflection of the true status of prevention efforts, offering deeper insight into the effectiveness of existing preventive measures as well as into priorities and opportunities for further preventive intervention.

AIM

The aim of the study was to assess the prevalence of major modifiable cardiovascular risk factors in patients who experienced myocardial infarction as their first ischemic cardiovascular event and to evaluate their distribution in relation to selected baseline characteristics, thereby providing a comprehensive overview of the current state and potential for improvement of primary cardiovascular prevention.

MATERIALS AND METHODS

A retrospective study was conducted at the Clinic of Emergency Medicine, Clinical Center of the University of Sarajevo, including 166 consecutive patients who presented over a nine-month period with a diagnosis of acute myocardial infarction, including ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI).

Inclusion criteria were: confirmed diagnosis of myocardial infarction (STEMI or NSTEMI), patients of both sexes, and age ≥40 years.

Exclusion criteria included: previous history of ischemic cardiovascular events, age <40 years, pregnancy, and incomplete medical documentation.

Data were extracted from medical records within the hospital information system (BIS), focusing on cardiovascular risk factors considered relevant according to ACC/AHA recommendations (3). Diabetes mellitus was analyzed as a categorical variable (presence/absence) based on documented clinical diagnosis, consistent with its classification in established cardiovascular risk assessment models and to ensure uniformity of data in this retrospective study (3).

Age, sex, and the presence or absence of diabetes mellitus were recorded as as baseline demographic and clinical characteristics. Major modifiable cardiovascular risk factors assessed included systolic and diastolic blood pressure, total cholesterol, LDL-cholesterol, HDL-cholesterol, smoking status, and the use of antihypertensive therapy, statins, and aspirin.

Data on major cardiovascular risk factors were obtained from measurements performed no more than three months prior to hospital presentation; blood pressure measurements recorded between one and six months before presentation were accepted. During data analysis, the prevalence of major modifiable cardiovascular risk factors in the study population was first assessed. Subsequently, subgroups were formed based on these factors, followed by comparative analyses within and between the defined subgroups.

In the analysis, the prevalence of modifiable risk factors in the overall study population was first assessed. Subsequently, subgroup analyses were performed according to baseline characteristics, including sex, age, and the presence of diabetes mellitus.

Statistical analysis was performed using the SPSS software package. Descriptive statistics were applied, and comparisons of categorical variables were conducted using the chi-square test. A p-value of <0.05 was considered statistically significant. The results are presented in tables and figures.

Definitions

Blood pressure categories were defined according to the ACC/AHA classification (3).
Systolic blood pressure was categorized as normal (<120 mmHg), elevated (120–129 mmHg), or hypertension (≥130 mmHg).
Diastolic blood pressure was categorized as normal (<80 mmHg) or elevated (hypertension) (≥80 mmHg).

Categories of total cholesterol and LDL cholesterol were established based on the target values proposed by the 2019 European Society of Cardiology and European Atherosclerosis Society Guidelines for the management of dyslipidaemias (5), as the ACC/AHA guidelines do not define specific lipid targets nor classify lipid levels into normal or abnormal ranges (3).

A cut-off value of 5.0 mmol/L for total cholesterol was selected as a reference population value. Accordingly, total cholesterol levels were categorized as normal (<5.0 mmol/L) and elevated (≥5.0 mmol/L).

LDL cholesterol cut-off values were defined as 3.0 mmol/L for patients without diabetes and 2.6 mmol/L for patients with diabetes, corresponding to the target values recommended for individuals without additional cardiovascular risk factors. These thresholds represent definitively elevated LDL-C levels, even in the absence of other risk factors. Based on these cut-offs, LDL-C levels were categorized as normal (<3.0 mmol/L in non-diabetic patients; <2.6 mmol/L in diabetic patients) and elevated (≥3.0 mmol/L in non-diabetic patients; ≥2.6 mmol/L in diabetic patients).

High-density lipoprotein cholesterol (HDL-C) was classified as low (<1.0 mmol/L in men and <1.2 mmol/L in women) or normal (≥1.0 mmol/L in men and ≥1.2 mmol/L in women), based on widely accepted thresholds consistent with definitions used in ACC/AHA guidelines (3).

RESULTS

Table 1 **Demographic characteristics of the patients and presence of diabetes mellitus.**

Characteristics				
Sex	Number		Percentage	
Male	115		69.3	
Female	51		30.7	
Total	166		100.00	
Age				
Mean	SD	Minimum	Maximum	Median
60.02	8.64	40	75	60
Presence of diabetes mellitus				
Characteristics	Number		Percentage	
Yes	44		26.5	
No	122		73.5	

A total of 166 patients were included in the analysis. Male patients predominated in the study cohort, accounting for 69.3% of the population, while females represented 30.7%. The mean age of the patients was 60.02 ± 8.64 years. Diabetes mellitus was present in 26.5% of patients. Detailed demographic characteristics are presented in Table 1.

Table 2 **Distribution of blood pressure and serum lipid levels (N=166).**

Characteristics	**Median**	**IQR**	**Min–Max**
Systolic blood pressure (mmHg)	140	121.5-150	100–185
Diastolic blood pressure (mmHg)	80	80-90	60–110
Total cholesterol (mmol/L)	4.9	4.2-5.7	3.1–8.2
HDL cholesterol (mmol/L)	1.01	0.89-1.19	0.6–2.3
LDL cholesterol (mmol/L)	3.1	2.7-4.0	1.8–5.3

Values are presented as median (interquartile range, 25th–75th percentile).

In the overall study sample, the median systolic and diastolic blood pressure values were 140 and 80 mmHg, respectively. The median levels of total cholesterol, HDL cholesterol, and LDL cholesterol were 4.9, 1.01, and 3.1 mmol/L. The detailed distribution of these parameters is presented in Table 2.

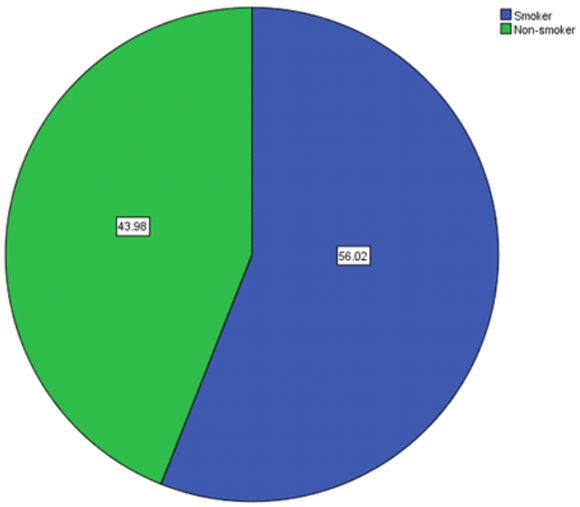

Figure 1 **Smoking status (N=166).**

Among the overall study population, 56.0% of patients were current smokers, while 44.0% were non-smokers. The distribution of smoking status is presented in Figure 1.

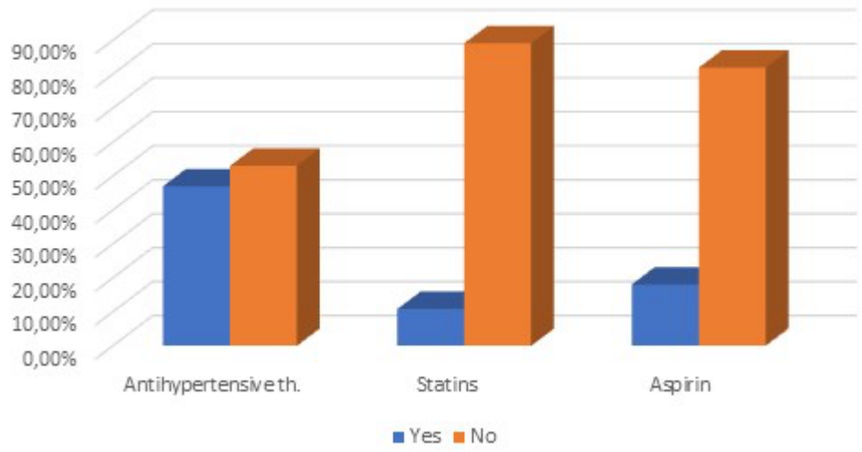

Figure 2 **Preventive therapy use (N=166).**

Table 3 **Distribution of elevated blood pressure according to baseline characteristics (age, sex, and diabetes mellitus).**

Variable	Sex			Age			Presence of diabetes			Total (n=166)
	Male (n=115)	Female (n=51)	p*	40–60 years (n=85)	>60 years (n=81)	P**	Yes (n=44)	No (n=122)	P***	
SBP <120 mmHg	16 (13.9)	8 (15.7)		8 (9.4)	16 (19.8)		7 (15.9)	17 (13.9)		24 (14.5)
SBP 120–129 mmHg	17 (14.8)	6 (11.8)		13 (15.3)	10 (12.3)		7 (15.9)	16 (13.1)		23 (13.9)
SBP >129 mmHg	82 (71.3)	37 (72.5)	0.854	64 (75.3)	55 (67.9)	0.162	30 (68.2)	89 (73.0)	0.830	119 (71.7)
DBP <80 mmHg	28 (24.3)	9 (17.6)		15 (17.6)	22 (27.2)		8 (18.2)	29 (23.8)		37 (22.3)
DBP ≥80 mmHg	87 (75.7)	42 (82.4)	0.339	70 (82.4)	59 (72.8)	0.141	36 (81.8)	93 (76.2)	0.445	129 (77.7)

Values are presented as N (% within group). Percentages are calculated within columns. P-values were derived using the Pearson chi-square test; p* - comparison between gender groups (male vs. female); p** - comparison between age groups (40–60 vs. >60 years); p*** - comparison according to diabetes status (yes vs. no).
SBP-Systolic blood pressure, DBP-diastolic blood pressure.

The majority of patients had elevated blood pressure values. Systolic blood pressure above 129 mmHg was observed in 71.7% of patients, while diastolic blood pressure ≥80 mmHg was present in 77.7% of the cohort. No statistically significant differences were observed in the prevalence of elevated systolic or diastolic blood pressure when comparing subgroups based on sex, age, or diabetes status. Detailed distributions and subgroup comparisons are presented in Table 3.

Table 4 **Distribution of elevated total cholesterol, LDL cholesterol and reduced HDL cholesterol according to baseline characteristics (sex, age, and diabetes mellitus).**

	Sex			Age			Presence of diabetes			
Variable	Male (n=115)	Female (n=51)	p*	40–60 years (n=85)	>60 years (n=81)	p**	Yes (n=44)	No (n=122)	p***	Total (n=166)
Normal TC	65 (56.5)	22 (43.1)		45 (52.9)	42 (51.9)		24 (54.5)	63 (51.6)		87 (52.4)
High TC	50 (43.5)	29 (56.9)	0.111	40 (47.1)	39 (48.1)	0.888	20 (45.5)	59 (48.4)	0.741	79 (47.6)
Normal HDL-C	75 (65.2)	21 (41.2)		51 (60.0)	45 (55.6)	0.562	19 (43.2)	77 (63.1)		96 (57.8)
Low HDL-C	40 (34.8)	30 (58.8)	0.004	34 (40.0)	36 (44.4)		25 (56.8)	45 (36.9)	0.022	70 (42.2)
Normal LDL-C	37 (32.2)	18 (35.3)		23 (27.1)	32 (39.5)		25 (56.8)	40 (32.8)		55 (33.1)
High LDL-C	78 (67.8)	33 (64.7)	0.694	62 (72.9)	49 (60.5)	0.089	29 (65.9)	82 (67.2)	0.875	111 (66.9)

Values are presented as N (% within group). Percentages are calculated within columns. P-values were derived using the Pearson chi-square test; p* - comparison between gender groups (male vs. female); p** - comparison between age groups (40–60 vs. >60 years); p*** - comparison according to diabetes status (yes vs. no).
TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Overall, 52.4% of patients had normal total cholesterol levels. No statistically significant differences were observed between age groups or between patients with and without diabetes, with normal total cholesterol predominating in all these subgroups. When stratified by sex, the majority of male patients had normal total cholesterol levels, whereas elevated total cholesterol was more frequent among female patients; however, this difference did not reach statistical significance ($p = 0.111$).

The majority of patients had normal HDL-C levels (57.8%), and a similar pattern was observed in both age-defined subgroups. Among patients with diabetes, most had reduced HDL-C levels (56.8%), in contrast to non-diabetic patients, in whom normal HDL-C levels predominated (63.1%); this difference was statistically significant (p=0.022). Among female patients, reduced HDL-C levels were more prevalent (58.8%), whereas male patients more frequently exhibited normal values of this parameter (65.2%); this difference was highly statistically significant (p=0.004).

Normal LDL-C levels were present in only 33.1% of the study population. Subgroup analyses revealed similar distributions of LDL-C categories, with no statistically significant differences observed between the respective subgroups. Elevated LDL-C was most prevalent in individuals aged 40–60 years (72.9%). The detailed results are summarized in Table 4.

Table 5 **Prevalence of smoking according to baseline characteristics (age, sex, and diabetes mellitus).**

	Gender			Age			Presence of diabetes		
Variable	Male (n=115)	Female (n=51)	p*	40–60 years (n=85)	>60 years (n=81)	p**	Yes (n=44)	No (n=122)	p***
Current smoker	70 (60.9)	23 (45.1)		56 (65.9)	37 (45.7)		19 (43.2)	74 (60.7)	
Non-smoker	45 (39.1)	28 (54.9)	0.059	29 (34.1)	44 (54.3)	0.009	25 (56.8)	48 (39.3)	0.045

Values are presented as N (% within group). Percentages are calculated within columns. P-values were derived using the Pearson chi-square test; p* - comparison between gender groups (male vs. female); p** - comparison between age groups (40–60 vs. >60 years); p*** - comparison according to diabetes status (yes vs. no).

Smoking was more prevalent among men (60.9%) than women, among whom non-smokers predominated (54.9%); however, this difference was not statistically significant (p = 0.059). Smoking was significantly more common among patients aged 40-60 years compared with those older than 60 years (65.9% vs. 45.7%; p = 0.009) and among patients without diabetes compared with those with diabetes (60.7% vs. 43.2%; p = 0.045) (Table 5).

Table 6 **Use of preventive therapy according to baseline characteristics (age, sex, and diabetes mellitus).**

ANTIHYPERTENSIVE TREATMENT									
	Sex			Age			Presence of diabetes		
Variable	Male (n=115)	Female (n=51)	p*	40–60 years (n=85)	>60 years (n=81)	p**	Yes (n=44)	No (n=122)	p***
Yes	49 (42.6)	29 (56.9)		33 (38.8)	45 (55.6)		30 (68.2)	48 (39.3)	
No	66 (57.4)	22 (43.1)	0.090	52 (61.2)	36 (44.4)	0.031	14 (31.8)	74 (60.7)	0.001
STATIN									
	Sex			Age			Presence of diabetes		
Variable	Male (n=115)	Female (n=51)	p*	40–60 years (n=85)	>60 years (n=81)	p**	Yes (n=44)	No (n=122)	p***
Yes	9 (7.8)	9 (17.6)		5 (5.9)	13 (16.0)		11 (25.0)	7 (5.7)	
No	106 (92.2)	42 (82.4)	0.060	80 (94.1)	68 (84.0)	0.035	33 (75.0)	115 (94.3)	<0.001
ASPIRIN									
	Sex			Age			Presence of diabetes		
Variable	Male (n=115)	Female (n=51)	p*	40–60 years (n=85)	>60 years (n=81)	p**	Yes (n=44)	No (n=122)	p***
Yes	21 (18.3)	9 (17.6)		15 (17.6)	15 (18.5)		13 (29.5)	17 (13.9)	
No	94 (81.7)	42 (82.4)	0.924	70 (82.4)	66 (81.5)	0.884	31 (70.5)	105 (86.1)	0.021

Values are presented as N (% within group). Percentages are calculated within columns. P-values were derived using the Pearson chi-square test; p* - comparison between sexes (male vs. female); p** - comparison between age groups (40–60 vs. >60 years); p*** - comparison according to diabetes status (yes vs. no).

Most male patients did not use antihypertensive therapy (57.4%), whereas the majority of female patients did (56.9%); however, this difference was not statistically significant (p = 0.090). Patients aged 40–60 years were less likely to use antihypertensive therapy (61.2% non-users), while those older than 60 years more frequently used this therapy (55.6%), with a statistically significant difference (p = 0.031). Antihypertensive therapy was significantly more common among patients with diabetes (68.2%) compared with those without diabetes, among whom non-use predominated (60.7%) (p = 0.001).

Across all subgroups, the majority of patients did not use statin therapy. Statin use was significantly more frequent among patients older than 60 years compared with those aged 40–60 years (16.0% vs. 5.9%; p = 0.035) and among patients with diabetes compared with those without diabetes (25.0% vs. 5.7%; p < 0.001).

The majority of patients did not use aspirin therapy (81.9%), a pattern observed across all subgroups. However, aspirin use was significantly more frequent among patients with diabetes compared with those without diabetes (29.5% vs. 13.9%; p = 0.021). These results are presented in Table 6.

DISCUSSION

In this cohort of patients experiencing a first myocardial infarction, a high prevalence of major modifiable cardiovascular risk factors was observed. The predominance of male and upper middle-aged patients further reflects established epidemiological patterns. These findings suggest a substantial burden of preventable cardiovascular risk despite existing primary prevention strategies.

The total number of patients, after applying the inclusion and exclusion criteria, was 166. Men predominated, accounting for 69.3% of the sample, which is consistent with findings from the Global Burden of Ischemic Heart Disease analysis (6). The mean age of 60.02 ± 8.64 years is noteworthy, as the same study reports that the highest number of cases worldwide occurs among individuals older than 70 years (6).

Despite the fact that our study included only patients with a first myocardial infarction, in contrast to the referenced study which also included recurrent events that are more common in older populations (6), it remains evident that a comparatively younger population was affected in our cohort. This finding is in line with the classification of Bosnia and Herzegovina as a high cardiovascular risk country according to European Society of Cardiology, where ischemic coronary events tend to occur at a younger age compared with the European average (5).

The median systolic and diastolic blood pressure values were 140 (121.5-150) and 80 (80-90) mmHg, respectively.. A more detailed insight into the extent of this issue is reflected in the fact that 71.7% of participants had systolic blood pressure >129 mmHg, and 77.7% had diastolic pressure ≥80 mmHg values that meet the ACC/AHA criteria for the diagnosis of hypertension (3).

According to a global meta-analysis, the prevalence of hyper tension in the general population is only 29.4% (7). However, it should be noted that the studies included in this meta-analysis involved diverse age groups and populations from different regions of the world, each with varying genetic and environmental influences. In contrast, the key inclusion criterion in the present study was a first-time myocardial infarction as a cardiovascular event. A more appropriate comparison might be drawn with a study conducted in a neighboring country on patients who had experienced ST-elevation myocardial infarction (STEMI), where a similar proportion of participants had hypertension (8).

Across all subgroups defined by sex, age, and the presence of diabetes the majority of participants were hypertensive, with no statistically significant differences observed between groups. This finding suggests that measured blood pressure levels in this cohort were largely independent of non-modifiable risk factors, indicating insufficient effectiveness of current preventive strategies and underscoring the need for improved blood pressure control across all patient groups, regardless of their inherent risk profile.

These results differ from expectations based on existing literature, which consistently demonstrates an age-related increase in the prevalence of hypertension as well as a higher burden of hypertension among individuals with diabetes (9). The absence of sex-related differences in our cohort is, however, plausible, as although men tend to exhibit a higher prevalence of hypertension at younger ages, this difference diminishes with advancing age and often equalizes after menopause (10). Given that the present study included individuals aged ≥40 years with a mean age of 60.02 years, such convergence of hypertension prevalence between sexes is consistent with established epidemiological patterns.

The median total cholesterol value of 4.9 (4.2-5.7) mmol/L indicates that the majority of participants had values within the desirable range; however, 47.6% exhibited elevated total cholesterol levels. A similar distribution, without statistically significant differences, was observed across subgroups defined by baseline characteristics.

Notably, women accounted for a majority (56.9%) of partici pants with elevated total cholesterol, a finding that can be explained by the age structure of the study population, in which peri- and postmenopausal women predominated (11).

The comparable distribution of total cholesterol across age groups is somewhat unexpected, given that total cholesterol levels are known to increase with advancing age, particularly during middle adulthood, as shown in previous studies (12).

The majority of the overall study population exhibited normal HDL-C levels [median value 1.01 (0.89–1.19) mmol/L], while 42.2% of patients had reduced HDL-C values. Subgroup analysis by age demonstrated comparable distributions of HDL-C categories, with no statistically significant differences between groups.

At first glance, the finding that women had a higher proportion of patients with reduced HDL-C levels compared to men may appear expected, given that women generally have lower HDL-C levels, largely attributable to estrogen-mediated effects on lipid metabolism. However, considering that the study population consisted of individuals aged ≥40 years, with a mean age of 60.02 years, and that the female cohort predominantly comprised peri- and postmenopausal women—in whom the protective hormonal influence on HDL-C levels is diminished—this result may, in fact, be surprising (13).

Patients with diabetes are generally characterized by lower HDL-C levels, a finding consistently documented in the literature (14). In the present study, the prevalence of reduced HDL-C was significantly higher among patients with diabetes compared with those without diabetes (56.8% vs. 36.9%, respectively). This observation may suggest that glycemic control in the diabetic subgroup was overall inadequate, given that poor glycemic control is well known to be associated with more pronounced reductions in HDL-C (15).

Regarding LDL-C, only about one third (33.1%) of patients had values within the normal range [median value 3.1 (2.7-4.0) mmol/L]. Although no statistically significant differences were observed across subgroups, individuals aged >60 years showed a lower prevalence of elevated LDL-C (60.5%) compared with those aged 40–60 years (72.9%) (p=0.089). This finding contrasts with the well-established age-associated increase in LDL-C reported in the literature (16), but is consistent with evidence from another study indicating that LDL-C levels rise until approximately 57 years of age and subsequently decline (17). This age threshold closely approximates the mean age of the study population.

The prevalence of elevated LDL-C was comparable between men and women (67.8% vs. 64.7%, respectively). This pattern aligns with the age structure of the cohort, in which women were predominantly peri- and postmenopausal. While younger women typically exhibit lower LDL-C concentrations than men, sex-related differences in LDL-C are known to diminish after menopause, resulting in comparable levels between sexes (13).

Patients with and without diabetes exhibited comparable proportions of individuals with elevated LDL-C levels (65.9% vs. 67.2%, respectively), despite lower cut-off values being applied for patients with diabetes compared to those without diabetes (2.6 mmol/L vs. 3.0 mmol/L, respectively). It should be noted, however, that the categories of elevated LDL-C were defined in a way that captured values clearly above normal thresholds for all participants, regardless of other risk factors.

This study was designed as a cross-sectional assessment of the prevalence of cardiovascular risk factors and did not account for their interplay or the calculation of overall cardiovascular risk. Nevertheless, given that all included patients had experienced myocardial infarction, it is evident that they represented a population with a high burden of risk factors.

Accordingly, it can be assumed that the use of lower LDL-C target values (e.g., 1.8 or 1.4 mmol/L), as recommended based on overall cardiovascular risk, would have resulted in a substantially higher proportion of patients being classified as having elevated LDL-C. This would likely have accentuated the difference between patients with and without diabetes, as individuals with diabetes are assigned lower LDL-C targets in the presence of comparable overall risk profiles, given that the presence of diabetes itself places them in a higher cardiovascular risk category (5).

Cigarette smoking represents a particularly concerning risk factor in the present cohort, as 56.0% of patients were current smokers. This prevalence markedly exceeds the global smoking prevalence of 15.4%, as reported in a recent comprehensive meta-analysis of cardiovascular risk factors (7) Direct comparison with these global data should, however, be interpreted with caution, as the present study exclusively included patients aged ≥40 years presenting with a first myocardial infarction. Notably, smoking prevalence in comparable clinical populations appears similarly high. In a Croatian study including patients with ST-elevation myocardial infarction (not necessarily limited to first events), the proportion of smokers reached 66.8%, further underscoring the substantial burden of tobacco use in acute coronary syndromes (8).

Subgroup analyses revealed statistically significant differences in smoking status across age categories. Individuals aged >60 years were predominantly non-smokers, whereas smoking was more prevalent among patients aged 40-60 years ($p = 0.009$). This age-related pattern may reflect greater health awareness among older individuals, reduced financial resources, and broader cultural and generational differences in smoking behavior.

In addition, non-smokers were significantly more prevalent among patients with diabetes compared with those without diabetes ($p = 0.045$). This observation may reflect greater risk awareness among individuals with diabetes or, alternatively, more intensive counseling and smoking cessation interventions provided by healthcare professionals to this patient group.

Furthermore, non-smokers were more prevalent among women than among men. Although this difference did not reach statistical significance, it approached significance ($p = 0.059$). As smoking represents a behavioral rather than a biologically determined sex-specific characteristic, this difference is likely attributable to sociocultural factors rather than inherent biological differences

The use of preventive therapy in the study population was low. Only 47.0% of participants were receiving antihypertensive treatment, despite 71.7% and 77.7% presenting with elevated systolic and diastolic blood pressure values, respectively, corresponding to hypertension according to ACC/AHA criteria (3). This suggests that a substantial proportion of patients may be unaware of their hypertensive status or do not initiate antihypertensive therapy despite a known diagnosis. Moreover, the prevalence of statin and aspirin use was notably low (10.8% and 18.1%, respectively), particularly when compared with data from a U.S. study conducted among patients aged ≥50 years, in which the use of these medications for primary prevention was reported to be 30.4% and 33.9%, respectively (18).

Patients with diabetes demonstrated significantly different patterns of preventive therapy use, with the majority receiving antihypertensive treatment compared with patients without diabetes. Although statin and aspirin use was low in both groups, patients with diabetes nonetheless exhibited a significantly higher prevalence of statin and aspirin therapy. This finding may reflect greater adherence to preventive measures among patients with diabetes, as well as more intensive clinical attention and risk factor management by treating physicians. This interpretation is further supported by the relatively good profiles observed for other cardiovascular risk factors within the diabetic subgroup.

Although no statistically significant difference was observed between the 40–60 and >60 year age groups regarding the prevalence of elevated systolic and diastolic blood pressure, the use of antihypertensive medication was significantly more frequent among participants older than 60 years. This finding likely reflects a higher prevalence of previously diagnosed hypertension in older individuals, as well as more frequent blood pressure screening and earlier initiation of pharmacological treatment in this age group. At the same time, the absence of differences in measured blood pressure values between age groups may indicate more effective blood pressure control among older participants due to higher treatment rates, thereby attenuating the expected age-related increase in hypertension prevalence. Patients aged over 60 years used statins significantly more frequently; however, aspirin use was comparable between the two age groups. This finding contrasts with previously published data indicating that the use of both therapies increases with advancing age (18).

Regarding sex-based subgroups, women were more frequently treated with antihypertensive therapy than men; however, this difference did not reach statistical significance. In both sexes, only a minority of patients received statin and aspirin therapy.

This study provides novel, region-specific insights into the prevalence and distribution of major cardiovascular risk factors among patients experiencing their first myocardial infarction, a population in which primary prevention is particularly relevant, as risk profiles are not influenced by prior cardiovascular events or secondary prevention therapy.

Conducted in Sarajevo, Bosnia and Herzegovina, the study fills a regional evidence gap, offering real-world data on a population that has been underrepresented in contemporary cardiovascular research. By distinguishing baseline characteristics—age, sex, and diabetes mellitus—from modifiable risk factors such as elevated blood pressure, dyslipidemia, and smoking, the analysis highlights the substantial contribution of preventable risk to overall cardiovascular burden. Furthermore, the observed low use of antihypertensive, statin, and aspirin therapy despite high prevalence of hypertension and dyslipidemia underscores critical gaps in primary prevention and guideline adherence. These findings identify actionable targets for improving primary prevention strategies and reducing the burden of ischemic cardiovascular disease.

CONCLUSION

Patients presenting with a first myocardial infarction exhibit a high prevalence of major modifiable cardiovascular risk factors and low use of preventive therapy, emphasizing the urgent need for improved primary prevention strategies and early risk factor management.

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Stability Testing of Captopril Solution, Concentration 2mg/ml

Test stabilnosti kaptopril otopine, koncentracije 2mg/ml

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ABSTRACT

The stability of pharmaceutical products can be described as the ability of pharmaceutical formulation to remain within its physical, chemical, microbiological and therapeutic specifications. Aim: to design a captopril solution for oral use that has greater stability than the official formulation. Preparation of formulation: The study included captopril oral solutions prepared from commercially available tablets and pure substance. Storage conditions: All formulations were stored in a refrigerator and in an incubator. Analytical methods: Organoleptic tests include visual examination of the solution with purified water as a control sample. Measurement of the pH value was performed separately. The spectrophotometric method was used to determine the content of captopril. Statistical analysis: Statistical comparison is performed by ANOVA to determine a significant difference between 2 groups or more groups. Results: changes in the pH value of captopril solutions, made from tablets and from pure captopril substance (solvent purified water + vit.C), room temperature storage, are accompanied by changes in the appearance and smell. Determination of the captopril content in solutions indicates a constant decrease at room temperature. The results of determining the captopril content in solutions stored in the refrigerator show that the content does not have a sudden quantitative drop in relation to the initial concentrations, for all three samples. Conclusion: based on the overall results of the research, it can be determined that the product is more stable if 0.1% Na2-EDTA solution is used instead of vit. C in the preparation and if the solution is buffered to pH 3.3.

Keywords: stability, captopril, pharmacy

SAŽETAK

Stabilnost farmaceutskih preparata se opisuje kao sposobnost farmaceutske formulacije da ostane unutar svojih fizikalnih, hemijskih, mikrobiloških i terapeutskih specifikacija. Cilj: dizajnirati otopinu kaptoprila za oralnu primjenu koja ima veću stabilnost od postojeće. Priprema formulacije: Istraživanje je obuhvatilo kaptopril otopine za oralnu upotrebu pripremljene od komercijalno dostupnih tableta i čiste supstance. Uslovi čuvanja:Sve formulacije čuvane su u hladnjaku i u sobi za ispitivanje stabilnosti. Analitičke metode: Organoleptički testovi uključuju vizuelni pregled otopina sa pročišćenom vodom kao kontrolnim uzorkom. Mjerenje pH vrijednosti provedeno je odvojeno. Spektrofotometrijska metoda korištena je za određivanje sadržaja kaptoprila. Statistička analiza:Statistička usporedba provedena je ANOVA testom kako bi se utvrdila značajna razlika između 2 ili više skupina. Rezultati: Promjena pH vrijednosti otopine kaptoprila, pripremljenih od tableta i od čiste tvari kaptoprila (otapalo pročišćena voda i vit. C amp), sobna temperature čuvanja, popraćene su promjenama izgleda i mirisa. Određivanje sadržaja kaptoprila u otopinama ukazuje na stalno smanjenje na sobnoj temperature. Rezultati određivanja sadržaja kaptoprila u otopinama pokazuju da sadržaj nema nagli kvantitativni pad u odnosu na početnu koncentraciju, za sva tri uzorka. Zaključak: na temelju ukupnih rezultata istraživanja može se utvrditi da je preparat stabilniji ako se pripremi u otopini 0.1% Na2-Edta umjesto uVit.C i ako je otopina puferizovana na pH 3.3.

Ključne riječi: stabilnost, kaptopril, farmacija

INTRODUCTION

The stability of pharmaceutical products can be described as the ability of pharmaceutical formulation in a particular closured container to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications (1). Stability testing of pharmaceutical products is a complex set of procedures. It involved considerable cost, time consumption and scientific expertise in order to build in quality, efficacy and safety in a drug formulation. The most important stages of stability testing include pharmaceutical analysis and stability studies. These analyses and studies are required to determine and assure the identity, potency and purity of ingredients, as well as those of the formulated products. The conclusions obtained from stability testing apply to all future batches of the tested pharmaceutical substance or pharmaceutical product (1). Stability testing should be performed on samples that are representative of the quality of the pharmaceutical substance or the pharmaceutical product that will be manufactured in regular production. For this purpose, it is necessary to ensure that the manufacturing process, formulation, dosage form and packaging are identical to or closely simulate those that will be used in the future (1).

Many factors influence the stability of pharmaceutical pre parations, including the stability of active ingredients, potential interaction between drug and inactive ingredients, the manufacturing process, dosage form, packing, as well as environmental conditions during transportation, storage, handling and the length of time between manufacture and use (2).

The factors affect the stability of pharmaceutical preparation can be divided into three groups:

The first group relates to the drug and inactive ingredients: chemical structure, contamination.

The second group relates to physical properties, moisture content, particle size, contact surface, morphology.

The third group relates to temperature, relative humidity, packaging, light and oxygen (2).

The evaluation of stability captopril solution

Before the planned pharmaceutical preparation is made, it is essential that the substances must be chemically and physically characterized. Pharmaceutical solutions for oral use must meet certain quality requirements; have excellent organoleptic properties, easy solubility of drug (active) components, easy redispersion and resistance to microbiological contamination. The characterization of the substance is carried out according to subjective feeling in relation to the control solution (3). The control solution was stored under the same conditions (stability test chamber or fridge). The control solution was solvent or was made from pure substance, the same concentration. Captopril is available in doses: 12.5 mg-100mg for adults. The dose for children is generally lower than 12.5 mg. For children, the recommended maximum dose is 300 □g/kg for premature infants. 6 mg/kg per day for children from one month to 12 years, used in divided doses (4).

In the solution, captopril undergoes rapid auto-oxidation of thiols. Captopril disulfide was forming. The reaction is catalyzed by metal ions. The rate of degradation depends on pH (3.6) and oxygen concentration. If the pH of the captopril solution is around 4.0, the degradation of captopril may be slowed.

Captopril (1-[(2S)-3-mercapto-2-methylpropionyl]-1-proline) is a sulfhydryl - containing angiotensin - converting enzyme (ACE) inhibitor. It is used in the treatment of hypertension, heart failure, post-myocardial infarction, and diabetic nephropathy. It was excreted primarily in the urine, 40-50% of captopril was excreted as an unchanged drug. The rest was excreted as captopril disulfide and other metabolites.

Disulfide products of captopril metabolism were formed reversibly in vivo and in vitro. The dynamic transformation of captopril and the largely inactive disulfide metabolites distinguishes captopril from other antihypertensive agents. It probably contributes to the prolonged pharmacological effect of captopril in the absence of measurable amounts of captopril in the blood. Renal failure results in only a small increase in blood levels of captopril. Some studies have shown that captopril disulfides may contribute to the antihypertensive effect of captopril. It has a weak, significant inhibition of angiotensin-converting enzyme activity. Therefore, it is of great importance to assess the amount and impact of these metabolites.

Oxidation can be prevented by using antioxidants. In solution, antioxidants are subject to oxidation faster than the active substance (medicine), thus protecting the active component from decomposition (5).

A long-term study shows the stabilizing effect of 0.1% EDTA-Na solution on captopril solution, concentration 1mg/ml. According to the results of this study, lower concentrations of EDTA-Na (0.01%) are not sufficient to stabilize captopril effectively (5).

MATERIALS AND METHODS

The study included captopril oral solutions prepared from commercially available captopril tablets (two different manufacturers) and captopril oral solutions prepared from commercially available captopril pure substance.

Captopril oral solutions were prepared according to the recipe from Pediatric Drug Formulations, 6th edition, Milap C. Nahata, Vinita B. Pai (6).

Preparation of captopril solution formulations, concentration 2mg/ml

Method of preparation of captopril solution for oral use, concentration 2mg/ml made from tablet:

Dissolve captopril tablets (25 mg) 8 tablets of both manu facturers (the amount needed to prepare captopril solution, concentration 2mg/ml) in 50 ml of purified water in a 100ml measuring vessel. Add the remaining amount of purified water to a volume of 100ml. The solution is not filtered (6).

Method of preparation of captopril solution for oral use, concentration 2mg/ml made from pure captopril substance:

Dissolve captopril pure substance (200mg) (the amount needed to prepare captopril solution, concentration 2mg/ml) in 50 ml of purified water in a 100ml measuring vessel. Add the remaining amount of purified water to a volume of 100 ml. The solution is not filtered (6).

Method of making captopril solution for oral use, concentration 2mg/ml made from tablets. A mixture of ascorbic acid and purified water was used as a solvent:

Dissolve captopril tablets (25 mg) 8 tablets from both manufacturers (the amount needed to make a captopril solution, concentration 2mg/ml) in 50 ml of purified water in a 100ml measuring cup. Add the contents of the 500mg/5ml ascorbic acid ampoule and mix well. Then add the remaining amount of purified water to a volume of 100 ml. The solution is not filtered (6).

Method of making captopril solution for oral use, concentration 2mg/ml made from tablets. A solution of EDTA-Na, concentration 1mg/ml in purified water was used as a solvent:

Dissolve captopril tablets (25 mg) 8 tablets in 50 ml of a 1mg/ml EDTA-Na solution in purified water. Then add the remaining amount of solution to a volume of 100ml. The solution is not filtered (6).

Method of preparing captopril solution for oral use, made from pure captopril substance and EDTA –Na:

Dissolve captopril pure substance (the amount required for a given concentration of solution for oral use) in 50 ml of a 1mg/ml EDTA-Na solution in purified water. Then add the remaining amount of solution to a volume of 100 ml. The solution is not filtered (6).

Stability test

Storage conditions

All formulations were stored in a refrigerator (2-8°C) for 60 days and in a stability test chamber (22°C-25°C) for 60 days. Captopril solutions were made in duplicate and stored in dark glass bottles.

Analytical methods

The physical and chemical stability of all formulations was tested at the initial time, 1 day, 4 days, 7 days, 14 days, 21 days, 28 days, 56 days and 60 days. The solutions were shaken vigorously before measuring the pH value and before monitoring the physical-chemical characteristics, in order to ensure the homogeneity of the solutions (7,8).

The physical characterics

Organoleptic tests include visual examination of the solution, homogeneity (observation of the solution against a dark background) and its opalescence (observation of the solution against a white background) with purified water as a control sample for comparison. Appearance, color, smell (changes in color intensity, reduction or increase of the characteristic smell, if any, change in the texture of the solution, appearance of sediment).

The smell of the solution was also recorded. The solvent was used as a control solution (/,8). The sense of smell is affected by : health condition, the duration of acttion of a certain smell, the concentration of the odours substance, the presence of another smell, the temperature of the presented sample.

Note: The smell is not mentioned in all medical monographs, except in special cases when the smell and taste have a specific form. In some cases, the release of odor is considered an important criterion of purity, as it may indicate the substance's degradation.

Odor testing procedure: a thin layer of 0.5 g to 2 g of the test substance is applied to a watch glass, 6 cm to 8 cm in diameter. The odor is tested from a distance of 4 cm to 6 cm. After 15 minutes, the odor is determined or the absence of odor is confirmed.

The physical and chemical tests include methods according to the European Pharmacopoeia protocol.

The evaluation of smell according to ISO 5492, the intensity of smell: 0 (odorless) → 1(a mild, barely noticeable smell) → 2 (medium smell) →3 (extremely strong, powerful, pungent odor)

Chemical testing of products:

Measurement of the pH value: was performed separately from the determination of the content of the active component. All solutions were measured immediately after preparation, and then dynamically according to the previously established protocol (9).

In the implementation of the experimental part of this work, a simple spectrophotometric method was used to determine the content of captopril as a representative example of thiol. The principle of the method is based on the reaction of the aromatic substitution of the thiol group of captopril with 2, 4-dinitrofluorobenzene in an aqueous borate buffer pH 8.0, resulting in a yellow-colored product. 2.4 – dinitrofluorobenzene reagent (DNFB) was prepared as a 0.2% solution in methanol (10).

The reaction was validated according to the USP 27 criteria and was found to be suitable for the determination of the captopril content in pharmaceutical preparations without the influence of common auxiliary medicinal substances.

Absorption spectrum: Captopril reacts with 2,4-dinitrofluo robenzene in aqueous borate buffer to form a yellow colored product that has a maximum absorption at 327 nm with a blank (10).

To assess the suitability and specificity of the chosen spectrophotometric method for measuring captopril content in captopril solution, absorption spectra were recorded from 200 to 400 nm.

Considering working with complex sample, it was necessary to prove that excipients did not interfere and do not show any absorption at the wavelength of the reaction product of captorpil and DNFB.

It was also necessary to demonstrate that the reagent itself did not have absorption at that wavelength. The spectrum of the mixture was recorded between 200 and 400 nm.

Method for dosage forms for oral use

For solutions for oral use: Take 0.1 ml of solution for oral use with 25 ml of methanol. Apply the general procedure to determine the concentration of the active component for oral use.

Statistical analysis

Statistical comparison was performed by ANOVA to determine a significant difference between 2 groups or more groups. (10).

Table **1a and b Visual inspection and pH profile of captopril solution for oral use, concentration 2mg/ml, made from tablets of both manufacturers and captopril solution, concentration 2mg/ml, made from pure captopril substance. Solvent purified water and vit.C. Stability test chamber storage.**

a)

	After preparation	1 st day	4 th day	7 th day	14 th day	21 th day	28 th day	56 th day	60 th day
Tabl. 1	white , cloudly solution. After 15 days turns yellow. Strong sulphur odor. Intesity 3.								
Tabl.2	white , cloudly solution. After 15 days turns yellow. Strong sulphur odor. Intesity 3.								
Standard	clear, colorless solution. After 15 days turn yellow. Strong sulphur odor. Intesity 2								

b)

	After preparation	I st day	4 th day	7 th day	14 th day	21 th day	28 th day	56 th day	60 th day
Tab1pH	4, 56	4, 66	4, 73	4, 72	4, 79	4,67	4, 67	4,57	4, 53
Tabl.2pH	4, 56	4, 69	4, 73	4, 75	4,79	4,71	4,72	4,76	4, 79
Standard	4,55	4, 69	4, 75	4,77	4,78	4,73	4,75	4, 75	4, 79

A statistical analysis was performed on samples of captopril solutions. The analysis of the samples did not reveal a significant statistical difference with F= 2.280 and p= 0.124 or p>0.05.

Table 2 **Presents a statistical analysis of captopril solutions, with a concentration of 2mg/ml, made from tablets of both manufacturers and the pure substance. Solvent: purified water and vit. C ampoule. Stability test chamber storage.**

	N	X	SD	SG	Minimum	Maksimum
Tbl. I	9	4.6556	.08691	.02897	4.53	4.79
Tbl. 2	9	4.7222	.06978	.02326	4.56	4.79
Standard	9	4.7211	.06954	.02318	4.55	4.78
Total	27	4.6996	.07949	.01530	4.53	4.79
	F			p		
Between groups	2.280			.124		

Table 3 **a and b Presentation of the pH profile of captopril solution for oral use, concentration 2mg/ml, made from tablets of both manufacturers and captopril solution, concentration 2mg/ml, made from pure captopril substance. Solvent purified water + vit.C. Refrigerator storage.**

a)

	After preparation	I st day	4 th day	7 th day	14 th day	21 th day	28 th day	56 th day	60 th day
tabl. I	white , cloudly solution. Strong sulphur odor. Intesity 2.								
tabl.2	white , cloudly solution. Strong sulphur odor. Intesity2.								
standard	clear, colorless solution. Strong sulphur odor. Intesity 2								

b)

	After preparation	I st day	4 th day	7 th day	14 th day	21 th day	28 th day	56 th day	60 th day
tabl.1pH	4, 60	4, 64	4, 74	4,8	4,82	4,83	4,69	4,867	4,81
tabl.2pH	4, 48	4,63	4,75	4,79	4,77	4,73	4,83	4,9	4,88
standard	4,55	4,65	4,77	4,84	4,86	4,78	4,81	4,88	4,9

Table 4 **Statistical analysis of the samples proved that there is no significant difference between the samples made from tablets of both manufacturers and the sample made from the pure substance with F= 0.204 and p= 0.817 or p> 0.05. The solvent is purified water and vitamin C. Refrigerator storage**

	N	X	SD	SG	Minimum	Maksimum
Tbl. 1	9	4.7544	.09194	.03065	4.60	4.86
Tbl. 2	9	4.7511	.13014	.04338	4.48	4.90
Standard	9	4.7822	.11465	.03822	4.55	4.90
Total	27	4.7626	.10981	.02113	4.48	4.90
	F			p		
Between groups	.204			.817		

Determination of captopril content based on reference solution (purified water) by spectrophotometric method

Table 5 **The comparison of total results (%) of samples for dtermination of captopril content in slotion, concentration 2mg/ml (solvent: purified water and vit.C) and (solvent: 0,1 % EDTA solution) in comparison to captopril solution, concentration 2mg/ml (solvent: purified water). Storage: room temperature, stability test chamber.**

Duration		After preparation	The first week	The second week	The third week	4 th week	5 th week
2mg/ml aqua purificata		100	97	71,17	74,19	78,05	74,13
2mg/ml aqua purificata+ Vit.C		99,99	72,29	43,85	38,38	54,72	39.61
2mg/ml 0,1 %EDTA		100, 05	101,24	93,8	111,94	105,14	113
F		0	8,383	13,901	25,079	28,831	26,921
p		1	0,018	0,006	0	0	0

Table 6 **The comparison of total results (%) of samples for determination of captopril content in solution, concentration 2mg/ml (solvent: purified water and vit.C) and (solvent: 0,1% EDTA solution) in comparisson to captopril solution, concentration 2mg/ml (solvent : purified water). Stability test: refrigerator storage.**

Duration		After preparation	The first week	The second week	The third week	4 th week	5 th week
2mg/ml aqua purificata		100	102,26	103,87	51,29	73,57	72,97
2mg/ml aqua purificata+ Vit.C		99,9	100,76	93,53	93,53	91,17	79,69
2mg/ml 0,1 %EDTA		99,99	98,43	96,9	89,27	140,85	103,83
F		0	1,543	0,747	5,201	1,365	18,699
p		1	0,288	0,513	0,049	0,325	0

Table 7 **Comparison of total results (%) of samples for determination of captopril content in solution, concentration 2mg/ml (solvent: purified water and vit.C) and (solvent: 0,1% EDTA solution) in comparison to captopril solution, concentration 2mg/ml (solvent purified water). Storage temperature, stability test chamber and refrigerator.**

Duration		After preparation	The first week	The second week	The third week	4 th week	5 th week
2mg/ml aqua purificata		100	99,63	87,53	62,74	75,81	73,55
2mg/ml aqua purificata+ Vit.C		99,95	86,53	68,69	65,95	72,94	59,65
2mg/ml 0,1 %EDTA		100,03	99,83	95,35	100,61	122,99	108,42
F		0	2,85	2,485	4,837	3,527	19,957
p		1	0,089	0,117	0,024	0,056	0

DISCUSSION

Changes in the pH value of captopril solutions, concentration 2mg/ml, made from tablets of both manufacturers and from pure captopril substance (solvent purified water + vit.C), room temperature storage, are accompanied by changes in the appearance and smell of these solutions. Namely, after 14 days of measuring the pH value, the maximum value of all solutions is measured. The solutions also turn yellow 15 days after the start of the study. The smell of these solutions is intense, like sulfur. The smell intensity is 3, on a scale of 1-5 (ISO 5492 scale) (11). Statistical analysis of the pH profile of captopril solutions made from tablets of both manufacturers in comparison to captopril solutions, made from pure substance, concentration 2mg/ml, no significant difference was recorded. All solutions behave the same. Considering that all three solutions behave the same, the changes occur at the same time, it is concluded that it is not necessary to use pure substance instead of tablets when preparing captopril solutions, concentration 2mg/ml.

Determination of the captopril content in captopril solutions and analysis of the measured intensities of the absorbances indicate a constant decrease in the captopril content in captopril solutions / solvent purified water and solvent purified water + vit. C ampoule /. This indicates a possible decomposition of captopril in the captopril solution, if stored at room temperature. Formulations made by dissolving captopril in 0.1% EDTA remain colorless, if they are made from the pure substance. Formulations made from tablets of both manufacturers. In 0.1% EDTA solutions, they are white, cloudy solutions. The appearance is constant, whether they are stored at room temperature or in the refrigerator for the entire duration of the study. The sulfur odor in formulations made by dissolving in 0.1% EDTA has an intensity of 1-2, if stored at room temperature. If it is stored in the refrigerator, it remains odorless or has an odor intensity of 1(11). When determining the captopril content in captopril solutions, the concentration of 2mg/ml in the EDTA solution as a solvent and the analysis of the measured absorbances, no significant deviations from the initial value were observed.

This may indicate good stability of these solutions during the research. Storage temperature is room temperature. An exception to this is the variable absorbance intensity measured for 3 weeks, with a sudden rise and fall of the absorbance intensity during the research, which may be the result of a systematic error. Systematic error can be understood as the influence of the presence of metal ions introduced through the solvent, sensitivity of captopril to oxidation, and non-uniformity of sample collection. Captopril solutions, concentration 2mg/ml, stored in the refrigerator, made from tablets of both manufacturers, with purified water and vit.C amp as solvents, do not experience any change in appearance and smell during the study.

Captopril solutions are white, cloudy solutions throughout the study. Statistical processing of data obtained by measuring the pH profile did not record any significant difference between the samples. The results of determining the captopril content in captopril solutions stored in the refrigerator show that the captopril content in captopril solutions does not have a sudden quantitative drop in relation to the initial captopril concentrations in the solutions, for all three samples, as was the case with these same samples stored at room temperature. This indicates the possibility of reducing the degradation of captopril in solutions, if they are stored in the refrigerator and thus increasing their stability. When determining the captopril content in captopril solutions, concentrations of 2mg/ml in 0.1% EDTA solution as a solvent and analyzing the measured absorbances, no significant deviations were observed compared to the initial value, which may indicate good stability of these solutions during the study. Storage temperature (refrigerator/room temperature) did not affect the dispersibility of captopril solutions. Spectrophotometric determination of captopril content determined that the occurrence of turbidity does not affect the captopril content. The spectrophotometric method itself does not show any interference with auxiliary materials from tablets, as well as substances used in the preparation of formulations, namely vit. C from the ampoule and Na2-EDTA.

In the literature, you can also find the results of research into the stability of captopril solutions in drinking water (12). The usability of such works is seriously limited by geographical differences in the composition of drinking water around the world (12,13). The recommended storage period varies from three days to 14 days when stored in the refrigerator (13).

Kadin measured the oxidation level of captopril at different pH values and came to the conclusion that the captopril solution is most stable at pH values below 3.5. Above pH 4, oxidation is inhibited (14). Copper and iron have been identified as the most effective catalysts. EDTA, citric acid and oxalic acid are labeled as stabilizing agents (15).

CONCLUSION

Based on the results of the tests conducted in this experiment, it can be recommended that captopril tablets be used for the preparation of oral captopril solutions with a concentration of 2mg/ml. Based on the overall results of the research, it can be determined that the product is more stable if 0.1% Na2-EDTA solution is used instead of vit. C in the preparation and if the solution is buffered to pH 3.3. The captopril content decreases during the storage period in all formulations. The decrease in captopril content is less pronounced in formulations that were stored in the refrigerator, as well as in formulations that were prepared by dissolving captopril tablets in 0.1% EDTA. In accordance with the obtained results of determining the captopril content by the spectrophotometric method, the product should be packaged in dark glass bottles and stored in the refrigerator for no longer than 2 weeks after preparation. Due to the fact that the excipients used in the preparation of the tablets precipitate, the product should be issued with the indication "Shake before use".

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Air Medical Evacuation in Bosnia and Herzegovina: Current State and Future Development Perspectives

Medicinska evakuacija zračnim putem u Bosni i Hercegovini: trenutno stanje i perspektive budućeg razvoja

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ABSTRACT

Aeromedical evacuation and Helicopter Emergency Medical Service (HEMS) represent an essential component of modern emergency medical systems, particularly in trauma care and time-critical medical conditions. Many European countries have established organized civilian HEMS systems in accordance with European Union regulatory and safety standards. In Bosnia and Herzegovina, however, aeromedical evacuation remains institutionally fragmented and largely dependent on military and ad hoc solutions. The aim of this paper is to analyze the current state, organizational framework, and development perspectives of aeromedical evacuation and HEMS in Bosnia and Herzegovina. This manuscript was designed as a scoping review using a narrative analytical approach, synthesizing relevant scientific literature and official institutional documents published between 2010 and 2025. Data sources included PubMed-indexed journals, publications of the World Health Organization, documentation of the European Union Aviation Safety Agency, and national legal and regulatory sources. The analyzed data indicate that the absence of a unified civilian HEMS system, legal fragmentation, and limited civil–military coordination significantly reduce the efficiency of emergency medical response. Comparative experiences from neighboring countries suggest that phased system development aligned with European standards could substantially improve emergency medical outcomes.

Keywords: Helicopter Emergency Medical Service (HEMS), aeromedical evacuation, trauma care, health system organization, Bosnia and Herzegovina

SAŽETAK

Medicinska evakuacija zračnim putem i Helikopterska hitna medicinska služba (HEMS) predstavljaju ključnu komponentu savremenih sistema hitne medicinske pomoći, naročito u zbrinjavanju traume i drugih vremenski kritičnih medicinskih stanja. Mnoge evropske zemlje uspostavile su organizovane civilne HEMS sisteme u skladu s regulatornim i sigurnosnim standardima Evropske unije. Međutim, u Bosni i Hercegovini medicinska evakuacija zračnim putem i dalje je institucionalno fragmentirana i u velikoj mjeri se oslanja na vojne i ad hoc kapacitete. Cilj ovog rada je analizirati trenutno stanje, organizacioni okvir i perspektive razvoja medicinske evakuacije zračnim putem i HEMS-a u Bosni i Hercegovini. Ovaj rad je koncipiran kao scoping pregled uz korištenje narativnog analitičkog pristupa, koji sintetizira relevantnu naučnu literaturu i zvanične institucionalne dokumente objavljene u periodu od 2010. do 2025. godine. Izvori podataka obuhvatili su PubMed-indeksirane časopise, publikacije Svjetske zdravstvene organizacije, dokumentaciju Evropske agencije za sigurnost zračnog saobraćaja, kao i važeće domaće zakonske i regulatorne izvore. Analizirani podaci ukazuju da nepostojanje jedinstvenog civilnog HEMS sistema, pravna fragmentacija i ograničena civilno-vojna koordinacija značajno umanjuju efikasnost hitnog medicinskog odgovora. Komparativna iskustva susjednih zemalja sugeriraju da bi postepeni razvoj sistema, usklađen s evropskim standardima, mogao značajno unaprijediti ishode hitnog medicinskog zbrinjavanja.

Ključne riječi: helikopterska hitna medicinska služba (HEMS), medicinska evakuacija zračnim putem, trauma, organizacija zdravstvenog sistema, Bosna i Hercegovina

INTRODUCTION

Aeromedical evacuation and Helicopter Emergency Medical Service (HEMS) represent a fundamental component of modern emergency medical systems, particularly in the management of severe trauma and other time-critical medical conditions. Rapid access to advanced prehospital care and expedited transport to definitive treatment facilities are directly associated with improved survival and reduced complications among critically injured patients (1).

In this context, HEMS has progressively developed into a standard element of integrated Emergency Medical Services in the European region, especially in countries with complex geography, dispersed populations, or limited road accessibility. International health policy documents emphasize that organized aeromedical evacuation contributes to reduced prehospital time, improved trauma outcomes, and greater overall system efficiency (2).

The operation of HEMS within the European Union is supported by a harmonized regulatory framework that defines safety, operational, and organizational standards for air medical missions. The European Union Aviation Safety Agency has established comprehensive rules governing air operations, including Helicopter Emergency Medical Service flights, with the objective of ensuring a high and uniform level of safety across all member states and associated countries (3).

Despite these regulatory advances and widespread implementation of civilian HEMS systems in many European countries, notable disparities remain. While several states operate dedicated civilian aeromedical services fully integrated into national emergency medical systems, others rely on mixed civil–military arrangements or ad hoc solutions. Bosnia and Herzegovina exemplifies a system characterized by institutional fragmentation, absence of a unified civilian HEMS framework, and predominant reliance on military assets for aeromedical evacuation (4).

Geographical and infrastructural characteristics further accentuate the importance of aeromedical evacuation in Bosnia and Herzegovina. Mountainous terrain, limited road connectivity, and prolonged ground transport times significantly hinder timely access to emergency medical care. These challenges are compounded by a high burden of road traffic injuries and residual mine contamination, which together increase the risk of preventable mortality in emergency situations (5).

Situating the national context within broader European experience may help identify feasible approaches for strengthening HEMS capabilities and aligning practice with contemporary European preparedness and response standards, particularly those focused on health system resilience and coordinated crisis management (6).

MATERIALS AND METHODS

This manuscript was designed as a scoping review with a narrative analytical approach, synthesizing available scientific literature and official institutional documents related to aeromedical evacuation and Helicopter Emergency Medical Service (HEMS) systems. A scoping review approach was selected to allow structured mapping of key concepts, organizational models, regulatory frameworks, and implementation practices in aeromedical emergency care, particularly in health systems characterized by administrative and institutional complexity (7).

A systematic literature search was conducted using biomedical and health policy databases. Peer-reviewed scientific publications were primarily identified through PubMed/MEDLINE, while policy papers, technical reports, and strategic documents were retrieved from international and European institutional sources, including the World Health Organization and the European Commission. This approach enabled comprehensive coverage of both clinical and organizational aspects of aeromedical evacuation systems (8).

Regulatory and aviation-specific documentation relevant to HEMS operations was obtained from European Union Aviation Safety Agency guidance materials accompanying the Air Operations Regulation. These documents were used to analyze detailed requirements related to operational approval procedures, crew qualifications, training standards, and safety oversight mechanisms applicable to Helicopter Emergency Medical Service missions within the European regulatory framework (9).

The review included documents and publications issued between January 2010 and December 2025. Inclusion criteria encompassed peer-reviewed articles, narrative and policy reviews, official institutional reports, regulatory texts, and national legal documents addressing aeromedical evacuation, emergency medical systems, and HEMS organization. Exclusion criteria comprised conference abstracts without full texts, non-official opinion articles, and publications not directly related to emergency medical transport systems (10).

No primary data collection was performed, and no human or animal subjects were involved in this study. Consequently, approval by an ethics committee was not required. This manuscript represents an updated and substantially revised narrative analysis based on previously published work by the same author, expanded with contemporary data, updated regulatory sources, and restructured in accordance with current journal guidelines to reflect recent developments in aeromedical evacuation and HEMS organization (11).

RESULTS

The organization of aeromedical evacuation in Bosnia and Herzegovina remains largely fragmented and institutionally decentralized, reflecting the complex constitutional and administrative structure of the country. Emergency medical care is primarily organized at the entity and cantonal levels, without a unified state-level civilian Helicopter Emergency Medical Service framework. As a result, aeromedical evacuation is not systematically integrated into the national emergency medical system, leading to variability in access, activation procedures, and operational readiness across different regions of the country (12).

In the absence of a dedicated civilian Helicopter Emergency Medical Service (HEMS) system, aeromedical evacuation in Bosnia and Herzegovina is largely supported by the helicopter assets of the Armed Forces of Bosnia and Herzegovina through cooperation with civilian authorities. Available evidence indicates that such missions are conducted on a case-by-case basis, depending on operational availability and institutional requests, rather than as part of a permanently integrated civilian emergency medical service. Although military crews demonstrate significant operational experience and regularly participate in military aeromedical training activities, aeromedical evacuation is not organized as a continuous 24/7 civilian service, which limits its effectiveness for routine emergency medical response (13).

The operational framework for military-supported aeromedical evacuation in Bosnia and Herzegovina is primarily aligned with NATO standards and procedures for aeromedical evacuation. Standardization Agreements (STANAGs) provide guidance on medical training, in-flight care, and operational coordination, contributing to a baseline level of medical and aviation safety during missions. However, these standards are designed primarily for military contexts and do not fully address the requirements of a civilian-led HEMS system integrated into everyday emergency medical services (14).

In parallel with military involvement, international forces have periodically contributed to aeromedical evacuation capabilities within Bosnia and Herzegovina. During and after the implementation of Operation ALTHEA, EUFOR assets were occasionally utilized for medical evacuation and emergency response missions, particularly in remote or difficult-to-access areas. These contributions demonstrated the potential effectiveness of helicopter-based medical transport but were limited in scope and dependent on the mandate and availability of international forces rather than national health system planning (15).

At the entity level, the Helicopter Service of Republika Srpska represents one of the few civilian aviation structures with limited involvement in emergency response activities. While primarily established for governmental and civil protection purposes, this service has occasionally been engaged in medical transport and rescue missions. Nevertheless, its role in aeromedical evacuation remains supplementary, lacking formal integration into a structured HEMS model with defined medical staffing, activation protocols, and clinical governance (16).

Furthermore, in the absence of a dedicated civilian Helicopter Emergency Medical Service, aeromedical evacuation in Bosnia and Herzegovina is conducted primarily through military and international support mechanisms. In addition to the Armed Forces of Bosnia and Herzegovina, international military forces such as EUFOR play a critical role in selected emergency medical evacuations upon formal request by national authorities. These operations are typically mission-specific, dependent on operational availability, and activated through administrative decision-making rather than integrated civilian emergency dispatch systems. Although such interventions have demonstrated high operational effectiveness in challenging terrain and time-critical conditions, they do not constitute a continuous, nationally organized civilian HEMS framework (17).

Legal and regulatory analysis indicates that the organization of health care in the Federation of Bosnia and Herzegovina, including emergency medical services, is regulated primarily at the entity and cantonal levels. The Law on Health Protection of the Federation of Bosnia and Herzegovina establishes the obligation to ensure the availability of emergency medical care and patient transport but does not specifically define helicopter emergency medical services as a separate organizational or operational category. As a result, aeromedical evacuation is not systematically integrated into the health care system, leading to unclear institutional responsibilities, fragmented financing arrangements, and the absence of standardized protocols for helicopter-based emergency medical response (18).

The lack of a fully operational emergency call activation system further complicates aeromedical response. Although the establishment of a unified emergency number (112) has been formally envisaged in accordance with European standards, its implementation across the entire territory of Bosnia and Herzegovina remains incomplete. Consequently, activation of aeromedical evacuation often depends on informal coordination between emergency medical services, civil protection authorities, and military structures, rather than a streamlined, time-efficient dispatch system (19).

Comparative analysis with neighboring countries highlights the extent of these structural limitations. Croatia has recently established a civilian Helicopter Emergency Medical Service integrated into its national emergency medical system, with defined operational bases, standardized medical crew composition, and clearly structured activation procedures. The Croatian HEMS model demonstrates how centralized coordination and formal system integration can enhance the availability and organization of aeromedical emergency care, providing a relevant regional reference for potential development pathways in Bosnia and Herzegovina (20).

Overall, the results demonstrate that aeromedical evacuation in Bosnia and Herzegovina currently operates through a combination of military support, ad hoc civilian involvement, and international assistance, rather than through a coherent civilian HEMS system. While existing capabilities provide essential life-saving interventions in selected cases, the absence of unified organization, legal clarity, and continuous operational readiness significantly limits the effectiveness of aeromedical emergency care when compared with contemporary European standards (21).

DISCUSSION

The findings of this review indicate that aeromedical evacuation in Bosnia and Herzegovina is marked by structural fragmentation and the absence of an integrated civilian Helicopter Emergency Medical Service framework. Contemporary emergency care system analyses emphasize that effective prehospital and aeromedical services depend on coordinated governance, early triage, standardized dispatch, and seamless integration across the patient care pathway. In contrast, the current Bosnian approach relies predominantly on ad hoc arrangements, largely dependent on military assets and institutional goodwill rather than on system-level health planning. Such fragmentation limits predictability, response time optimization, and continuity of care in time-critical emergencies (22).

The dominant role of the Armed Forces of Bosnia and Herzego vina in aeromedical evacuation reflects both operational capability and systemic necessity rather than deliberate system design. While military involvement ensures a minimum level of aeromedical support in emergencies, this model is inherently constrained by administrative approval procedures, competing operational priorities, and the absence of permanent integration with civilian medical dispatch systems. Evidence from international experience indicates that civil–military cooperation can significantly enhance emergency response capacity during crises; however, reliance on military structures alone cannot substitute for a dedicated civilian Helicopter Emergency Medical Service designed around public health needs, continuous availability, and rapid activation mechanisms (23).

Regulatory analysis further demonstrates that the lack of explicit legal recognition of HEMS as a distinct component of emergency medical services contributes significantly to organizational inefficiency. Existing health protection laws mandate emergency transport but do not define helicopter medical operations, crew requirements, funding mechanisms, or accountability structures. This legal ambiguity contrasts with European regulatory frameworks, where HEMS is clearly regulated as a specialized medical and aviation activity, enabling safer and more efficient service delivery (24).

The incomplete implementation of a unified emergency activation and dispatch system represents an additional operational limitation. Efficient HEMS systems depend on centralized dispatch, rapid triage, and seamless coordination between ground and air medical services. Evidence from low- and middle-income settings indicates that fragmented prehospital emergency medical systems are associated with prolonged response times and reduced effectiveness of time-critical care. In Bosnia and Herzegovina, similar structural fragmentation and the absence of fully integrated nationwide dispatch mechanisms may limit the clinical benefits of aeromedical evacuation, particularly in trauma and critical care scenarios (25).

Comparative experience from neighboring Croatia illustrates the potential benefits of a structured civilian Helicopter Emergency Medical Service model. With the support of the European Union, Croatia established a nationally coordinated HEMS system characterized by clearly defined operational bases, standardized medical crew composition, and integration with the national emergency medical services. This organizational approach has improved accessibility to emergency medical care, particularly in remote, rural, and insular areas, while enhancing response efficiency and system transparency. The Croatian example demonstrates that a centralized civilian HEMS framework can be successfully implemented even in geographically and administratively complex settings, offering a relevant reference point for potential system development in Bosnia and Herzegovina (26).

From a clinical perspective, timely access to advanced medical care remains a critical determinant of morbidity and mortality in trauma and other life-threatening conditions. Evidence from well-organized trauma systems indicates that helicopter emergency medical services can confer a survival advantage for severely injured patients, even when associated with longer prehospital time intervals. These benefits are most pronounced when aeromedical transport is integrated within a coordinated emergency care framework that enables appropriate triage, advanced prehospital interventions, and rapid access to definitive hospital care. In Bosnia and Herzegovina, aeromedical evacuation is available only in selected circumstances and lacks systematic integration into civilian emergency medical services, limiting its potential clinical impact despite demonstrated benefits in comparable health systems (27).

Overall, the discussion indicates that the principal limitation in Bosnia and Herzegovina does not lie in the absence of aviation assets or medical expertise, but in insufficient systemic integration, fragmented governance, and the lack of a coherent strategic framework. European-level performance and monitoring mechanisms, such as those reflected in the Performance Review Body Monitoring Report 2021, emphasize the importance of coordination, standardization, and performance-based oversight in complex air service systems. Within this context, strengthening aeromedical evacuation in Bosnia and Herzegovina would not necessarily require the immediate creation of a new aviation infrastructure, but rather the gradual development of an integrated civilian HEMS framework aligned with broader European performance, safety, and coordination principles, and adapted to national institutional realities (28).

Notably, similar structural and organizational challenges in aeromedical evacuation were identified more than a decade ago in earlier national analyses, which highlighted the reliance on military assets, the absence of a civilian HEMS framework, and the lack of systemic integration within emergency medical services in Bosnia and Herzegovina. The persistence of these findings over time underscores the need for strategic continuity and long-term policy commitment in the development of aeromedical emergency care (29).

CONCLUSION

Aeromedical evacuation and Helicopter Emergency Medical Services represent a critical component of modern emergency medical care, particularly in geographically complex and administratively fragmented settings. The findings of this review indicate that Bosnia and Herzegovina currently lacks a unified civilian HEMS system, resulting in reliance on ad hoc and predominantly military-based aeromedical support. While such arrangements provide essential life-saving capabilities, they do not ensure consistent availability, rapid activation, or full integration with emergency medical services. The primary limitations identified are institutional fragmentation, insufficient legal recognition of HEMS within health system legislation, and incomplete emergency dispatch integration. These factors collectively reduce the potential clinical benefits of aeromedical evacuation, especially in time-critical trauma and medical emergencies. The gradual development of a coordinated civilian HEMS framework, aligned with European operational and safety standards and adapted to national administrative realities, represents a feasible and sustainable pathway forward. Strengthening civil–military cooperation, improving regulatory clarity, and ensuring centralized emergency activation would significantly enhance the effectiveness of aeromedical emergency care in Bosnia and Herzegovina.

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Immunopathological Connection Between Multiple Sclerosis and Epstein-Barr Virus Infection

Imunopatološka povezanost između multiple skleroze i infekcije epstein-barr virusom

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ABSTRACT

Multiple sclerosis (MS) is a progressive inflammatory neurological disease caused by up-regulation of proinflammatory cytokines produced by Th1 and Th17 cells resulting in oligodendrocyte phagocytosis and subsequent demyelination. The speculated etiology of the condition is autoinflammatory, with certain exogenous factors triggering a complex immunological cascade, prompting cross-reactivity with particular biomolecules and inducing the following condition. The aforedescribed connection between exogenous trigger and endogenous inflammatory sequence was shown to be most prominent in its connection with Epstein-Barr virus (EBV). The immunopathological link ought to be percipitated by homogenous amino-acid sequences shared between basic myelin protein (BMP) and EBV, so called "molecular mimicry", with strongest correlation being exhibited in EBV latency proteins, leading to the production of matrixmetalloproteinase (MMP) and blood-brain barrier (BBB) breakdown, followed by phagocyte influx and BMP disintegration. Additional molecular cross-reacitivity has been associated with different lythic proteins adjacent to HLADR-15 locus and its high avidity TCR engagement with RASGRP2, with CD+ activation and inflammatory cascade, and between CRYAB proteins and EBNA, leading to pathological up-regulation of autoinflammatory response and subsequently to MS.

Keywords: inflammation, cross-reactivity, virus

SAŽETAK

Multipla skleroza (MS) je progresivna inflamatorna neurološka bolest uzrokovana pojačanom regulacijom proinflamatornih citokina koje proizvode Th1 i Th17 ćelije, što rezultira fagocitozom oligodendrocita i naknadnom demijelinizacijom. Pretpostavlja se da je etiologija stanja autoinflamatorna, pri čemu određeni egzogeni faktori pokreću složenu imunološku kaskadu, podstičući unakrsnu reaktivnost s određenim biomolekulama i inducirajući sljedeće stanje. Pokazalo se da je prethodno opisana veza između egzogenog okidača i endogene upalne sekvence najizraženija u vezi s Epstein-Barr virusom (EBV). Imunopatološku vezu trebale bi izazvati homogene aminokiselinske sekvence koje dijele bazni mijelinski protein (BMP) i EBV, takozvana „molekularna mimikrija", s najjačom korelacijom koja se pokazuje u proteinima latencije EBV-a, što dovodi do proizvodnje matriks metaloproteinaze (MMP) i razgradnje krvno-moždane barijere (KMB), nakon čega slijedi priliv fagocita i dezintegracija BMP-a. Dodatna molekularna unakrsna reaktivnost povezana je s različitim litičkim proteinima uz lokus HLADR-15 i njegovom visokoavidnom TCR angažmanom s RASGRP2, s aktivacijom CD+ i upalnom kaskadom, te između CRYAB proteina i EBNA, što dovodi do patološke regulacije autoinflamatornog odgovora i potom do MS.

Ključne riječi: upala, unakrsna reaktivnost, virus

INTRODUCTION

Multiple sclerosis (MS) is defined as an acquired disabling neurological disease with inflammatory basis. The condition shows the biggest prevalence in North America (140 per 100000) and Europe (108 per 100000), with total world cases adding up to approximately 2.3 million worldwide (1,2,3). The disease primarily affects younger population ranging between the ages 20 to 50 years, with most presentations occuring during early adult life, with greater prevalence in females (4,5).

Since its discovery by Jean-Martin Charcot in 1868, MS has proven itself to be complex neurological disorder owing to its wide array of affected systems, including motor and sensory disruptions, visual impairment, corticobulbar and cranial lesions, as well as cerebral and cerebellar atrophy.

Behind the condition lies a pathophysiological process that affects an essential component of the central nervous system: the compacted myelin. This proteolipid membrane serves as a conductor and electrical insulator of nerve cells, which then enables fast and directed conduction of impluses through saltatory fashion, using voltage-gated Na+ channels found in the nodes of Ranvier. Voltage-induced signal travels from one node to the next avoding the isolated internodal segment of neurons. With demyelinating diseases such as MS the aforementioned signal transmission is interrupted with prolonged refractory period that inhibits repetitory signal volleys from being conducted through neuropathways.

Ethiopathophysiological basis of MS is complex and multilayered, with genetical and enviromental influences. Various risk factors, such as tobacco consumtion and obesity in early life are linked with increased MS incidence, with overweight individuals showing 200% risk increase (6,7). Particular viral infections show strong association with multiple sclerosis, primarily Epstein-Barr virus, where early life exposure increases relative risk (RR) to 3.0 MS incidence shows a certain degree of heredity (8). The human leukocyte antigen (HLA) region is commonly known as a hotspot for various disorders, including autoimmune ones, with gene HLA-DRB1*15:01-DQB1*06:02, located in the MHC region at (6p21.3) on the short arm of chromosome 6, showing strongest link with MS. According to recent studies, the aforementioned allele increases individuals susceptibilty by three-fold when compared with non-carriers (9).

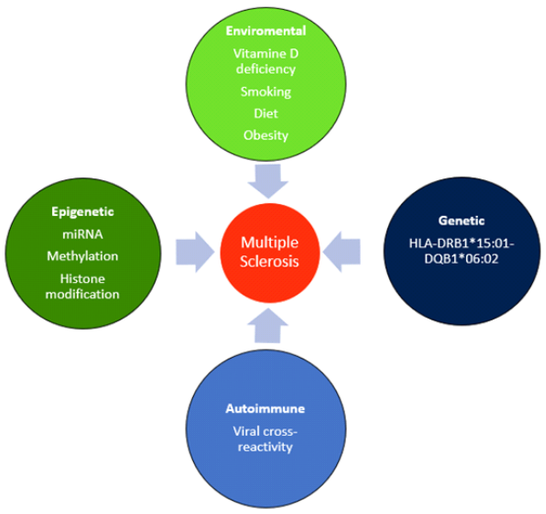

Figure 1 **Precipitating and risk factors for the development of Multiple Sclerosis.**

PATHOGENESIS OF MS

At its core, MS is an autoimmune disease that causes a casade of autoantigen-induced neurological damage, starting with inflammation, followed by demyelination, gliosis and consequently neuronal loss. Cross-reactivity between exogenous antigen of particular viruses e.g. Epstein-Barr virus and self-antigen of myelin basic protein is suggested to cause a certain degree of "molecular mimicry", prompting an anti-myelin basic protein (MBP) response. The target antigen is presented by Antigen-presenting cells (APCs), such as dendritic cells, macrophages and oligodendrocyte precursors, through major histocompatibilty complex (MHC) type II to naive T cells. The activation also requires cluster differentation interaction between CD28 on T-cells and CD7 on APCs. This action is followed by activation of T cells, specifically T-helper 1 cells (Th1) or CD4+ cells. Activated cells spread into lymphatic tissues to replicate in the proccess known as clonal expansion. Thereafter they get triggered by sphingosine-1-phosphate, lysophospholipid responsible amongst other functions for cell growth and proliferation, and enter circulation. The activated Th1 cells travel to the brain-blood barrier (BBB) and roll on its endothelial cells. After some time integrin $\alpha_4\beta_1$ (VLD4) binds with Vascular Cell Adhesion Molecule 1 (VCAM1) on endothelial cells of BBB. Post-activation Th1 cells produce matrixmetalloproteinase (MMP), which breaks down the BBB. This process forms openings and the activated Th1 cells enter brain parenchyme. Along with Th1 cells another subtype of CD4+ cells, Th17 cells, are involved in MS pathogenesis. Their classification derives from the cytokine they produce: IL17. Th1 and Th17 cells produce multiple cytokines: IL1, IL2, IL12, IL17, IL23, tumor necrosis alpha ($TNF\alpha$) and interferon gamma ($INF\eta$), resulting in more extensive BBB destruction and openings for additional Th1 cells and macrophages that migrate to the brain parenchyma due to chemotaxis. The core process of MS pathogenesis is the interaction between resident macrophages, astrocytes and microglia, with cytokines produced by Th1 and Th17 cells activating additional cellular mechanisms. $INF\eta$ activates B-cells, which convert to plasma cells and start the production of autoantibodies targeting myelin basic protein, it also activates macrophages, while IL-2 activates CD8 cells. The production of proinflammatory cytokines and subsequent activation of cellular immune response results in oligodendrocyte phagocytosis on the base of genetic sequence homology. Astrocytes form scar tissue that plaques (scleras), from which the name of the disease originates. It is important to note that 2 types of T cells are activated: proinflammatory Th1 cells (producing the aforemetioned infalmmatory cytokines) and antiinflammatory Th2 cells that produce IL10 (antinflammatory cytokine), attacking macrophages amd microglial cells, leading to partial remyelination followed by relapse

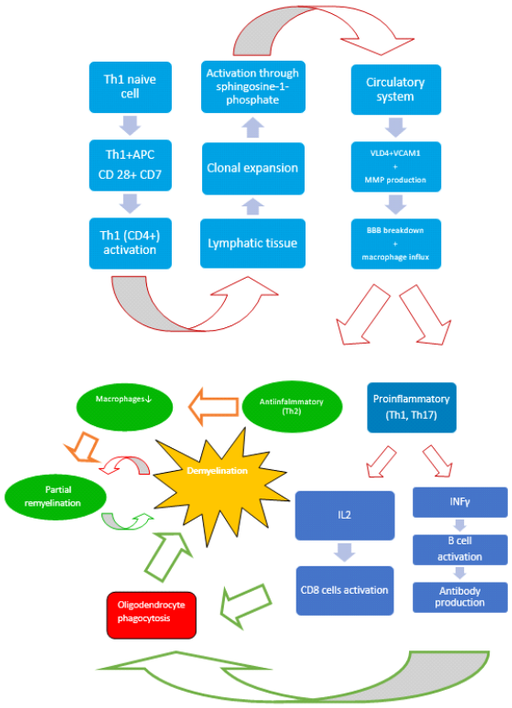

Figure 2 **Possible pathogenesis of Multiple Sclerosis.**

EBV AND MS

Direct causal connection between Epstein-Barr virus and MS has long been discussed, with newest findings supporting this link. Epstein-Barr virus (EBV) or formaly known as Human gammaherpes virus 4 (184 kb dsDNA), is a ubiquitous virus that causes lifelong infection in more than 95% of adults worldwide. It has been linked with various types of cancers, including but not limited to nasopharyngal cancer, Hodgkin and non-Hodgkin lymphoma. The most common disease caused by EBV is infective mononucleosis (10, 11,12,13). EBV represents one of the the eight human herpesviruses, large double-strained DNA genome (173-kb), with 100 protein-coding genes, non coding RNA and microRNA (miRNA) (14,15).The virus is packaged as a linear genome in the infectious viral particle. The aforementioned particle enters the targeted cell and infects it and aquires a different name during the latent infection: "episome". "Episome" is one of a group of extrachromosomal genetic elements called plasmids. They perform a synchronized replication with the genome of the infected cell and associacte with metaphase chromosomes during mytosis.

In the context of autoimmune diseases, such as multiple sclerosis, EBV performs a cascade of specific processes that directly affect cell's immune response and serves as one of most upfront scientific theories in regards to MS pathogenesis. Naive B-cells become the prime target for EBV and through its infection and subsequent genetic reprogramming that mimics the occuring processes in the germinal centre of lymph nodes, they are transformed into long lived memory B cells with EBV episomes.This process "immortalizes" the particular B-cell phenotype (16,17,18). EBV contains various viral proteins (gp 350/gp 220 and gp 42) that enable it the entrance into lymphocytes and genetic interaction required for genome alteration. The fusion of viral protein gp350 with CD21 (complement receptor 21) enables endocytosis of viral particle, facilited by glycoproteins B, H and L (gB, gH, gL) (19).

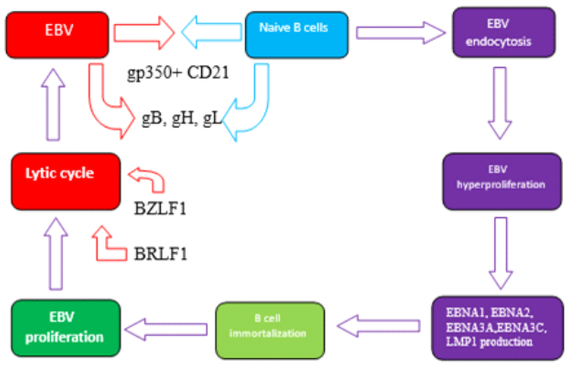

Figure 3 **EBV endocytosis and lytic cycle. The process is percipitated by latency genes expressed in type 3 latency and antigenes (EBNA1, EBNA2, EBNA3A, EBNA3C and LMP1) causing lytic cycle of continuous virus proliferation.**

Subsequently glycoprotein H binds with gp42 and interacts with MHC class II. During viruses hyperproliferative phase it adopts a type 3 latency during which various latency associated genes are expressed.These genes include EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, EBNA-LP, LMP1 and LMP-2 (20). The aforementioned "immortalization" is enabled by 5 latent antigenes: EBNA1, EBNA2, EBNA3A, EBNA3C and LMP1 (21). After EBV entered the B-cell it triggers a "lytic cycle", activated by various pathways, many of which related to immune cell signalling, such as B cell activation receptor (BCR). For its activation two encoded transcription factors are required, namely BZLF1 (ZEBRA) and BRLF1 (RTA and R). This expression changes latent phase to a lythic phase. Through lythic gene expression, transcription and translation specific lythic proteins are activated. These particular proteins are suggested to have a corellation with MS pathogenesis, but with controversial results in the actual reserach (22,23,24).

Most probable connection between Epstein-Barr virus and MS pathogenesis is exhibited by EBV latency proteins (EBNA 2, EBNA 3a, EBNA 3b, EBNA 3c and LMP2), which are recognized by CD8+ T cells responsible for olygodendrocyte phagocytosis, while latency protein EBNA1 is recognized by CD4+ T cells resulting with the production of matrix metalloproteinase (MMPs), consequently breaking down the blood-brain barrier (BBB) and enabling the onset of autoimmune processes (25,26). Strongest inflammatory potential is exhibited by latency gene EBNA1 through its cross-reactivity.The gene mimics self-antigenes and triggers the inflammation cascade through the activation of Th1 CD4+ cells, followed by MMBs production and BBB breakdown, as well as production of proinfammatory cytokines and phagocyte cell activation.The basis of "molecular mimicry" is the interaction between naive Th1 CD4+ T cells and viral peptide, leading to the production of antiEBNA411-426 antigenes and antiMBP response, through cross-reactivity with myelin proteins on the basis of amino-acid sequence (411-426).

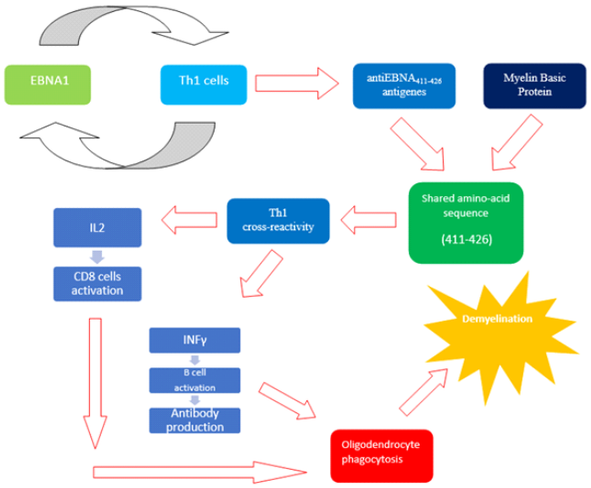

Figure 4 **EBNA1 and Th1 cross-reacitivity and the subsequent inflammatory cascade caused by shared amino-acid sequence between EBNA1 and MBP.**

Similiar autoreactivity was found between Anoctamin 2 and Crystalin B (CRYAB) and T cells, with most recent being Glial Cell Adhesion Molecule, as well as between BHRF1 and BPLF1 (27,28,29,30). These viral lytic proteins were found adjacent to HLADR15 halotype and crossreacted with RASGRP-2, which plays a specific role in MS pathogenesis. The key to this process lies in the difference between high- and low-avidity TCR engagement. HLADR-15 respresents an MS-associated locus responsible for the maintance of DR SP-specific T-cells. HLA-D proteins, composed of an alpha- and a betachain heterodimers, are MHC II class molecules, responsible for the presentation of processed peptide fragments of foreign antigenes or circumstantialy autoantigenes to CD4+ T cells. The alpha heterodimers, originating from the merger of alpha and beta antigenes (DR2a and DR2b) are confirmed to bind with their respective molecules on T-cells, creating a B-T cell connection for antigene presentation (31). Physiologically, HLA SPs should exhibit low-avidity, however in the case of crossreactive pathogenic small peptides originating from RASGRP2 (expressed both by B cells and neurons in the cortical gray matter), the replacement of DR2a and DR2b small peptides ensues high-avidity TCR engagement, followed by the activation of pathogenic-peptide CD4+ T-cells. After CD4+ cells migrate to CNS, antigene-presenting cells that express DR2a and DR2b may present the corresponding pathogenic peptide to CD4+T cells (32).The proceeding cascade of inflammatory events is triggered, concluding with myelin related damage associated with Multiple Sclerosis

Alpha-crystallin beta chain is part of the small heat shock protein (sHsps or HspBs) family coded by CRYAB gene. It functions as a molecular chaperone and operates as a protective agent through the downregulation of proinflammatory responses of innate immune cells and blocking the aggregation of denatured proteins (33). However, the physiological function of mentioned gene is under the influence of proinflammatory cytokines when targeted by adaptive immunity, prompting the reversal of its protective characteristics (33,34). The gene involved in the aforementioned cascade of proinflammatory processes shows strong crossreactive link between epitopes found in viral EBNA1 protein. Studies have shown increased anti-CRYAB IgG antibodies targeting specific aminoacid sequences, including CRYAB7-21, CRYAB2-16, with similar trends found in CRYAB1-15 and CRYAB8-22 peptides.

Link between the two most prominent amino acid sequences indicates a single MS-associated epitope (HPWIRREFP). Correlation between EBNA1 protein and CRYAB epitopes is shown through overlapping peptides of CRYAB8-20 and EBNA399-408, particulary sequence homology involving CRYAB 11-15 and EBNA1402-406. Amino-acid sequence "RRPFF" is responsible for crossreactive procces and inflammatory response against autoantigenes found in CRYAB peptides through shared sequence.

CONCLUSION

Multiple sclerosis still represents an enigmatic neurological condition with definitive pathological nuances yet to be discovered. Nevertheless Epstein-Barr virus has been proved to be a positive factor in the immunopathogenesis of MS through peptide sequence homology between antiEBNA antigenes and MBP, as well as between EBNA and CRYAB epitopes and the consequent cross-reactivity and proinflammatory cascade resulting in demyelination. Specifics of all the above-described processes lies in the fundamental genetic amin-acid sequence located on resident biomolecules and its identical counterparts found on EBV episomes, suggesting that the key of future MS patients infected with EBV virus lies in medicamentous manipulation of homologue sequence shared between MBP and EBV episomes. Further studies are warranted to fully grasp the extent of the viral influence on the progression and genetic as well as epidemiological basis of this neurodegenerative condition and future therapeutic procedures.

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How, When, and in What Way: An Early Rehabilitation Program After Total Shoulder Arthroplasty

Kako, kada i na koji način - rani rehabilitacijski program nakon totalne artroplastike ramena

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ABSTRACT

A 67- year- old female patient was admitted to the Clinic of Physical Medicine and Rehabilitation of the Clinical Center University of Sarajevo after total shoulder arthroplasty (TSA). TSA was done after a fracture of the proximal part of the right humerus. Early rehabilitation program started after the surgery and was continued at the Clinic of Physical Medicine and Rehabilitation. This paper discusses rehabilitation strategies for the successful management of patient after the total shoulder arthroplasty. Strategies included: pendular exercise, ROM exercises, occupational therapy, and electrotherapy.

Keywords: total shoulder arthroplasty (TSA), fracture, rehabilitation, ROM exercises

SAŽETAK

Pacijentica u dobi od 67 godina primljena je na Kliniku za fizikalnu medicinu i rehabilitaciju Kliničkog centra Univerziteta u Sarajevu nakon totalne artroplastike ramena. TSA je učinjena nakon prijeloma proksimalnog dijela desnog humerusa. Rani rehabilitacijski program započeo je nakon operacije i nastavljen je na Klinici za fizikalnu medicinu i rehabilitaciju. Ovaj rad razmatra rehabilitacijske strategije za uspješno liječenje pacijentice nakon totalne artroplastike ramena. Provedeni fizikalni tretman je uključivao pendularne vježne, vježbe obima pokreta, radnu terapiju te elektroterapiju.

Ključne riječi: totalna artroplastika ramena (TSA), prijelom, rehabilitacija, vježbe obima pokreta

INTRODUCTION

Proximal humerus fractures (PHFs) are the third most common upper extremity fractures in skeletally mature individuals (1) and typically result from low-energy falls in older adults (2). Most PHFs are minimally displaced and are thus managed conservatively. Shoulder replacement surgery is indicated in patients with afflictions such as primary osteoarthritis, posttraumatic arthritis, osteonecrosis and severe fractures of the proximal humerus. There are three types of surgeries for shoulder replacement: hemiarthroplasty, total shoulder replacement (TSA) and reverse shoulder arthroplasty (rTSA) (3). Total shoulder replacement is a type of surgery where both the humeral head and the glenoid are replaced by a metal and polyethylene prosthesis, a ball and socket replacement. The incidence of Total Shoulder Arthroplasty (TSA) is constantly growing, with a subsequently increased post-surgery rehabilitation demand (4). The specialist who develops the rehabilitation program must take into consideration the comorbidities of the patient and the type of surgery that was performed. For regular total shoulder arthroplasty (TSA), subscapularis integrity is necessary for a full rehabilitation (5). Restoration of shoulder strength has shown to be a determinant of functional outcomes, shoulder range of motion (ROM) and satisfaction following TSA (6).

CASE REPORT

A 67- years-old female patient was treated at the Clinic of Physical Medicine and Rehabilitation of the Clinical Center University of Sarajevo (CCUS) after total right shoulder arthroplasty and the proximal part of the right humerus fracture. The patient sustained injuries following a fall. Initial management was conservative; however, due to an inadequate response, surgical treatment was indicated. The patient subsequently underwent total shoulder arthroplasty of the right shoulder.

Postoperatively, the patient was enrolled in an early rehabilitation program. Further rehabilitation was continued at the Clinic of Physical Medicine and Rehabilitation of the CCUS. On admission, the patient reported limited mobility of the right shoulder with associated pain rated VAS 2–3/10. The Barthel Index score on admission was 16/20, indicating moderate dependence in activities of daily living. Circumferential measurements revealed the following: the right shoulder circumference increased by 2 cm compared to the left (measured from the acromion to the axillary fold). Right upper arm circumference increased by 3 cm compared to the left (measured 10 cm below the acromion and 10 cm above the olecranon). Range of Motion (ROM): the right shoulder (active ROM): Anteflexion: 20°, Retroflexion: 25°, Abduction: 35°, External and internal rotation: limited to the first third of the normal range. Right elbow: flexion: 125°, Extension: deficit of 10°, Pronation and supination: approximately 50% of normal range. The patient was unable to form a full fist or cylindrical grip. Mild edema of the right hand was present. Gross muscle strength was reduced. Manual Muscle Testing (MMT) revealed muscle strength graded 2- to 3-/5. Deep tendon reflexes were symmetrical. Sensory examination was normal. Following assessment, the patient was included in an individual medical rehabilitation program consisting of: cryotherapy as preparation for exercise therapy, kinesiotherapy focused on improving right shoulder range of motion, electrotherapy (Interferential Therapy - IFS), occupational therapy. During hospitalization, the progress was monitored using MMT, the Barthel Index, right shoulder ROM measurements, and circumferential measurements of the shoulder, upper arm, forearm, and hand. During rehabilitation, the patient achieved an improvement in right shoulder ROM of approximately 5-10 degrees in anteflexion, retroflexion, and abduction.

Full range of motion was restored in the right elbow joint, with improved functional use of the right hand. At discharge, the Barthel Index score improved to 18/20, corresponding to continued moderate dependence in activities of daily living.

Following discharge, the patient continued outpatient physical therapy, resulting in further functional improvement. Final active ROM of the right shoulder was: Anteflexion: 65° Retroflexion: 40° Abduction: 60° External and internal rotation: approximately 50% of normal range, the patient was able to fully form a fist and cylindrical grip.

Table1 **Pre Rehab Manual Muscle testing.**

Muscle Test	Right	Left
m. trapezius	2+	3+
m. deltoideus	2+	3
m. pectoralis maior	2-	3
mm. flexores antebrachii	2	3
m. triceps brachii	2	3
m. supinator	3-	3+
mm. pronator teres et quadrtus	3-	3-
mm. flexores carpi	3-	3
mm. extensores carpi	2+	3+
mm. oponens pillicis et digiti V	2+	3

Table 2 **Post Rehab Manual Muscle testing.**

Muscle Test	Right	Left
m. trapezius	3	4-
m. deltoideus	3	4-
m. pectoralis maior	3	4-
mm. flexores antebrachii	3	4-
m. triceps brachii	3	4
m. supinator	3	4
mm. pronator teres et quadrtus	3+	4
mm. flexores carpi	3+	4
mm. extensores carpi	3-	4
mm. oponens pillicis et digiti V	3-	4

DISCUSSION

Total shoulder arthroplasty (TSA) is increasingly used in elderly patients with complex proximal humerus fractures when conservative treatment fails to provide satisfactory functional outcomes (12). Although surgical technique is crucial, postoperative rehabilitation plays a key role in restoring shoulder function and patient independence (13). This case report demonstrated the clinical relevance of an early and individualized rehabilitation program following TSA.

In the presented case, early rehabilitation was initiated with careful consideration of soft tissue healing and prosthetic stability. The rehabilitation protocol focused on pain and edema reduction, gradual restoration of shoulder range of motion, improvement of muscle strength, and functional reintegration of the upper extremity (14). Such an approach is consistent with current recommendations suggesting that early, controlled mobilization after TSA may improve functional outcomes without increasing postoperative complications (15).

Functional improvement in this patient was confirmed by objective clinical measures. Manual Muscle Testing showed progressive improvement in muscle strength of the shoulder and upper extremity, while range of motion measurements demonstrated gradual gains in anteflexion, retroflexion, and abduction (16). Additionally, the improvement in the Barthel Index reflects enhanced independence in activities of daily living, which represents a key goal of rehabilitation in elderly patients (17).

Pain control and edema management were essential in enabling active participation in therapy. The use of cryotherapy and interferential electrotherapy contributed to pain reduction and improved tolerance of exercise therapy. Occupational therapy played an important role in restoring hand function and grip strength, which significantly influenced the patient's ability to perform daily tasks and improved overall functional outcomes (18).

The main limitation of this report was a single-case design, which limited applicability. Furthermore, long-term follow-up was not available to assess sustained functional outcomes. Nevertheless, this case supports the concept that early, structured, and multidisciplinary rehabilitation after TSA can be safe and effective.

This case report suggests that early rehabilitation following total shoulder arthroplasty, when carefully planned and individualized, contributes to improved shoulder mobility, muscle strength, and functional independence. These findings support the inclusion of early rehabilitation protocols in the postoperative management of patients undergoing TSA.

CONCLUSION

For patients with TSA postoperative rehabilitation is crucial. We believe that rehabilitation reduces complications and maximizes patient improvement in performing basic activities. Postoperative rehabilitation program should be established with strong collaboration between the physiatrist, physical therapist and referring surgeon.

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Cesarean Section in a Patient with Complex Uterine Anomaly Following Long-Term Infertility

Carski rez kod kompleksne anomalije uterusa nakon dugogodišnjeg infertiliteta

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ABSTRACT

Bicornuate unicollis uterus is a rare congenital anomaly characterized by two endometrial cavities and a single cervix. It occurs in approximately 0.1-0.2% of women evaluated during pregnancy or infertility. Although live birth rates are lower compared to women with normal uterine anatomy, successful pregnancies are possible, particularly with assisted reproductive techniques. Case report: the report related to a 37-year-old woman with bicornuate unicollis uterus and a long history of infertility. Her reproductive history included eight IVF attempts, three spontaneous miscarriages, and two ectopic pregnancies treated with bilateral salpingectomy. The current pregnancy was achieved through IVF and embryo transfer. A cervical cerclage was placed at 19 weeks, and pregnancy-induced hypertension was controlled with methyldopa. At 36+6 weeks, the patient was admitted for elective cesarean section due to breech presentation. On admission, she was normotensive and in good general condition. Ultrasound revealed breech presentation, posterior placentation, normal amniotic fluid, estimated fetal weight of 2320 g, and uterine artery resistance index of 0.52. Following standard preparation, a transverse lower uterine segment incision was performed. On 7 January 2026, a live male neonate weighing 2650 g, length 48 cm, Apgar score 9/10, was delivered from the left uterine horn. The right horn was rudimentary but practicable. Hemostasis was achieved, the uterus closed in a single layer, and the abdominal wall closed in standard fashion. The patient tolerated the procedure well, with uneventful postoperative recovery. Conclusion: this case illustrates the potential for favorable outcomes in women with bicornuate unicollis uterus despite significant reproductive challenges. The patient's eventual live birth after multiple IVF failures, miscarriages, and ectopic pregnancies underscores the importance of persistence, individualized management, and planned delivery strategies in complex uterine anomalies.

Keywords: bicornuate uterus, congenital uterine anomaly, in vitro fertilization, delivery, Cesarean section

SAŽETAK

Uterus bikornis unikolis je rijetka kongenitalna anomalija koju karakteriziraju dvije endometrijske šupljine i jedan cerviks. Javlja se kod otprilike 0,1-0,2% žena koje se procjenjuju tokom trudnoće ili infertiliteta. Iako su stope živorođenosti niže u poređenju sa ženama sa normalnom anatomijom uterusa, uspješne trudnoće su moguće, naročito uz pomoć asistiranih reproduktivnih tehnika. Prikaz slučaja: prikazujemo slučaj 37-godišnje pacijentice sa anomalijom po tipu uterus biokrnis unikolis i dugom historijom infertiliteta. Njena reproduktivna anamneza uključivala je osam pokušaja IVF-a, tri spontana pobačaja i dvije ektopične trudnoće liječene bilateralnom salpingektomijom. Trenutna trudnoća je ostvarena putem IVF-a i embriotransfera. Cervikalni serklaž je postavljen u 19. sedmici, a hipertenzija inducirana trudnoćom kontrolisana je metildopom. U 36+6 sedmica pacijentica je primljena radi elektivnog carskog reza zbog karlične prezentacije. Pri prijemu je bila normotenzivna i u dobrom općem stanju. Ultrazvučni nalaz pokazao je karličnu prezentaciju, posteriornu lokalizaciju placente, normalnu količinu amnionske tečnosti, procijenjenu težinu fetusa od 2320 g i indeks otpora uterine arterije od 0,52. Nakon standardne pripreme, izveden je transverzalni rez donjeg segmenta uterusa. Dana 7. januara 2026. godine iz lijevog roga uterusa je porođeno živo muško novorođenče težine 2650 g, dužine 48 cm, Apgar skor 9/10. Desni rog je bio rudimentaran, ali prohodan. Hemostaza je postignuta, uterus zatvoren u jednom sloju, a trbušni zid zatvoren standardnom tehnikom. Pacijentica je dobro podnijela zahvat, sa urednim postoperativnim oporavkom. Zaključak: ovaj slučaj naglašava mogućnost povoljnog ishoda kod žena sa uterus bikornis unikolis uprkos značajnim reproduktivnim izazovima. Uspješno iznesena trudnoća nakon višestrukih neuspjelih IVF pokušaja, pobačaja i ektopičnih trudnoća ističe važnost upornosti, individualiziranog pristupa i planiranih strategija porođaja kod kompleksnih anomalija uterusa.

Ključne riječi: bicornuatni uterus, kongenitalna anomalija uterusa, in vitro fertilizacija, porođaj, carski rez

INTRODUCTION

Bicornuate unicollis uterus is a congenital uterine anomaly characterized by two endometrial cavities and a single cervix. Congenital uterine malformations occur in approximately 0.5% of the general population, with bicornuate uteri accounting for nearly 40% of cases . The estimated prevalence of bicornuate unicollis uterus in population-based studies is around 0.1–0.2% among women evaluated during pregnancy or for infertility . Although live birth rates in women with bicornuate uterus are lower compared to those with normal uterine anatomy, successful pregnancies are possible, with reported cumulative live birth rates approaching 78% after repeated attempts .

We presented a case of successful live birth in a woman with bicornuate unicollis uterus following eight attempts of in vitro fertilization (IVF), repeated spontaneous miscarriages, and two ectopic pregnancies.

CASE REPORT

A 37-year-old woman was admitted to the Department of Obstetrics at 36+6 weeks of gestation for planned termination of pregnancy. A cervical cerclage had been performed at 19 weeks of gestation. During the course of pregnancy, she developed pregnancy-induced hypertension, which was well controlled with methyldopa at a dose of 500 mg three times daily (TID).

The pregnancy was achieved following IVF and embryo transfer, in the context of a long history of infertility. Her past medical history included eight IVF attempts, three spontaneous miscarriages, two dilatation and curettage procedures, and bilateral salpingectomy after ectopic pregnancies in 2016 and 2018. She was known to have a congenital uterine anomaly of the bicornuate unicollis type.

No other significant findings were noted in her personal medical history. Family history was unremarkable for gynecological or obstetric disorders; her father was known to have hypertension.

On 6 January 2026, the patient was admitted for elective Cesarean section. On admission, the patient was in good general condition, with unremarkable systemic examination and normotensive. Her height was 165 cm; pre-pregnancy weight 66 kg, current weight 80 kg; abdominal circumference measured 117 cm. Ultrasound examination demonstrated breech presentation with fetal legs presenting, normal amniotic fluid volume, posterior placentation, estimated fetal weight of approximately 2320 g, and a uterine artery resistance index of 0.52.

Following standard preparation and induction of anesthesia, the abdominal cavity was opened in layers. The vesicouterine fold was incised and the bladder mobilized inferiorly. A transverse incision was made at the lower uterine segment and digitally extended. On 7 January 2026 at 09:09 hours, the fetal legs were delivered, followed by a live male neonate weighing 2650 g, length 48 cm, with an Apgar score of 9/10. The newborn was handed over to the pediatric team. The pregnancy was located in the left uterine horn, while the right horn was rudimentary but practicable. The placenta was extracted, and the uterine cavity inspected, confirming it was empty (Figure 1 and 2). Hemostasis was achieved with corner clamps and individual hemostatic sutures. The uterus was closed in a single layer with a continuous suture, reinforced with several interrupted stitches. The abdominal wall was closed in layers in the usual manner, with a continuous fascial suture and intradermal skin closure. Urine in the catheter was clear, and all instruments, sponges, and swabs were accounted for. The patient tolerated the procedure well and was transferred in stable condition to postoperative care with standard therapy.

Figure 1 **Uterine inspection.**

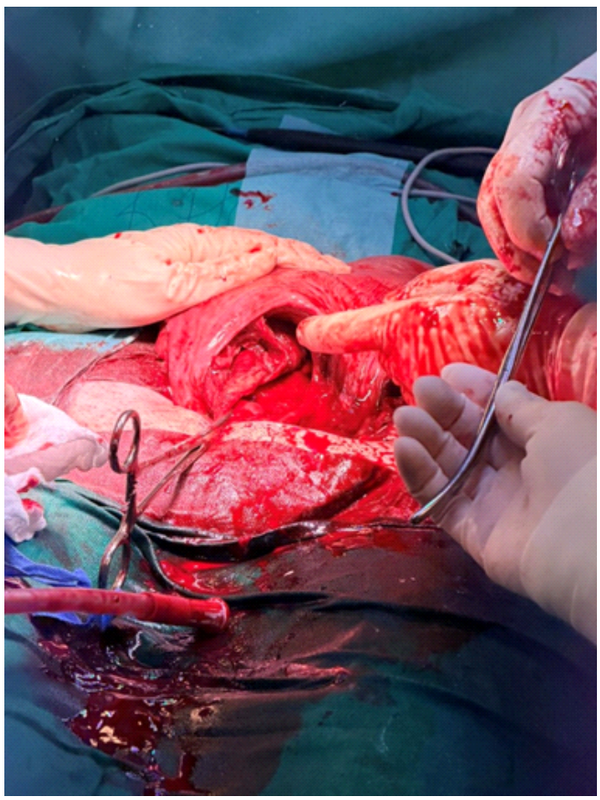

Figure 2 **Uterine inspection with visible uterine septum.**

DISCUSSION

The embryogenesis of a bicornuate unicollis uterus involves incomplete fusion of the upper portion of the Müllerian ducts, resulting in two distinct endometrial cavities with a single cervix. Diagnosis is typically established through imaging modalities such as ultrasound, hysterosalpingography, or magnetic resonance imaging, with an intercornual angle greater than 105° serving as a key criterion . In most cases, treatment is not required unless complications arise, such as recurrent miscarriage or abnormal uterine bleeding.

Over time, several systems have been developed to classify müllerian anomalies. Early frameworks, such as those by Buttram and Gibbons and later the American Fertility Society (AFS), grouped anomalies according to developmental failures and their clinical consequences . More recent approaches, including the VCUAM system, the embryology-based classification by Acién, and the ESHRE/ESGE model, aimed to provide more precise descriptions of uterine, cervical, and vaginal malformations .The latest update, the ASRM Müllerian Anomalies Classification 2021 (MAC2021), distinguishes nine categories and clearly identifies bicornuate uterus as a separate entity .

In the present case, the anomaly was associated with a long history of infertility, multiple miscarriages, and ectopic pregnancies, yet ultimately culminated in a successful live birth following assisted reproductive techniques. Interestingly, retrospective cohort study has demonstrated that there are no significant differences in ovarian responses, pregnancy outcomes, or obstetric outcomes after IVF/ICSI treatment between women with bicornuate uterus and those with normal uterine anatomy . These findings suggest that bicornuate uterus does not exert a significant negative effect on cumulative IVF/ICSI outcomes in infertile patients. Nevertheless, our patient achieved live birth only after the eighth IVF attempt, underscoring the variability of individual reproductive trajectories and the importance of persistence and tailored management in complex cases.

Another retrospective cohort study with a larger sample provided further insight into reproductive outcomes in women with congenital uterine anomalies . Compared to matched controls, women with a bicorporeal uterus demonstrated significantly lower live birth rates (24.4% vs. 34.8%), along with non-significant trends toward higher miscarriage and preterm delivery rates. Moreover, the bicorporeal group had lower gestational age at delivery, higher cesarean section rates, and reduced birthweight. These findings supported the decision to perform an elective cesarean section in our patient.

CONSLUSION

The patient's history of eight IVF attempts, multiple miscarriages, and ectopic pregnancies underscores the significant reproductive challenges typically encountered in this setting of uterine congenital malformations. The achievement of a viable pregnancy and delivery by Cesarean section highlights both the potential for favorable outcomes despite complex uterine anatomy and the importance of individualized management strategies. Reporting such cases contributes to a better understanding of reproductive outcomes in women with complex uterine malformations and may guide clinical decision-making.

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Tracheal Atresia: A Rare but Fatal Congenital Anomaly

Atrezija traheje: rijetka, ali smrtonosna kongenitalna anomalija

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ABSTRACT

Tracheal atresia is an extremely rare and fatal congenital malformation of the airways characterized by the complete or partial absence of the trachea. We report the case of a male infant born at 34+6 weeks of gestation to a 23-year-old mother with polyhydramnios detected during prenatal ultrasound. Immediately after birth, the infant presented with cyanosis, lack of audible crying, and severe respiratory distress. Attempts at endotracheal intubation were unsuccessful due to the lack of visible tracheal lumen. A contrast-enhanced chest radiograph showed absence of bronchial aeration and airway termination in the proximal third of the trachea, confirming the diagnosis of tracheal atresia. Despite resuscitation measures, the infant died within an hour of birth. An autopsy was not performed. Tracheal atresia is classified into three types according to Floyd's system, with type II - the complete absence of the trachea and bronchus communicating with the esophagus - being the most common. The etiology is thought to involve abnormal separation of the tracheoesophageal septum during early pregnancy. Although surgical and experimental approaches have been attempted, survival beyond the neonatal period remains extremely rare. Early prenatal suspicion via ultrasound or fetal magnetic resonance imaging is essential for counseling, birth planning, and multidisciplinary perinatal management. Continued advances in fetal imaging and regenerative airway reconstruction may offer hope for better outcomes in the future.

Keywords: tracheal atresia, congenital airway obstruction, CHAOS syndrome, neonatal respiratory failure, prenatal diagnosis

SAŽETAK

Atrezija traheje iznimno je rijetka i fatalna kongenitalna malformacija dišnih puteva koju karakteriše potpuna ili djelimična odsutnost traheje. Izvještavamo o slučaju muškog novorođenčeta rođenog u 34+6 nedelja gestacije 23-godišnje majke s polihidramnionom otkrivenim tokom prenatalnog ultrazvuka. Odmah nakon rođenja, novorođenče se javilo s cijanozom, nedostatkom čujnog plača i teškim respiratornim distresom. Pokušaji endotrahealne intubacije bili su neuspješni zbog nedostatka vidljivog lumena traheje. Radiografija prsnog koša s kontrastom pokazala je odsutnost bronhijalne aeracije i završetak dišnih putova u proksimalnoj trećini traheje, potvrđujući dijagnozu trahealne atrezije. Unatoč mjerama oživljavanja, novorođenče je umrlo unutar jednog sata od rođenja. Budući da je dijagnoza atrezije postavljena na osnovu RTG snimka učinjenog u prvom satu života, daljnje dijagnostičke procedure nisu provođene, kao ni obdukcija. Trahealna atrezija klasificira se u tri tipa prema Floydovom sistemu, pri čemu je tip II - potpuni odsutnost traheje i bronha koji komuniciraju s jednjakom - najčešći. Smatra se da etiologija uključuje abnormalno odvajanje traheoezofagealnog septuma tijekom rane trudnoće. Iako su pokušani hirurški i eksperimentalni pristupi, preživljavanje nakon neonatalnog razdoblja ostaje izuzetno rijetko. Rana prenatalna sumnja putem ultrazvuka ili fetalne magnetne rezonancije ključna je za savjetovanje, planiranje poroda i multidisciplinarno perinatalno liječenje. Kontinuirani napredak u fetalnom snimanju i regenerativnoj rekonstrukciji dišnih puteva može ponuditi nadu za bolje ishode u budućnosti.

Ključne riječi: trahealna atrezija, kongenitalna opstrukcija dišnih putova, CHAOS sindrom, neonatalno respiratorno zatajenje, prenatalna dijagnoza

INTRODUCTION

Tracheal atresia is a rare and fatal congenital anomaly in which the trachea is completely interrupted or absent. Since it was first described in 1900, very few cases have been reported worldwide. The prevalence of tracheal atresia is less than 1:50,000, with a male-to-female ratio of 2:1. Overall, 52% of cases are associated with prematurity and approximately half of cases are associated with polyhydramnios (1). Congenital high obstructive airway syndrome or sequence (CHAOS) refers to a rare, often fatal, congenital condition of the larynx and trachea, and is primarily characterized by obstruction of the fetal upper airway. The pathology of CHAOS can be one of three possible types: complete laryngeal atresia without esophageal fistula, complete laryngeal atresia with tracheo-esophageal fistula, and near-total upper airway obstruction (2). Radiographic features: ultrasound may show some or all of the following: dilated trachea/bronchi: distal to obstruction increased echogenic lung parenchyma diaphragmatic inversion and/or flattening fetal ascites; fetal heart may appear medially displaced and compressed. Additional ultrasound features include fetal anasarcapolyhydramnios. Fetal MRI can confirm features detected by ultrasound and more accurately demonstrate the extent of obstruction. The lung signal is increased (3,4). The Floyd classification is sometimes used, which is as follows: type I: agenesis of the proximal trachea with a short segment of normal distal trachea, carina, and bronchi; fistula between the distal trachea and esophagus is present type II: agenesis of the entire trachea; may be accompanied by communication between the esophagus and carina, from which bronchi arise type III: atresia of the entire trachea and carina; bronchi arise individually from the esophagus (5). A newborn with tracheal atresia presents with polyhydramnios, respiratory distress, aphonia, cyanosis, and the impossibility of endotracheal intubation. At present, the etiology of tracheal atresia is unknown (6). In general, prenatal diagnoses can only be made by ultrasound, and the outcome is poor (7,8). This case report describes an unusual situation in which tracheal atresia was diagnosed independently of any other congenital anomalies.

CASE REPORT

A male newborn of a 23-year-old mother born in 34+6NG. Uncontrolled pregnancy, spontaneous delivery. Polydroamnion. Admitted to the Neonatal Intensive Care Unit immediately after birth. After emergency delivery, he does not make a sound cry, cyanotic, tries to breathe, bradycardic, frequency 80/min, Apgar score 1/1 (Apgar score is a rapid, standardized assessment performed on newborns at 1 and 5 minutes after birth to evaluate their immediate health and transition to life outside the womb, it scores five criteria—Appearance (skin color), Pulse, Grimace (reflex irritability), Activity (muscle tone), and Respiration—on a scale of 0 to 2, for a maximum total of 10). Intubation was approached, he was intubated, the entrance to the larynx was clearly visible, but there was no ventilation. From the initial part of the trachea, the tube had an elastic resistance for deeper placement. Tracheal atresia was suspected, and the esophagus was intubated in an attempt to ventilate if tracheoesophageal communication was present. It was ventilated with an ambu balloon, which resulted in minimal movements of the chest. Still bradycardic. An X-ray of the chest with contrast was performed, and it was identified on the front third of the trachea of the lung without aeration, the bronchi were not identified (image number 1 and image number 2). Ventilation was not established and an lethal outcome occured. Dg: Neonatus praetemporarius 34+6NG, Atraesio trachea, Polyhydramnios.

Based on this radiological examination, tracheal atresia was diagnosed. The newborn died within 1 hour of birth. An autopsy was not performed.

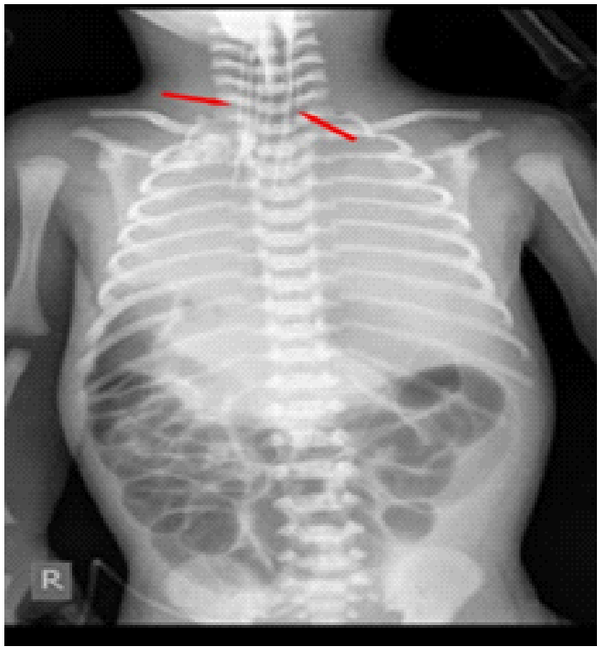

Figure 1 **Chest X-ray after intubation.**

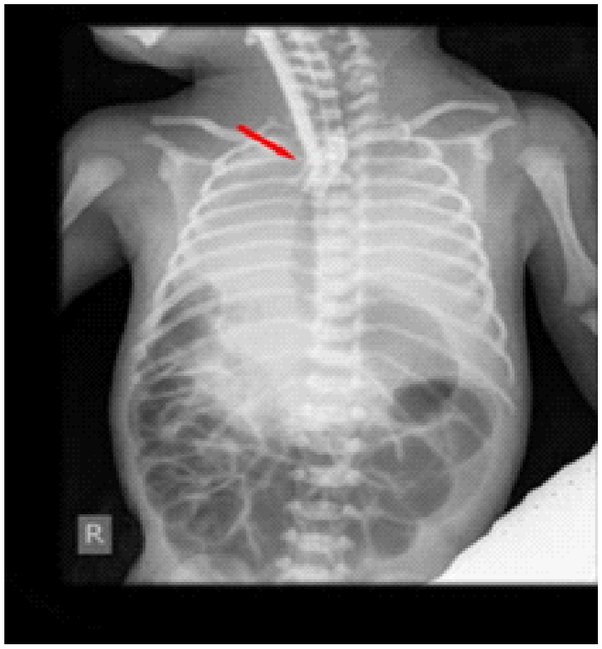

Figure 2 **X-ray of the chest after the application of the second tube and contrast agent.**

DISCUSSION

Floyd J, et al. defined type II tracheal atresia as the complete absence of the trachea with the presence of normal bifurcated main bronchi. In 94% of cases, other congenital defects are also present, including congenital cardiac, genitourinary, gastrointestinal, pulmonary, CNS, and musculoskeletal anomalies (8,9). Although the embryogenic mechanisms underlying type II tracheal agenesis remain controversial, it has been suggested that tracheal agenesis results from the cessation of lung bud growth and delayed formation of the bronchi and lungs by residual primordial mesenchyme that is often associated with the esophagus (9). Also, although no significant genetic karyotype has been found that correlates with tracheal atresia, homozygous Shhnull mutant mice show foregut defects similar to those seen in tracheal atresia (10). Tracheal atresia should be included in the differential diagnosis when the following clinical signs are manifested: neonate with polyhydramnios, lack of audible crying at birth, inability to intubate beyond the vocal cords, and respiratory distress (11). In patients with tracheal atresia, surgical correction is suggested as a corrective measure. To date, only one patient with atresia has survived beyond the neonatal period. However, that surviving patient had a proximal tracheoesophageal fistula in addition to a bronchoesophageal fistula, which allowed successful creation of a permanent airway by performing a tracheotomy and inserting a long T-tube to create a patent airway (12). Although temporary management may include insertion of an esophageal tube, depending on the extent of the fistula, a newborn with a complete absence of the trachea usually dies within a few hours of birth, as a permanent airway cannot be created. Mask ventilation can also be used to temporarily prolong life. Currently, this anomaly is incompatible with life, and future hopes for survival will depend on surgical advances.

CONCLUSION

Tracheal atresia is an extremely rare and almost always fatal congenital airway anomaly. It should be suspected in newborns with polyhydramnios, absent crying, severe respiratory distress, and failed endotracheal intubation. Prenatal detection using ultrasound and fetal MRI is crucial for parental counseling and perinatal planning. Despite advances in neonatal and surgical care, tracheal atresia remains largely incompatible with life.

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Preserved Fertility and Two Full-Term Pregnancies after Allogeneic Stem Cell Transplantation

Očuvana plodnost i dvije uredne trudnoće nakon alogene transplantacije matičnih ćelija

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ABSTRACT

Aplastic anemia (AA) is a severe but rare hematologic disorder associated with bone marrow failure, leading to decreased or totally absent hematopoietic precursor cells in the bone marrow. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the first-line treatment for AA and offers a curative approach in eligible patients, but fertility potential is commonly diminished by allo-HSCT conditioning regimens. Case report: we described a rare case of preserved fertility and successful natural conception resulting in two healthy, full-term pregnancies following allo-HSCT. Both pregnancies were uncomplicated and resulted in cesarean deliveries. The intraoperative and postoperative courses were uneventful, proceeding smoothly without complications. Conclusion: pregnancy after allo-HSCT is uncommon but possible, especially in younger patients treated for non-malignant disorders such as AA and with conditioning regimens that exclude total body irradiation. Although pregnancy after allo-HSCT is uncommon, this case demonstrates that it is indeed possible.

Keywords: aplastic anemia, allogeneic hematopoietic stem cell transplantation, healthy pregnancies, case report

SAŽETAK

Aplastična anemija je rijedak, ali ozbiljan hematološki poremećaj koji se karakteriše zatajivanjem koštane srži, što dovodi do smanjenja ili potpunog odsustva hematopoetskih matičnih ćelija u koštanoj srži. Alogena transplantacija hematopoetskih matičnih ćelija je terapija prvog izbora i nudi potencijalno izlječenje kod odabranih pacijentica. Međutim, zbog pripremnih terapija koje se koriste prije transplantacije, plodnost je često smanjena ili u potpunosti izgubljena. Prikaz slučaja: prikazujemo rijedak slučaj očuvane plodnosti i uspješne spontane trudnoće, koja je rezultirala s dvije zdrave, terminske trudnoće nakon alogene transplantacije matičnih ćelija. Obje trudnoće su protekle bez komplikacija i završene su carskim rezom. Intraoperativni i postoperativni tok bili su uredni i bez komplikacija. Zaključak: trudnoća nakon alogene transplantacije hematopoetskih matičnih ćelija je rijetka, ali moguća, naročito kod mlađih pacijentica koje su liječene zbog nemalignih oboljenja kao što je aplastična anemija, te koje su primile hemoterapijske protokole bez totalnog zračenja. Ovaj slučaj pokazuje da trudnoća nakon transplantacije, iako neuobičajena, ipak može biti ostvariva.

Ključne riječi: aplastična anemija, alogena hematopoetska transplantacija matičnih ćelija, zdrava trudnoća, prikaz slučaja

INTRODUCTION

Aplastic anemia (AA) is a severe but rare hematologic disorder associated with bone marrow failure leading to decreased or totally absent hematopoietic precursor cells in the bone marrow (1). The condition commonly occurs in individuals aged 10-25 and in those older than 60 (2). It is diagnosed through complete blood count, bone marrow biopsy, and clinical evaluation of symptoms such as fatigue, infections and bleeding tendencies, dyspnea, skin color paler than usual, dizziness, headache and fever (3,4).

Allo-HSCT is the first-line treatment for AA and offers a curative approach in eligible patients (5). Fertility potential is commonly diminished by allo-HSCT conditioning regimens, largely as a result of prior intensive chemotherapy and radiotherapy (6), which frequently leads to gonadal dysfunction and irreversible infertility (7).

Advancing age at the time of allo-HSCT is significantly associated with an increased risk of developing ovarian insufficiency (5). Factors associated with infertility following transplant included being female, undergoing allo-HSCT at an age greater than 30, and receiving total body irradiation as part of the conditioning regimen (8). Pregnancy after allo-HSCT is considered high risk and is associated with increased rates of miscarriage, preterm delivery, and low birth weight (9). Available data on pregnancy outcomes following allo-HSCT remain limited (7).

In this report, we described a rare case of preserved fertility and successful natural conception resulting in two healthy, full-term pregnancies following allo-HSCT.

CASE REPORT

The patient was diagnosed with AA in 2016. In the same year, she underwent an allo-HSCT in Turkey, with her HLA-identical sister serving as a 100% matched donor. As part of the conditioning regimen, she received four doses of chemotherapy in combination with cyclosporine. Total body irradiation was not included in the preparative protocol. Throughout the treatment period, her menstrual cycles remained regular and uninterrupted.

Approximately three years following the allo-HSCT, the treating medical team assessed the patient's condition and determined that it was medically safe for her to attempt conception. This decision was based on her stable clinical status, absence of transplant-related complications, and preserved ovarian function.

Five years following allo-HSCT, the patient conceived spontaneously for the first time without prior preconception counseling or preparation. The pregnancy progressed uneventfully, with the patient receiving routine vitamin and iron supplementation. In the third trimester, ultrasound examination revealed a low-lying placenta, requiring increased surveillance. At 39 weeks gestation, the patient presented with vaginal bleeding and was diagnosed with partial placental abruption.

Pelvimetry indicated cephalopelvic disproportion, prompting an emergency cesarean section. The procedure was uncomplicated, resulting in the delivery of a healthy female newborn weighing 3.6 kg and measuring 52 cm. The infant's Apgar scores were 8 at one minute and 9 at five minutes.

Postoperative laboratory tests showed anemia with reduced hemoglobin and hematocrit levels, for which she received two units of packed red blood cells and two units of fresh frozen plasma. The patient's hematologic parameters subsequently improved, and her postoperative course was uneventful. Both mother and newborn were discharged home in stable condition five days postoperatively.

Four years after her first delivery, the patient conceived naturally for the second time. The pregnancy was uneventful, with continued vitamin and iron supplementation.

Due to the prior cesarean delivery, delivery was again performed by cesarean section. The patient delivered a healthy male newborn weighing 3.6 kg and measuring 53 cm. The infant's Apgar scores were 10 at both one and five minutes. Both surgical and postoperative courses were uneventful, and the patient and her newborn were discharged home four days after delivery.

DISCUSSION

Pregnancy after allo-HSCT is uncommon but possible, especially in younger patients treated for non-malignant disorders such as AA and with conditioning regimens that exclude total body irradiation(7).

After allo-HSCT, ovarian failure occurs in over 90% of women when myeloablative chemotherapy or total body irraditon is used, particularly in patients older than 25 (9). Current data primarily come from small cohorts where recoveries of fertility and spontaneous conception have been documented, typically within 4 to 6 years post-transplant (10). Larger and long-term studies are still needed to better understand reproductive outcomes following various conditioning regimens.

In our patient, regular menstrual cycles persisted post-transplant, likely reflecting preserved ovarian reserve due to the absence of total body irradiation and relatively young age at allo-HSCT. Both pregnancies occurred spontaneously at five and nine years post-transplant, with no conception assistance or fertility preservation measures required. This aligns with evidence that AA patients treated with reduced-intensity or chemotherapy-only regimens have a higher chance of fertility recovery (9).

In summary, this case underscores that natural fertility may persist in selected AA survivors following allo-HSCT when high-risk conditioning modalities are avoided. It highlights the need for multidisciplinary long-term follow-up and individualized fertility counseling for female transplant recipients. Although pregnancy after allo-HSCT is uncommon, this case demonstrates that it is indeed possible.

CONCLUSION

This case illustrates that natural fertility can be preserved in selected female patients following allo-HSCT, particularly when irradiation is avoided and the patient is younger at the time of transplantation. From a gynecological perspective, preserved regular menstrual cycles after transplant are an encouraging sign of ovarian function retention. Nevertheless, pregnancies after allo-HSCT should be considered high-risk and require multidisciplinary management to address potential obstetric complications such as placental abnormalities and postpartum hematologic challenges. Awareness and individualized counseling regarding reproductive potential and pregnancy risks are critical components of post-transplant care.

MESSAGE FROM THE AUTHORS

Through this case report, we hope to inspire and encourage all women who have undergone allo-HSCT and still dream of becoming mothers.

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Treatment of Metastatic Colon Cancer

Laparoskopsko-onkološki tretman metastatskog karcinoma kolona

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ABSTRACT

Colorectal cancer (CRC) is the third most common cancer worldwide (right after breast and lung cancer), of which males rank third and females rank second. CRC is also the second most common cause of cancer mortality worldwide with more than 1.9 million new cases and 0.9 million deaths registered in 2020. Case report: patient EA (61) arrived to the Clinic of Abdominal Surgery of the Clinical Center University of Sarajevo (CCUS) for the surgical treatment of sigmoid colon cancer, following the recommendation of the surgical-oncology council. Given the diffuse lesions in the liver, a laparoscopic resection of the rectosigmoid colon according to Hartman was performed and the patient was referred to oncological treatment, during which the number and size of metastatic changes in the liver gradually decreased, which was confirmed radiologically through 3 CT scans of the abdomen during the year following the operation. Changes in the liver would primarily correspond to metastatic lesions that were less than 20% according to the RECT criteria. The patient was presented again at the surgical-oncology council, and metastasectomy of the above-mentioned changes was indicated. The operation was also performed laparoscopically and the changes were sent for PH analysis; the definitive finding did not establish the presence of tumor tissue, but non-specific granulomatous inflammation of the liver - complete oncological response to adequate therapy. Following the irrigography, which did not confirm the presence of a recurrence, a laparoscopic Hartman's reversal was performed. Conclusion: a review of the institutional surgical database indicated that laparoscopic Hatrman's reversion had not previously been performed at the CCUS. Combined systemic therapy (CAPEIRI + bevacizumab) and selective liver metastasectomy could lead to partial regression or complete resected status of metastases in patients with mCRC.

Keywords: colorectal cancer, metastases, Hartman's operation, laparoscopic Hartman's reversion

SAŽETAK

Rak debelog crijeva (KRK) treći je najčešći rak u svijetu (odmah nakon raka dojke i pluća), od čega su muškarci treći, a žene drugi. KRK je također drugi najčešći uzrok smrtnosti od raka u svijetu s više od 1,9 milijuna novih slučajeva i 0,9 milijuna smrtnih slučajeva registriranih u 2020. godini. Prikaz slučaja: pacijent E.A (61) dolazi u Kliniku za abdominalnu kirurgiju Kliničkog centra Univerziteta u Sarajevu (KCUS) radi kirurškog liječenja raka sigmoidnog debelog crijeva, na preporuku kirurško-onkološkog konzilija. S obzirom na difuzne lezije u jetri, izvodi se laparoskopska resekcija rektosigmoidnog debelog crijeva po Hartmanu i pacijentica se upućuje na onkološko liječenje, tijekom kojeg se broj i veličina metastatskih promjena u jetri postupno smanjuju, što se radiološki potvrđuje kroz 3 CT snimke abdomena tijekom sljedeće godine od operacije. Promjene u jetri prvenstveno će odgovarati metastatskim lezijama koje su manje od 20% prema RECT kriterijima. Pacijent se ponovno predstavlja na kirurško-onkološkom konzilijumu, te je indicirana metastazektomija gore navedenih promjena. Operacija se također izvodi laparoskopski i promjene se šalju na PH analizu; definitivan nalaz ne utvrđuje prisutnost tumorskog tkiva, već nespecifične granulomatozne upale jetre - potpuni onkološki odgovor na adekvatnu terapiju. Nakon provedene irigografije, koja ne potvrđuje prisutnost recidiva, izvodi se laparoskopska reverzija Hartmana. Zaključak: pretraživanjem hirurške baze podataka u našoj ustanovi sugeriše da laparoskopska reverzija Hartmana nije ranije rađena u KCUS-u. Kombinirana sistemska terapija (CAPEIRI + bevacizumab) i selektivna metastazektomija jetre mogu dovesti do djelomične regresije ili potpunog reseciranog statusa metastaza u bolesnika s mCRC-om.

Ključne riječi: kolorektalni karcinom, metastaze, Hartmanova operacija, laparoskopska reverzija Hartmana

INTRODUCTION

Epidemiology: colorectal cancer (CRC) is the third most common cancer worldwide (right after breast and lung cancer), of which males rank third and females rank second. CRC is also the second most common cause of cancer mortality worldwide with more than 1.9 million new cases and 0.9 million deaths registered in 2020. When it comes to the incidence, the differences both in distribution and time variations between different countries are noted, which could be a result of the differences in risk factors among populations (1). Recent studies show that the incidence and mortality of CRC increases with age - both indicators are low until the age of 45 and increase significantly after that, peaking in the age group over 80. Still, a significant number of cases occur also in the younger population (2,3,4).

Symptoms: recent studies show that not all patients are sympto matic at diagnosis, but the most common presenting symptoms are usually changes in bowel habits and rectal bleeding followed by abdominal pain. Some of the other red flag signs and symptoms which CRC patients present with are diarrhea and iron deficiency anemia (5).

Diagnosis and follow-up: diagnostic evaluation of colorectal cancer typically involves a combination of endoscopic, imaging, and laboratory procedures. Structural examinations such as colonoscopy remain the gold standard for initial tumor detection and histopathological confirmation, while cross-sectional imaging with contrast-enhanced computed tomography (CT) plays a central role in staging and assessment of disease extent, including evaluation for regional and distant metastases. CT imaging provides valuable information on tumor size, local invasion, lymphadenopathy, and metastatic spread, and is routinely used in treatment planning and follow-up (6,7).

Treatment: modern treatment strategies for CRC include elimination of underlying infections, surgical intervention, chemotherapy, radiation therapy and targeted therapy. Surgery though serves as the cornerstone of curative treatment and is commonly complemented by chemotherapy, especially in patients with verified liver metastases. It can be performed through either open or laparoscopic techniques, but it is essential to assess the tumor extent prior to the surgical procedure (4).

CASE REPORT

Patient EA (61) reported to the Clinic of Abdominal Surgery of the CCUS for the surgical treatment of sigmoid colon cancer, on the recommendation of the surgical-oncology council.

The tumor was discovered "accidentally", 2 months before the admission The patient fell from a height, and as part of the diagnostic work-up, a CT scan of the abdomen and pelvis was performed, which indicated an infiltrative tumor process in the central part of the sigmoid colon and a diffuse focal lesion in the enlarged liver, of a secondary nature. As part of the further diagnostic workup, a colonoscopy with biopsy was performed and a pathohistological diagnosis of moderately differentiated adenocarcinoma, G2, was obtained.

Given the diffuse lesions in the liver, a laparoscopic resection of the rectosigmoid colon according to Hartman was performed and the patient was referred to oncological treatment, during which the number and size of metastatic changes in the liver gradually decrease, which was confirmed radiologically through 3 CT scans of the abdomen during the following year from the operation. Changes in the liver primarily corresponded to metastatic lesions that were less than 20% according to the RECIST criteria.The patient was presented again at the surgical-oncology council, and metastasectomy of the above-mentioned changes was indicated. The operation was also performed laparoscopically and the changes were sent for PH analysis; the definitive finding did not establish the presence of tumor tissue, but non-specific granulomatous inflammation of the liver - complete oncological response to adequate therapy, in sense of complete reduction of the metastases.

After the performed irrigography, which did not confirm the presence of a recurrence, a laparoscopic Hartman reversal was performed (a BIS search does not find information about a previously performed laparoscopic Hartmann reversal at the CCUS). The postoperative course was smooth. Intestinal passage was established spontaneously and the patient was discharged from hospital.

Figure 1 **Carcinoma with the part of left colon.**

Patient's oncologicals status and follow-up

Following histopathological verification in September 2024, oncological treatment was initiated. After the primary surgical resection of the rectosigmoid colon, followed by adjuvant chemotherapy and targeted biological therapy, a second surgical procedure was performed in October 2025: Exploratio LAP; Metastasectomia segmenti III,VI,VIII at the Department of Abdominal Surgery, KCUS.

Postoperative evaluation, primarily based on CT imaging of the abdomen and pelvis, was conducted within one month of the surgery (CT performed on 21 November 2025) and demonstrated radiological regression of the disease. The CT scan showed no evidence of recurrence in the rectal stump. Additionally, previously identified metastatic lesions remained stable in size and number compared with the preoperative CT scan of the abdomen and pelvis (CT performed on 2 September 2025). Postoperative chest CT revealed normal pulmonary parenchymal structures, with no nodular or micronodular density changes observed.

Following surgical and systemic treatment, a favorable biochemical response was observed, corresponding with the documented radiological regression. Carcinoembryonic antigen (CEA) decreased from 5,455.72 ng/mL to 2.99 ng/mL, while CA 19-9 (9.40 U/mL) and CA-125 (12.9 U/mL) remained within low, reference-range values.

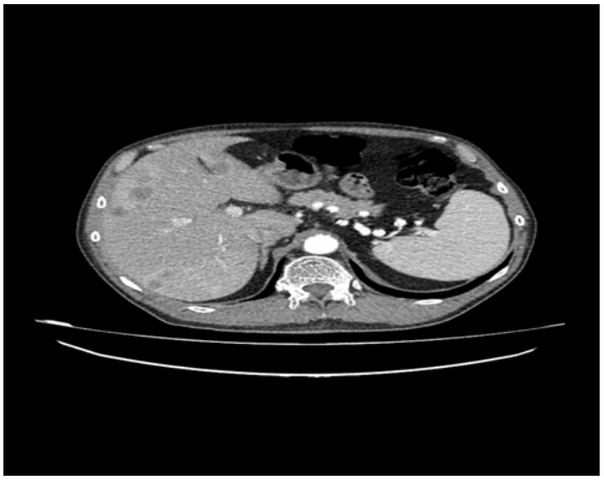

Figure 2 **CT scan of the liver with multiple metastasis (before the first operation).**

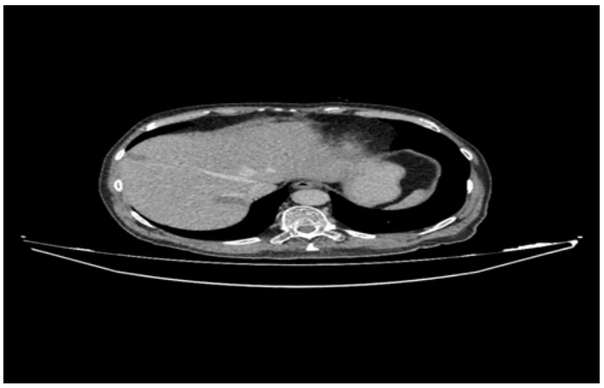

Figure 3 **CT scan of the liver with reduction of metastasis in size and numbe (after oncological tretment, prior to mestastasectomy).**

DISCUSSION

Only 40-60% of patients who undergo Hartman's operation ever have their stoma reversed. Among patients undergoing reversal, laparoscopy is used in approximately 30-70% of cases in modern tertiary centers. Conversion to open surgery occurs in 10-25% of cases. The adoption of laparoscopy has increased steadily over the past 15-20 years due to improved surgical expertise and equipment. Several large retrospective cohort studies and meta-analyses demonstrate increasing use of minimally invasive approaches, particularly in high-volume colorectal units. Compared with open reversionl, laparoscopic reversion offers multiple advantages; reduced postoperative pain, shorter hospital stay, lower wound infection rate, reduced adhesion-related trauma, faster return of bowel function, cosmetic advantage. Meta-analyses demonstrate lower morbidity rates, comparable anastomotic leak rates, similar mortality compared to open surgery (8-13).

CONCLUSION

Laparoscopic Hartman's reversion is a safe and effective technique in appropriately selected patients. A review of the institutional surgical database indicated that laparoscopic Hatrman's reversion had not been previously performed at the CCUS. Combined systemic therapy (CAPEIRI + bevacizumab) and selective liver metastasectomy can lead to partial regression or complete resected status of metastases in patients with mCRC

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Giant ovarian cyst

Velika cista jajnika

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ABSTRACT

Ovarian cyst is a relatively common gynecological pathology that occurs in women of all ages. Most cysts are benign, small in size and asymptomatic, regress spontaneously, and are discovered incidentally during gynecological examinations or radiological diagnostics for other reasons. However, in rare cases, the cyst can reach exceptional dimensions and cause serious symptoms due to compression of surrounding organs. Giant ovarian cysts are most often defined in the literature as those whose diameter is greater than 10 cm or that extend beyond the pelvic cavity into the abdomen, often occupying a large part of the abdominal cavity. Rare cases have been reported in which ovarian cysts contain over 20 liters of fluid. In developed health systems, giant ovarian cysts have become rare due to routine ultrasound diagnostics and early treatment. Case report: neglect of symptoms, socioeconomic barriers, fear of medical intervention or limited access to health care contribute to the late appearance and progression of such lesions. Due to their rarity and serious clinical consequences, the presentation of such cases has educational and clinical value. This case report documents a rare case of a giant ovarian cyst with over 35 liters of fluid. It manifests with marked abdominal distension and respiratory failure in a patient who has not sought medical help for years. Conclusion: This case highlights the importance of early diagnosis, careful preoperative preparation, and the key role of a multidisciplinary approach in the care of such patients. It is precisely the anesthetic challenges that were the dominant aspects of care, and emphasizes the importance of planning induction, controlled decompression, and postoperative monitoring.

Keywords: giant ovarian cyst, rare case

SAŽETAK

Ovarijalna cista je relativno česta ginekološka patologija koja se javlja kod žena svih životnih dobi. Većina cista je benigne prirode, malih dimenzija i asiptomatska, spontano regredira, te se otkriju slučajno prilikom ginekoloških pregleda ili radiološke dijagnostike iz drugih razloga. Međutim, u rijetkim slučajevima, cista može dostići izuzetne dimenzije i uzrokovati ozbiljne simptome usljed kompresije okolnih organa. Gigantske ovarijalne ciste su u literaturi najčešće definisane kao one čiji je prečnik veći od 10 cm ili koje se šire izvan zdjelične šupljine u abdomen, često zauzimajući veći dio trbušne šupljine. Rijetki su zabilježeni slučajevi u kojima ovarijalne ciste sadrže preko 20 litara tečnosti. U razvijenim zdravstvenim sistemima, gigantske ovarijalne ciste postale su rijetkost zahvaljujući rutinskoj ultrazvučnoj dijagnostici i ranom liječenju. Prikaz slučaja: zanemarivanje simptoma, socioekonomske barijere, strah od medicinske intervencije ili ograničen pristup zdravstvenoj zaštiti doprinose kasnom javljanju i progresiji ovakvih lezija. Zbog rijetkosti i ozbiljnih kliničkih posljedica prikaz ovakvih slučajeva ima edukativnu i kliničku vrijednost. Ovaj prikaz slučaja dokumentuje rijedak slučaj gigantske ovarijalne ciste sa preko 35 litara tečnosti. Manifestuje se izraženom abdominalnom distenzijom i respiratornom insuficijencijom kod pacijentkinje koja godinama nije tražila medicinsku pomoć. Zaključak: ovaj slučaj naglašava važnost rane dijagnostike, pažljive preoperativne pripreme, kao ključne uloge multidisciplinarnog pristupa u zbrinjavanju ovakvih pacijenta. Upravo anesteziološki izazovi bili su dominantni aspekti zbrinjavanja, te naglašava važnost planiranja indukcije, kontrolisane dekompresije i postoperativnog praćenja.

Ključne riječi: gigantska ovarijalna cista, rijetki slučaj.

CASE REPORT

A 72-year-old female patient, with no significant previous medical or surgical history, presented to the Clinic of Emergency Medicine, Clinical Center University of Sarajevo (CCUS), due to several years of progressive abdominal distension and recent worsening of difficulty breathing, especially in the supine position. The history was taken with the help of an escort, a cousin who stated that the patient had occasional "hallucinations". She stated that she had never previously sought medical help due to an increase in abdominal circumference, because she had no pronounced symptoms, she refused to be examined by a doctor. On examination, she was conscious, communicative, oriented, afebrile, dyspnoeic at rest, bilaterally weakened breath sounds, RR 140/90 mmHg, abdomen significantly above the level of the chest, tense anterior abdominal walls, with pronounced venous pattern, pretibial edema with hypostatic changes. A chest X-ray was performed, showing marked abdominal distension, which caused elevation of the diaphragm and reduced respiratory expansion capacity (Figure 1).

Figure 1 **Markedly enlarged shadows of the heart with widened tracheal hili, accentuated bronchovascular drawing with discrete patchy changes in the lung parenchyma, possibly of tracheal nature.**

A computerized tomography (CT) of the abdomen and pelvis was attempted, but the examination was not possible because the patient's abdominal dimensions could not pass through the gantry of the apparatus. The patient is referred to a gynecologist who, on ultrasound examination, finds that the abdomen and small pelvis are filled with a tumorous mass filled with thick contents with septa. Uterus of regular dimensions, thin endometrium, no adnexa, and cavum Douglas free. The patient had no births or abortions. Stools rare, difficulty urinating. Denies food and drug allergies. Laboratory findings in reference values, except: INR 0.83; D-Dimer 2.72 mg/L; Troponin 52 ng/L; CK 1260 U/L; CKMB 185 U/L; urea 8.1 mmol/L; AST 40 U/L; Ca 2.16 mmol/L; LDH 313 U/L; CRP 18.7 mg/L. ABS capillary, without oxygen support: pH 7.421; BE 4.8 mmol/L; HCO3-act 30.2 mmol/L; pCO2 6.28 kPa; pO2 6.18 kPa; SO2 80.5%.

The patient was hospitalized at the Clinic of Gynecology and Obstetrics of the CCUS. Preoperative preparation included a multidisciplinary approach with consultations with a cardiologist and a pulmonologist to assess perioperative risk and optimize the patient's general health (Figure 2).

Figure 2 **Preoperatively distended abdomen, giant ovarian cyst.**

The cardiologist confirmed stable hypertension, without signs of heart failure. The patient was initiated by the pulmonologist on systemic corticosteroid and bronchodilator therapy with antibiotics and anticoagulant prophylaxis. The anesthesiologist assessed the perioperative risk as high due to possible ventilatory and hemodynamic complications. Gradual decompression of the cyst during surgery, continuous hemodynamic monitoring, and preparation for postoperative admission to the intensive care unit were recommended.

After positioning and disinfection of the operative field, general endotracheal anesthesia was initiated with special care, with prior preoxygenation and prepared vasopressors and cardiotonics. A laparotomy was performed, and the entire abdomen and pelvis were filled with a smooth capsule tumor originating from the left adnexa. Controlled aspiration of approximately 35 L of dark gelatinous fluid from the ovarian cyst was performed, which enabled safer manipulation and extraction of the entire tumor mass, extirpation of the uterus and right adnexa, morphologically normal. The intraoperative course was without significant complications, with stable hemodynamic parameters. The patient was extubated in the operating room and transferred to the intensive care unit for continuous non-invasive monitoring of vital parameters, heart rate approximately 90/min, normotensive, oxygen support via nasal catheter 2-4 L/min.

Postoperative CT scan of the thoracic organs indicates extensive atelectasis of the lower lobe on the right, which is completely involved, and marginal atelectasis also of the posterior parts of the lower lobe on the left, bilateral pleural effusion visible in the pleural space on the left up to 27 mm AP diameter, on the right up to 25 mm AP diameter.

Pathological and histological examination: mucinous cystadenoma of intestinal type, borderline malignant potential, with a focus of intraepithelial carcinoma and a focus of well-differentiated mucinous adenocarcinoma (G1) of the left ovary measuring 385x275x180 mm, smooth outer surface with pronounced vascular pattern without papillary proliferations. AJCCTNM: pT1a Nx Mx Ro.

The postoperative course of our patient was initially stable, hemodynamically compensated, with the need for oxygen support. Supportive therapy, parenteral fluid replacement, correction of hypoproteinemia (serum albumin 27g/L), antibiotic and anticoagulant prophylaxis, and gastroprotectants were included. Drainage of pleural effusions was not indicated, and treatment was focused on diuretics and respiratory physical therapy.

Despite the multidisciplinary approach, the patient died on the 6th postoperative day, which emphasizes the seriousness of the clinical picture and the long-term consequences of massive abdominal pathology on cardiorespiratory function. Complications are rapid and fatal.

DISCUSSION

Giant ovarian cysts represent a particular challenge not only for anesthesiologists, but also for gynecologists and the entire multidisciplinary team. Their massive size can significantly compromise the respiratory function, hemodynamics and metabolic status of the patient. Elevation of the diaphragm leads to reduced lung volumes, hypoventilation and an increased risk of hypoxia during induction and maintenance of general anesthesia. From a hemodynamic perspective, compression of large blood vessels can cause reduced venous return and large blood pressure oscillations during intraoperative manipulation and decompression of the cyst. Abrupt evacuation of a large amount of fluid can cause acute circulatory oscillations including hypotension and collapse. In addition, in these patients, the anesthesiologist must anticipate difficulties in positioning the patient on the operating table and postoperative monitoring in the intensive care unit. Therefore, preoperative evaluation by a cardiologist, pulmonologist, and anesthesiologist is essential for safe perioperative management (5,6). According to the literature, although most patients with benign ovarian cysts have a favorable outcome, in giant cysts the perioperative mortality rate can be significantly higher (estimated at 5-15% in cases with a mass greater than 20 L) depending on the tumor volume. The most common causes of mortality are related to respiratory complications, sepsis, and hemodynamic decompensation.

These operations carry an exceptionally high perioperative risk and a great challenge for the anesthesiologist, due to chronic abdominal compression, problems with ventilation, diaphragmatic displacement due to redistribution of intra-abdominal and thoracic pressure, reduction of lung volumes, as well as sudden hemodynamic changes are possible.

From an anesthesiological aspect, our case clearly indicates the complexity of perioperative care of patients with giant intra-abdominal tumors. This case emphasizes that anesthesia supervision in the postoperative period is as important as intraoperative anesthesia management.

The importance of early diagnosis and timely treatment, although technically the operation can proceed without intraoperative complications, as well as stable early postoperative recovery (7-9).

CONCLUSION

In conclusion, this outcome points to the important fact that despite carefully administered anesthesia, stable intraoperative and immediate postoperative course, and timely multidisciplinary support, the outcome of such cases largely depends on timely diagnostic and therapeutic approach. Early recognition and earlier surgical treatment of giant ovarian cysts could significantly reduce the mortality rate, which in the literature remains high in patients with advanced clinical stage and respiratory complications.

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PREPARATION OF MANUSCRIPT

Article should be no longer than 10 computer pages, including figures, graphs, tables and references. The article may be submitted as a CD disk (Word Windows), or e-mail.
Spacing: 1,5: left margin: 2,5 cm; right margin: 2,5 cm; top and bottom margin: 2,5 cm.
Graphs, tables, figures and drawings should be incorporated in the text, precisely in the text, where these will be published, regardless of the program in which they are prepared. Articles are written in-extenso in English language.
The manuscript should be submitted on a good quality CD disc, or by e-mail, together with two printed copies (if possible). Sent CD disks will not be returned to the authors.

ARTICLE CONTAINS:

TITLE OF THE ARTICLE IN ENGLISH LANGUAGE
TITLE OF THE ARTICLE IN BOSNIAN/SERBIAN/CROATIAN (B/S/C) LANGUAGE
First and last name of the author/co-author(s)

Name and address of the institution in which author/co-authors is employed (same for all authors) in B/S/C and English language as well as the address of corresponding author at the end of the article.

Summary in B/S/C language with the precise translation in English. Abstract of approximately 200-250 words should concisely describe the contents of the article.
Key words (in B/S/C and in English language): up to five words should be listed under the Abstract.

ARTICLE BODY

The main body of the article should be systematically ordered under the following headings:
- **INTRODUCTION**
- **MATERIALS AND METHODS**
- **RESULTS**
- **DISCUSSION**

- **CONCLUSION**
- **REFERENCES**

INTRODUCTION

Introduction is a concise, short part of the article, and it contains purpose of the article relating to other published articles with the same topic. It is necessary to quote the main problem, aim of investigation, and/or main hypothesis which is investigated.

MATERIALS AND METHODS

This part should contain description of original or modification of known methods. If there is a method that has previously been described, it would be sufficient to include it in the reference list. In clinical and epidemiological studies the following should be described: sample, protocol and type of clinical investigation, place and period of investigation. Main characteristics of investigation should be described (randomization, double-blind test, cross test, placebo test), standard values for tests, time framework (prospective, retrospective study), selection and number of patients – criteria for inclusion and exclusion from the study.

RESULTS

Main results of investigation and level of its statistical significance should be quoted. Results should be presented in tables, graphs, figures, and directly incorporated in the text, at the exact place, with ordinal number and concise heading. Table should have at least two columns and explanation; figures clean and contrasted, graphs clear, with visible text and explanation.

DISCUSSION

Discussion is concise and refers to own results, in comparison with the other authors' results. Citation of references should follow Vancouver rules. Discussion should be concluded by the confirmation of the stated aim or hypothesis, or by its negation.

CONCLUSION

Conclusion should be concise and should contain most important facts, which were obtained during investigation and its eventual clinical application, as well as the additional studies for the completed application. Affirmative and negative conclusions should be stated.

REFERENCES – Instructions for writing references

References should follow the format of the requirements of **Vancouver rules**.

Every statement, knowledge and idea should be confirmed by reference. Each reference in the text is given its own sentence case in Arabic number in parenthesis at the end of the sentence according to the order of entering. Every further referring to the same reference, number of the first referring in the text should be stated. References are to be placed at the end of the article, and are to be numbered by ordinal numbers in the order of entering in the text (entering reference number). Journal's title is abbreviated using Index Medicus abbreviations. The names of the first six authors of each reference item should be provided, followed by "et al.".

It is very important to properly design references according to instructions that may be downloaded from addresses National Library of Medicine Citing Medicine http://www.ncbi.nlm.nih.gov/books/bv.fcg?rid=citmed.TOC&depth=2,

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Radovi ne smiju biti duži od deset stranica na računaru, ubrajajući slike, grafikone, tabele i literaturu. CD zapis teksta je obavezan (Word of Windows), ili e-mail.
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RAD SADRŽI:

NASLOV RADA NA ENGLESKOM JEZIKU
NASLOV RADA NA NAŠEM JEZIKU

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Sažetak na našem jeziku, kao i na engleskom - max. 200–250 riječi, s najznačajnijim činjenicama i podatcima iz kojih se može dobiti uvid u kompletan rad.
Ključne riječi - Key words, na našem jeziku i na engleskom, ukupno do pet riječi, navode se ispod Sažetka, odnosno Abstracta.

SADRŽAJ

Sadržaj rada mora biti sistematično i strukturno pripremljen i podijeljen u poglavlja i to:
- **UVOD**
- **MATERIJAL I METODE**
- **REZULTATI**
- **DISKUSIJA**
- **ZAKLJUČAK**
- **LITERATURA**

UVOD

Uvod je kratak, koncizan dio rada i u njemu se navodi svrha rada u odnosu na druge objavljene radove sa istom tematikom. Potrebno je navesti glavni problem, cilj istraživanja i/ili glavnu hipotezu koja se provjerava.

MATERIJAL I METODE

Potrebno je da sadrži opis originalnih ili modifikaciju poznatih metoda. Ukoliko se radi o ranije opisanoj metodi dovoljno je dati reference u literaturi. U kliničko-epidemiološkim studijama opisuju se: uzorak, protokol i tip kliničkog istraživanja, mjesto i vrijeme istraživanja. Potrebno je opisati glavne karakteristike istraživanja (npr. randomizacija, dvostruko slijepi pokus, unakrsno testiranje, testiranje s placebom itd.), standardne vrijednosti za testove, vremenski odnos (prospektivna, retrospektivna studija), izbor i broj ispitanika – kriterije za uključivanje i isključivanje u istraživanje.

REZULTATI

Navode se glavni rezultati istraživanja i nivo njihove statističke značajnosti. Rezultati se prikazuju tabelarno, grafički, slikom i direktno se unose u tekst gdje im je mjesto, s rednim brojem i konciznim naslovom. Tabela treba imati najmanje dva stupca s obrazloženjem što prikazuje; slika čista i kontrastna, a grafikon jasan, s vidljivim tekstom i obrazloženjem.

DISKUSIJA

Piše se koncizno i odnosi se prvenstveno na vlastite rezultate, a potom se nastavlja upoređivanje vlastitih rezultata s rezultatima drugih autora, pri čemu se citiranje literature navodi po važećim Vankuverskim pravilima. Diskusija se završava potvrdom zadatog cilja ili hipoteze, odnosno njihovim negiranjem.

ZAKLJUČAK

Treba da bude kratak, da sadrži najbitnije činjenice do kojih se došlo u radu tokom istraživanja i njihovu eventualnu kliničku primjenu, kao i potrebne dodatne studije za potpuniju aplikaciju. Obavezno navesti i afirmativne i negirajuće zaključke.

LITERATURA - Upute za citiranje - pisanje literature

Literatura se obavezno citira po **Vankuverskim pravilima**.

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